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INTRAMOLECULAR DISTRIBUTION OF URIC ACID-N^{15} AFTER ADMINISTRATION OF GLYCINE-N^{15} AND AMMONIUM-N^{15} CHLORIDE TO GOUTY AND NONGOUTY SUBJECTS *

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Thus far, administration of N^{15}- or C^{14}-labeled uric acid precursors to patients with primary gout has revealed an unequivocal metabolic aberration (namely, grossly increased total uric acid-N^{15} or -C^{14}) only in some cases (3–8), estimated to represent roughly 30 per cent of the gouty population (9). This group of gouty subjects is further characterized by greatly enhanced uric acid excretion while maintaining enlarged uric acid pools—clear evidence of production of uric acid considerably in excess of the normal. In the remaining gouty subjects studied, apparently representative of the majority of cases of primary gout, the available isotope data either reveal lesser increases in utilization of isotope-labeled precursors for uric acid biosynthesis, manifest only when appropriate corrections are made for enlarged uric acid pools and increased uric acid excretion into the gut (10), or fail to disclose any distinct metabolic abnormality whatever.

In the isotope studies made to date, measurements have been limited almost exclusively to total uric acid-N^{15} or -C^{14}; i.e., the mean isotope enrichment of all four nitrogens or all five carbons, collectively, of the uric acid molecule. The present study extends such measurements to analysis of the intramolecular distribution of uric acid-N^{15}, and explores further the possibility of abnormal distribution of isotope within the uric acid molecule even when the total uric acid isotope abundance approximates that found in nongouty subjects. Dissection of the uric acid molecule in this way can be particularly revealing in gout now that the sequential enzymatic reactions effecting placement of each atom in purine biosynthesis are securely established (11).

Data on the intramolecular distribution of the isotope incorporated into uric acid in normal man were recorded in one subject given glycine-N^{15} by Shemin and Rittenberg (12) who found that most of the uric acid-N^{15} appeared at N–7 but that a substantial proportion, increasing from 23 per cent on day 1 to 41 per cent on day 14, was present also in the remaining nitrogens, 1, 3, and 9 (Figure 1).[1] The predominant N^{15} enrichment of N-7 indicated that glycine contributes N-7 (and C-4 and C-5) of the uric acid molecule (Figure 1), by a reaction now known to combine intact glycine with 5-phosphoribosyl-1-amine to form glycinamide ribonucleotide (11). The N^{15} enrichment of the three remaining nitrogen atoms implied that appreciable quantities of nitrogen derived from glycine are utilized in normal man to provide N-9 and N-3 by way of the amide nitrogen of glutamine, and N-1 through the amino nitrogen of aspartic acid (11) (Figure 1).

Recent studies with glycine-1-C^{14}-N^{15} suggest that these latter more indirect metabolic pathways for utilization of glycine nitrogen—i.e., involving other than direct incorporation of intact glycine—may be of even greater quantitative importance in uric acid formation in gout (8). When glycine-1-C^{14}-N^{15} was administered to two gouty uric acid "overexcretors," not only was incorporation of both N^{15} and C^{14} greater than normal but the N^{15}/C^{14} ratios obtained ranged from about 2.0 to 3.0, as compared with normal ratios of 1.3 to 1.7 calculated from the data of Shemin and Rittenberg (12) [2] and 1.4 to 1.7 and 1.6 to 2.0 found in two nongouty subjects (8). N^{15}/C^{14}

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[1] Seegmiller, Laster and Stetten (J. biol. Chem. 1955, **216**, 653) likewise demonstrated N^{15}-(7+9)/N^{15}-(1+3) ratios of about 5 on the second day after glycine-N^{15} administration to two normal human subjects.

[2] This calculation assumes that all C-4 is derived from the carboxyl carbon of intact glycine in equimolecular proportion to the N^{15}-labeling of N-7.

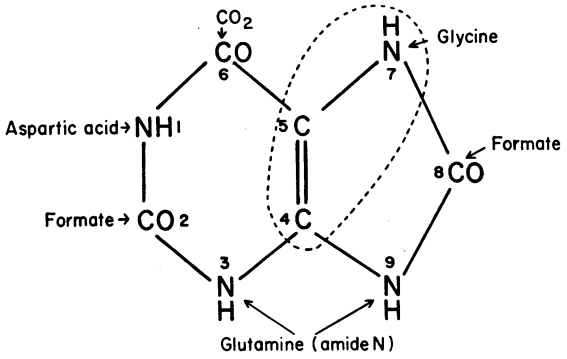

FIG. 1. URIC ACID, SHOWING SOURCE OF EACH NITROGEN AND CARBON ATOM. N-7, C-5, and C-4 are enclosed by a broken line to indicate that glycine is the common source.

ratios of 2.0 to 3.0 imply (8) that the disproportion between glycine nitrogen and glycine carbon utilized for purine biosynthesis in these gouty overexcretors was even greater than in normal man, because more of the nitrogen was incorporated into N–(1 + 3 + 9), or recycled back into N–7, or both. The present analysis indicates that both of these processes are in fact operative.

Glycine-1-C^{14}-N^{15} has been given also to a gouty subject whose urinary uric acid excretion was within normal limits (8). The uric acid-N^{15} and -C^{14} incorporation, too, appeared to be within the limits of normal variations, but the N^{15}/C^{14} ratios again were high, approximating 2.0. In this instance, uric acid samples were degraded and N^{15}-7 and N^{15}–(1 + 3 +9) separately determined. Increased initial N^{15}–(1 + 3 + 9)/total uric acid-N^{15} ratios and high N^{15}-7/total C^{14} ratios suggested that in this gouty "normal excretor" too, more than normal glycine nitrogen was being diverted into pathways leading to N-9, N-3, and N-1, and recycled back into N-7.

The rather arbitrary classification of gouty subjects according to urinary uric acid output is based on the following criteria (8) : gouty "overexcretors" are defined as those whose 24-hour urinary uric acid excretion on a basal diet exceeded our normal mean plus 3 SD (i.e., > 628 mg) ; those whose daily output was less are designated "normal excretors."

The present report includes data on the intramolecular distribution of uric acid-N^{15} in four normal subjects, four gouty uric acid overexcretors and one additional gouty normal excretor, all given glycine-N^{15}. Summarized also are data on uric acid-N^{15} and its intramolecular distribution after administration of ammonium-N^{15} chloride to two normal subjects, to three of the four gouty overexcretors given glycine-N^{15} some years before, and to three gouty normal excretors of whom two had previously received glycine-N^{15}. The objective in giving ammonium-N^{15} chloride was to study incorporation of N^{15} into N-9 and N-3 of the uric acid molecule more directly, since ammonia nitrogen is rapidly and extensively utilized for the synthesis of glutamine (13) through the action of glutamic dehydrogenase and glutamine synthetase.

METHODS

Data on uric acid-N^{15} and uric acid-C^{14} after administration of glycine-C^{14}-N^{15} have been previously recorded (8) for gouty subjects C.W., P.C., H.G., and A.R., and nongouty subjects T.G. and L.C.; also the details of the clinical status and dietary regulation of these subjects, the dosage of isotope-labeled glycine given, and the methods of isolation of uric acid from the urine and of measurements of specific activity. Similar procedures were followed in gouty subjects D.R. and I.S. and normal subjects L.B. and M.A., who were given glycine-N^{15} and are added to the present series. N^{15}-7 and N^{15}–(1+3+9) were determined after acid hydrolysis of uric acid samples (14).

Six gouty subjects (C.W., P.C., H.G., A.R., I.S., G.H.) were given a single oral dose of 6.5 g ammonium-N^{15} chloride (N^{15} = 64 atom per cent excess), the approximate N^{15} equivalent of the glycine-N^{15} dosage they had received 2 or 3 years before. For G.H., who weighed 127 kg, this dose was too low and he was therefore subsequently given 13.0 g ammonium-N^{15} chloride. Normal subjects R.W. and N.J. received 6.5 g ammonium-N^{15} chloride. Urinary uric acid samples were obtained under the same conditions as for the glycine-N^{15} experiments (8). In addition to N^{15}-7 and N^{15}–(1+3+9) determinations (14), N^{15}–(1+3) and N^{15}–(7+9) were estimated after oxidation of uric acid samples with potassium chlorate in acid solution (14). From the data so obtained the N^{15} atom per cent excess of each of the four uric acid nitrogens could be calculated.

The daily urinary excretion of total nitrogen, urea nitrogen, ammonia (and the corresponding urinary total N^{15}, urea-N^{15}, and ammonium-N^{15}), uric acid, and creatinine was measured by the methods previously employed (3, 4, 8).

RESULTS

1. Glycine-N^{15} incorporation into uric acid

A. Total uric acid-N^{15}. In four normal subjects, T.G., L.C., L.B., and M.A., the uric acid

total N^{15} incorporation rose to a small peak of 0.07 to 0.14 atom per cent excess on day 2 or 3 after administration of glycine-N^{15}. The values then declined somewhat to a plateau that persisted throughout the 2 weeks of observation (Table I, Figure 2). The cumulative urinary uric acid-N^{15} over the first week averaged 0.12 per cent of dose.

Patients D.R., C.W., P.C., and H.G., all gouty overexcretors, showed the characteristic high uric acid-N^{15} on day 1, varying from 0.20 to 0.29 atom per cent excess, with somewhat higher peak levels on day 2 (Table I, Figure 2). A more or less precipitous decline in N^{15} abundance then ensued, but after the first week uric acid-N^{15} atom per cent excess still substantially exceeded the normal. The cumulative urinary uric acid-N^{15} over the first week ranged from 0.34 to 0.56 per cent of dose.

Subjects A.R. and I.S., both gouty normal excretors, gave uric acid-N^{15} curves throughout within the upper limits of normal (Table I, Figure 2). The respective cumulative urinary uric acid-N^{15} over the first week was 0.12 and 0.14 per cent of dose. Taking into account their enlarged miscible uric acid pools, however (2.75 and 2.3 g, respectively; some two or three times the normal), and the consequent greater dilution of labeled uric acid by exchange with unlabeled uric acid, the true figures for glycine-N^{15} incorporation in these cases may be assumed to be somewhat higher than normal. Moreover, the figures for cumulative uric acid-N^{15} do not include uric acid excretion into the gut, which may be increased in hyperuricemic subjects (10).

The data for uric acid total N^{15} thus far referred to are those obtained by direct measurement of uric acid-N^{15}, and conform to previous reports. Table I also gives values for uric acid total N^{15} calculated from N^{15}-7 and N^{15}-(1 + 3 + 9), separately determined. Except for some unexplained discrepancies, notably in Patient P.C., there is in general reasonably good agreement between the two sets of figures in the several categories of subjects studied.

B. Uric acid N^{15}-7 and N^{15}-(1 + 3 + 9). In Table I and Figure 2 the day-by-day distribution of uric acid total N^{15} between N-7 and N-(1 + 3 + 9) is expressed again as atom per cent N^{15} excess. N-7 consistently was enriched by glycine-N^{15} far more than was any other one uric acid nitrogen atom. This relative N^{15}-7 abundance reflects the derivation of N-7 by direct incorporation of intact glycine, and the limited dilution of the glycine-N^{15} administered (100 mg glycine containing 60 atom per cent excess N^{15} per kg body weight) by the relatively small endogenous free glycine metabolic pool, estimated to approximate 80 mg per kg body weight in normal man (15).

Of the total N^{15} incorporated into uric acid in the four normal subjects studied, 66 to 75 per cent appeared at N-7 on day 1 and 25 to 34 per cent at N-(1 + 3 + 9). On day 2 the proportion of uric acid total N^{15} in N-(1 + 3 + 9) rose somewhat, and thereafter increased further to 39 to 50 per cent at the end of the first week (Table I, Figure 2). The results are in general agreement with those previously reported in normal man by Shemin and Rittenberg (12).

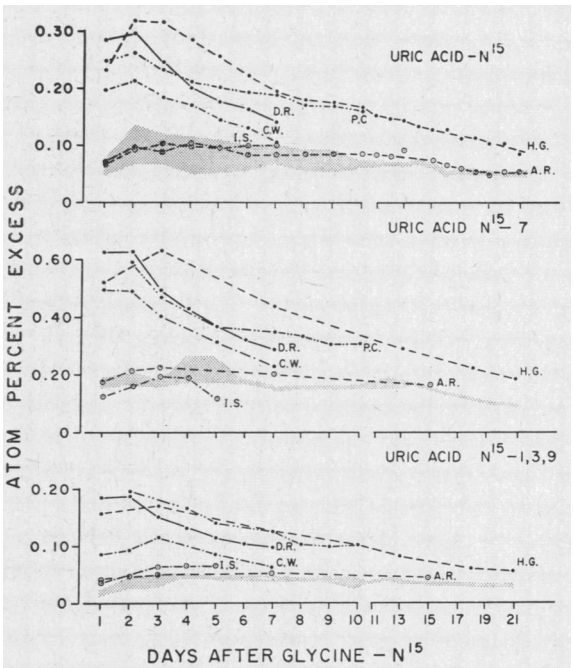

FIG. 2. URIC ACID-N^{15} (TOP), URIC ACID N^{15}-7 (MIDDLE), AND URIC ACID N^{15}-(1+3+9) (BOTTOM), EXPRESSED AS ATOM % EXCESS N^{15}, AFTER ADMINISTRATION OF GLYCINE-N^{15} TO GOUTY OVEREXCRETORS (SOLID CIRCLES) AND GOUTY NORMAL EXCRETORS (OPEN CIRCLES). Approximate normal range of variation is indicated by shaded area. Note that ordinate for N^{15}-7 is drawn to twice the scale of the others.

TABLE I

Incorporation of glycine-N^{15} into uric acid in nongouty and gouty subjects *

Subject, Age, Weight (*kg*) Control urinary uric acid (*mg/24 hrs*) Serum uric acid (*mg%*)	Day	Urinary uric acid	Urinary uric acid-N^{15}		N^{15}-7	N^{15}-(1+3+9)	$\frac{3C+B}{4}$	$\frac{3C}{4D}$
		mg/24 hrs	*atom % excess* A	*% dose*	*atom % excess* B	*atom % excess* C	*atom % excess* D	%
Nongouty								
T.G., 21	1	504	0.048	0.012	0.126	0.014	0.042	25
60	2	489	0.067	0.016	0.163	0.027	0.061	33
557	3	615	0.062	0.019	0.157	0.030	0.067	34
4.2	4	538	0.049	0.013				
	5	607	0.054	0.018				
	6	541	0.059	0.016				
	7	473	0.066	0.016				
L.C., 28	1	576	0.072	0.016	0.191	0.033	0.073	34
76	2	421	0.138	0.022				
525	3	460	0.122	0.022				
5.7	4	540	0.104	0.022	0.263	0.052	0.105	37
	5	406	0.113	0.018	0.262	0.066	0.115	43
	6	517	0.103	0.021				
	7	477	0.103	0.109	0.236	0.053	0.103	39
L.B., 34	1	517	0.054	0.010	0.129	0.018	0.046	29
84	2	533	0.075	0.015	0.206	0.039	0.088	33
465	3	435	0.121	0.019	0.182	0.039	0.075	39
5.7	4	517	0.084	0.015	0.210	0.045	0.086	39
	5	512	0.085	0.015	0.200	0.046	0.085	41
	6	607	0.093	0.020				
	7	583	0.093	0.019				
M A., 58	1	743	0.051	0.015	0.144†	0.020		29‡
76	2	695	0.080	0.022	0.185†	0.045		42‡
601	4	657	0.080	0.021	0.179†	0.047		44‡
5.8	5	930	0.075	0.027	0.168†	0.044		44‡
	6	665	0.070	0.018	0.165†	0.045		48‡
	7	662	0.071	0.018	0.143†	0.047		50‡
Gouty								
D.R., 41	1	1,288	0.286	0.114	0.630	0.187	0.298	47
96	2	1,265	0.297	0.116	0.630	0.189	0.299	47
1,198	3	1,284	0.247	0.098	0.497	0.152	0.238	48
10.3	4	1,000	0.203	0.063	0.427	0.135	0.208	49
	5	1,235	0.179	0.068	0.397	0.115	0.186	46
	6	1,150	0.166	0.059	0.327	0.106	0.161	49
	7	1,132	0.143	0.050	0.295	0.099	0.148	50
C.W., 33	1	1,288	0.199	0.056	0.496	0.085	0.188	34
98	2	1,023	0.213	0.068	0.495	0.097	0.197	38
1,110	3	1,100	0.186	0.065	0.407	0.116	0.189	46
10.0	4	988	0.171	0.051				
	5	960	0.148	0.046	0.320	0.088	0.146	45
	6	836	0.133	0.034				
	7	890	0.108	0.033	0.235	0.061	0.105	44
P.C., 61	1	555	0.249	0.059				
70	2	700	0.264	0.079	0.599	0.198	0.298	50
693	3	644	0.238	0.066				
9.5	4	560	0.212	0.051	0.476	0.184	0.257	54
	5	668	0.203	0.058	0.424	0.143	0.249	43
	6	577	0.192	0.047				
	7	665	0.184	0.053	0.359	0.127	0.185	52

* Results given only for first week after administration of glycine-N^{15}; see Figure 2 for additional data.
† Calculated as 4A − 3C.
‡ Calculated as 3C/4A.

TABLE I—(*Continued*)

Subject, Age, Weight (*kg*) Control urinary uric acid (*mg/24 hrs*) Serum uric acid (*mg%*)	Day	Urinary uric acid	Urinary uric acid—N^{15}		$N^{15}-7$	$N^{15}-(1+3+9)$	$\frac{3C+B}{4}$	$\frac{3C}{4D}$
		mg/24 hrs	*atom % excess* A	*% dose*	*atom % excess* B	*atom % excess* C	*atom % excess* D	*%*
H.G., 44	1	684	0.231	0.042	0.521	0.158	0.249	48
104	2	630	0.318	0.054				
680	3	630	0.317	0.054	0.638	0.170	0.287	44
10.5	4	630	0.288	0.049				
	5	762	0.259	0.053				
	6	947	0.222	0.056				
	7	735	0.197	0.039	0.442	0.127	0.206	46
A.R., 47	1	824	0.069	0.019	0.180	0.041	0.076	40
86	2	618	0.092	0.019	0.218	0.048	0.091	40
601	3	658	0.103	0.022	0.223	0.051	0.094	41
9.0	4	565	0.100	0.018				
	5	642	0.099	0.021				
	6	710	0.086	0.020				
	7	608	0.086	0.017	0.205	0.053	0.091	44
I.S., 54	1	658	0.074	0.017	0.121	0.036	0.057	47
83	2	532	0.095	0.018				
542	3	488	0.090	0.016	0.195	0.063	0.096	49
10.0	4	444	0.104	0.017	0.192	0.064	0.096	40
	5	445	0.099	0.016	0.180	0.063	0.092	51
	6	441	0.101	0.016				
	7	455	0.104	0.017				

In the gouty overexcretors, D.R., C.W., P.C., and H.G. (Table I, Figure 2), all of whom incorporated glycine-N^{15} into uric acid in gross excess, the uric acid N^{15}-*7* atoms per cent excess rose to 0.5 to > 0.6 on day 1, as compared with about 0.13 to 0.19 in the normal subjects. The curves for N^{15} incorporation into N-*7* closely paralleled throughout those for uric acid total N^{15} (Figure 2).

More of the excess N^{15} appropriated from glycine-N^{15} in these gouty overexcretors appeared in N-*7* than in any other one uric acid nitrogen. N^{15}-*7* enrichment of this magnitude could hardly be accounted for plausibly by postulating a reduction in the free glycine metabolic pool in gouty subjects, particularly in view of the comparatively large glycine dose administered. It follows, therefore, that in such gouty overexcretors more than the normal quantity of intact glycine reacts directly with what must then be more than the normal quantity of 5-phosphoribosyl-1-amine; and evidence will be presented that more 5-phosphoribosyl-1-amine is in fact generated in these subjects.

About half of the excess N^{15} incorporated into uric acid in these cases appeared in the aggregate N^{15}–(1 + 3 + 9), although the mean N^{15} abundance of each of the three nitrogen atoms was relatively low compared with N^{15}-*7* (peaks at some 0.10 to 0.20 atom per cent excess). The curves for the composite over-all pattern for N^{15} incorporation into N–(1 + 3 + 9) did not vary much from those for uric acid total N^{15} (Figure 2).

In contrast to the nongouty subjects, this marked enrichment of N^{15}–(1 + 3 + 9) in the gouty overexcretors occurred even on day 1, with initial N^{15}–(1 + 3 + 9)/total uric acid-N^{15} ratios of 34, 47, 48, and 50 per cent as compared with 25, 29, 29, and 34 per cent in the normal subjects (Table I). The initial greater enrichment of N^{15}–(1 + 3 + 9) presumably results from fuller utilization of N^{15} derived from glycine-N^{15} by the action of such enzymes as glycine oxidase, serine dehydrase, transaminases. (To attempt to account for these differences by postulating a substantial contraction of the endogenous metabolic nitrogen pool seems unwarranted in view of the specificity of the aberration in uric acid metabolism in primary gout.) If more than nor-

TABLE II

Uric acid N^{15}/C^{14} and N^{15}–7/C^{14} ratios after administration of glycine-1-C^{14}–N^{15} to nongouty and gouty subjects

Subject	Day	$\frac{N^{15}}{C^{14}}$	$\frac{N^{15}-7}{C^{14}}$
Nongouty			
T.G.	1	1.50	1.13
	2	1.63	1.09
	3	1.73	1.15
	10	1.56	1.02
L.C.	1	1.70	1.12
	4	1.69	1.06
	5	1.64	0.94
	7	1.96	1.20
	8	1.79	1.07
	9	1.67	0.99
Gouty			
C.W.	1	1.75	1.16
	2	2.43	1.52
	3	2.83	1.53
	5	2.88	1.58
	7	2.91	1.65
H.G.	1	2.33	1.22
	3	2.41	1.34
	7	2.82	1.51
	13	2.67	1.46
	18	2.66	1.46
A.R.	1	1.72	1.03
	2	2.05	1.24
	3	2.12	1.26
	7	2.10	1.13
	15	2.46	1.33

mal glutamine amide nitrogen, derived in these circumstances from degraded glycine, were utilized for the reaction with 5-phosphoribosyl-1-pyrophosphate, more 5-phosphoribosyl-1-amine would be made available to combine with the increased quantities of intact glycine implied by the high N^{15}-7 enrichment.

Interestingly enough, in the gouty normal excretors A.R. and I.S., the intramolecular distribution of N^{15} also was abnormal: the ratios N^{15}–(1 + 3 + 9)/total uric acid-N^{15} were high on day 1 (40 and 47 per cent, respectively) and remained high throughout. Thus, as in the overt gouty overexcretors, more than the normal proportion of nitrogen derived from degraded glycine was utilized for purine biosynthesis.

C. Uric acid N^{15}-7/C^{14} ratios. The abnormally high N^{15}/C^{14} ratios in patients with gout (8), previously referred to, are accounted for in part by the demonstration of high N^{15}–(1 + 3 + 9)/uric acid total N^{15} ratios in such subjects. There remains, however, the possibility that recycling of glycine-N^{15} back into N-7 of the uric acid molecule might also be contributory. Recycling can be identified when glycine-1-C^{14}-N^{15} is administered, by establishing N^{15}-7/C^{14} ratios > 1.0.[3]

In nongouty subjects T.G. and L.C., N^{15}–7/C^{14} ratios of 1.0 to 1.1 were the rule (Table II). In gouty overexcretors C.W. and H.G. and gouty normal excretor A.R., the N^{15}-7/C^{14} ratios were higher: 1.2 to 1.6, 1.2 to 1.5, and 1.0 to 1.3, respectively (Table II). These results imply more recycling of labeled reconstituted glycine back into purine biosynthesis in gouty than in normal subjects, particularly after day 1.

In gouty overexcretor P.C., given glycine-2-C^{14}-N^{15}, lower N^{15}-7/C^{14} ratios were found, varying from 0.7 on day 1 to 0.8 on days 4 and 5, and 1.0 on day 7. These lower ratios are consistent with the wider distribution within the uric acid molecule of the α-carbon of glycine, which contributes C-2 and C-8 as well as C-5.

2. Ammonium-N^{15} incorporation into uric acid

When ammonium-N^{15} chloride was given, the general pattern of day-by-day isotope incorporation into uric acid in both gouty and nongouty subjects was similar to that after glycine-N^{15} administration, although in approximately equivalent N^{15} dosage the uric acid-N^{15} atom per cent excess was consistently lower (Table III, Figure

[3] The N^{15}-7/C^{14} ratio would be unity if the carboxyl carbon of glycine-1-C^{14} incorporated into uric acid appeared solely at C-4; actually, ratios slightly less than unity would be anticipated in view of weak specific activity found at C-6 and other carbon atoms. N^{15}-7/C^{14} ratios > 1.0 are generated as a result of recycling and direct incorporation of glycine which has been metabolized and reconstituted (16). The higher ratios are a consequence, among other things, of the disparity in the magnitude of the metabolic nitrogen and carbon pools from which the constituents for resynthesis of glycine are drawn: presumably, N^{15} derived from glycine-1-C^{14}-N^{15} has a smaller volume of distribution than C^{14}, hence the donors of nitrogen to glycine resynthesis would be expected to retain a greater isotope abundance than the carbon donors. It has been established (17, 18) that the chief source of glycine in biosynthesis is serine, which is itself formed from hydroxypyruvate and (by way of phosphoserine) from 3-phosphohydroxypyruvate, with alanine and glutamic acid, respectively, chiefly contributing nitrogen by transamination. There is also, of course, rapid loss of C^{14} as $C^{14}O_2$.

3). Significant N^{15} enrichment was present on day 1, there was usually a demonstrable small peak in uric acid-N^{15} atom per cent excess on day 2 or 3, and for some time thereafter a prolonged plateau, even in the gouty overexcretors in whom the decline in uric acid-N^{15} abundance was less pronounced in the first week than after glycine-N^{15} administration. In individual cases

TABLE III

Incorporation of ammonium-N^{15} into uric acid in nongouty and gouty subjects

Subject	Day	Urinary uric acid-N^{15}		N^{15}-7	N^{15}-(1+3+9)	$\frac{3C+B}{4}$	$\frac{3C}{4D}$
		atom % excess A	*% dose*	*atom % excess* B	*atom % excess* C	*atom % excess* D	%
Nongouty							
R.W.	1	0.034	0.0058	0.047	0.035	0.038	69
	2	0.033	0.0059	0.030	0.044	0.041	82
	3	0.046	0.0082	0.034	0.049	0.045	81
	4	0.043	0.0095	0.043	0.060	0.056	81
	5	0.043	0.0075	0.040	0.055	0.051	81
	6	0.046	0.0078	0.025	0.048	0.042	85
	7	0.045	0.0078	0.032	0.050	0.046	83
N.J.	1	0.036	0.0087	0.027	0.045	0.040	83
	2	0.038	0.0095	0.029	0.058	0.051	86
	3	0.043	0.0097	0.053	0.068	0.064	79
	4	0.048	0.0140	0.035	0.052	0.048	82
	5	0.050	0.0124	0.027	0.053	0.047	86
	6	0.050	0.0117	0.032	0.054	0.049	84
	7	0.038	0.0114	0.024	0.040	0.036	83
Gouty							
C.W.	1	0.109	0.0399	0.058	0.117	0.102	86
	2	0.130	0.0478	0.057	0.149	0.126	89
	3	0.105	0.0397	0.055	0.116	0.101	86
	4	0.103	0.0435	0.047	0.103	0.099	84
	5	0.085	0.0321	0.045	0.103	0.086	87
	6	0.087	0.0387	0.064	0.111	0.099	84
	7	0.090	0.0408	0.057	0.099	0.089	84
P.C.	1	0.110	0.0193	0.072	0.117	0.106	83
	2	0.130	0.0220	0.084	0.142	0.128	84
	3	0.111	0.0186	0.076	0.123	0.111	83
	4	0.113	0.0191	0.068	0.128	0.113	85
	5	0.104	0.0199	0.070	0.118	0.106	84
	6	0.100	0.0163	0.066	0.110	0.099	83
	7	0.084	0.0200	0.056	0.095	0.085	84
H.G.	1	0.057	0.0119	0.065	0.056	0.058	73
	2	0.074	0.0161	0.065	0.062	0.063	74
	3	0.075	0.0164	0.058	0.077	0.072	80
	4	0.077	0.0180	0.070	0.074	0.073	76
	5	0.071	0.0149	0.072	0.081	0.079	77
	6	0.068	0.0159	0.070	0.074	0.073	76
	7	0.062	0.0161	0.078	0.066	0.069	72
A.R.	1	0.051	0.0086	0.042	0.089	0.077	86
	2	0.057	0.0107	0.042	0.073	0.065	84
	3	0.066	0.0113	0.054	0.072	0.067	80
	4	0.065	0.0146	0.048	0.075	0.068	82
	5	0.064	0.0122	0.032	0.078	0.067	88
	6	0.058	0.0126	0.032	0.062	0.055	85
	7	0.051	0.0085	0.031	0.061	0.054	85
I.S.	1	0.036	0.0073	0.021	0.041	0.037	84
	2	0.034	0.0058	0.018	0.037	0.032	86
	3	0.037	0.0059	0.023	0.037	0.036	83
	4	0.040	0.0070	0.024	0.039	0.036	83
	5	0.038	0.0062	0.025	0.042	0.038	83
	6	0.036	0.0067	0.023	0.039	0.035	84
	7	0.034	0.0065	0.024	0.039	0.035	83

TABLE III—(*Continued*)

Subject	Day	Urinary uric acid-N^{15}		N^{15}-7	N^{15}-(1+3+9)	$\frac{3C+5}{4}$	$\frac{3C}{4D}$
		atom % excess A	*% dose*	*atom % excess* B	*atom % excess* C	*atom % excess* D	%
G.H.(a)*	1	0.025	0.0062	0.020	0.026	0.025	80
	2	0.027	0.0073	0.020	0.032	0.029	83
	3	0.028	0.0096	0.022	0.030	0.028	83
	4	0.030	0.0075	0.024	0.030	0.028	83
	5	0.028	0.0065	0.019	0.030	0.027	84
	6	0.032	0.0078	0.023	0.028	0.027	84
	7	0.027	0.0080	0.023	0.030	0.028	81
G.H.(b)†	1	0.039	0.0052	0.028	0.045	0.041	83
	2	0.057	0.0080	0.038	0.058	0.053	83
	3	0.063	0.0094	0.040	0.063	0.057	80
	4	0.063	0.0091	0.040	0.065	0.059	79
	5	0.064	0.0074	0.038	0.069	0.061	82
	6	0.061	0.0090	0.039	0.066	0.059	79
	7	0.062	0.0076	0.037	0.069	0.061	80

* Ingested 6.5 g ammonium-N^{15} chloride, the same dose as all other subjects.
† Ingested 13.0 g ammonium-N^{15} chloride (body weight, 127 kg).

of gout there were, as will be noted subsequently, interesting differences in the abundance and intramolecular distribution of uric acid-N^{15} after administration of ammonium-N^{15} chloride, as compared with glycine-N^{15}.

A. Total uric acid-N^{15}. In normal subjects R.W. and N.J. the uric acid-N^{15} averaged 0.035 atom per cent excess on day 1, with peak levels of about 0.045 to 0.050. The cumulative urinary uric acid-N^{15} over the first week averaged 0.065 per cent of the N^{15} dose.

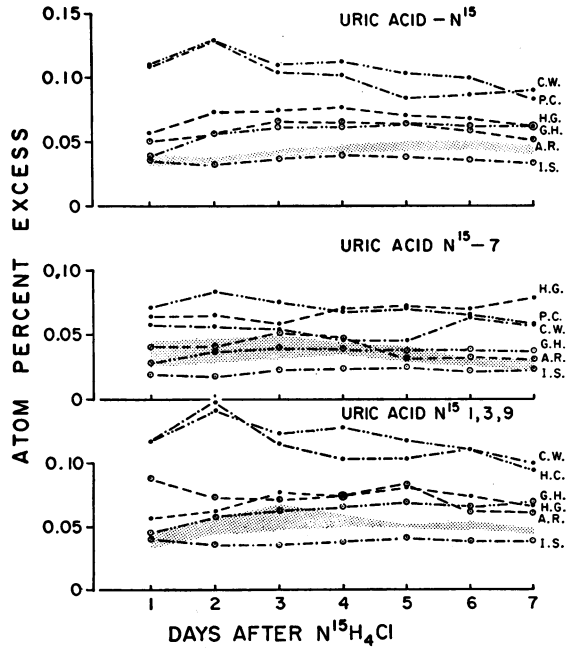

FIG. 3. INCORPORATION OF AMMONIUM-N^{15} INTO URIC ACID-N^{15}, URIC ACID N^{15}-7, AND URIC ACID N^{15}-(1+3+9). Symbols as in Figure 2.

In gouty overexcretors C.W. and P.C. the uric acid-N^{15} was three times normal: 0.11 atom per cent excess on day 1, and 0.13 on day 2, gradually declining thereafter. Gouty overexcretor H.G. incorporated less ammonium-N^{15} than his counterparts: 0.057 atom per cent excess uric acid-N^{15} on day 1, rising to a peak of 0.077. This is in contrast to H.G.'s excessive incorporation of glycine-N^{15}, which was greater than that of any of the other gouty overexcretors and out of proportion to the quantity of urinary uric acid he excreted daily. In C.W. the high uric acid-N^{15} after ammonium-N^{15} chloride also was more consistent with his very high daily urinary uric acid output than after glycine-N^{15}, which yielded lower uric acid-N^{15} figures than those found in any of the other gouty overexcretors. (The day-to-day relative abundance of uric acid-N^{15} after glycine-N^{15} as compared with that after ammonium-N^{15} chloride averaged 3.8 for H.G., 1.6 for C.W., and 2.1 for P.C.) The cumulative urinary uric acid-N^{15} incorporation at the end of the first week was 0.28 per cent of dose for C.W., 0.14 for P.C., and 0.11 for H.G.

Gouty normal excretor A.R. incorporated more ammonium-N^{15} (0.051 atom per cent excess N^{15}) into uric acid on day 1 than did the two normal

subjects and he continued to do so over the period of observation; in fact his uric acid-N^{15} curve approaches that of gouty overexcretor H.G. (Figure 3). I.S., on the other hand, yielded uric acid-N^{15} data within or slightly below the normal range, at least before correction for his expanded uric acid pool. (In A.R. the mean relative abundance of uric acid-N^{15} after glycine-N^{15} as compared with that after ammonium-N^{15} chloride was 1.6; in I.S. it was higher, 2.6; in both instances these ratios gradually rose, in contrast to the gradual decline in the ratios in the gouty overexcretors.) The cumulative urinary uric acid-N^{15} over the first week was 0.08 per cent of dose in A.R. and 0.05 in I.S.

Two experiments were conducted in gouty normal excretor G.H., who weighed 127 kg and whose uric acid pool was 3.0 g (serum uric acid 12.4 mg per 100 ml). In the first experiment 1.0 g N^{15} was given as ammonium-N^{15} chloride; in the second, a few months later, twice this dosage was given. As shown in Table III, the uric acid-N^{15} atom per cent excess throughout the second experiment was just about double that in the first. The percentage of N^{15} dose incorporated into urinary uric acid consequently remained relatively constant at 0.05 to 0.06.

B. Uric acid N^{15}-(1 + 3 + 9) and N^{15}-7. As anticipated, more of the uric acid-N^{15} was found in N-(1 + 3 + 9) after administration of ammonium-N^{15} chloride than after glycine-N^{15}; consequently, N^{15}-(1 + 3 + 9)/total uric acid-N^{15} ratios consistently were high. No clear distinction between gouty and nongouty subjects could be made in these ratios on day 1 (as was the case after glycine-N^{15}) or subsequently.

In normal subjects R.W. and N.J., N^{15}-(1 + 3 + 9) was 0.035 and 0.045 atom per cent excess, respectively, on day 1 (69 and 83 per cent of the total uric acid-N^{15}), rising somewhat subsequently (Table III, Figure 3) to continue at 80 to 86 per cent of the total uric acid-N^{15}. In gouty overexcretors C.W. and P.C. the excessive incorporation of N^{15} into uric acid already noted was found to be the result chiefly of an abnormal N^{15}-(1 + 3 + 9) abundance, for the most part > 0.10 atom per cent excess N^{15}, constituting 83 to 89 per cent of the total uric acid-N^{15} throughout the first week. In gouty overexcretor H.G. the incorporation of N^{15} into N-(1 + 3 + 9) on day 1 (0.056 atom per cent excess, rising to a peak of 0.081) was less pronounced than in his fellows, hence the total uric acid-N^{15} also was not so high; nevertheless, N^{15}-(1 + 3 + 9) constituted 72 to 80 per cent of the total uric acid-N^{15} throughout the first week. In gouty normal excretors A.R., I.S., and G.H. the N^{15} abundance of N-(1 + 3 + 9) varied in correspondence with their varied total uric acid-N^{15}, of which, as in the others, it constituted by far the greatest part.

In contrast to glycine-N^{15}, administration of ammonium-N^{15} chloride resulted in generally less N^{15} enrichment of N-7 than of any other nitrogen atom, and least of the large surplus of uric acid-N^{15} in gouty overexcretors could be ascribed to N^{15}-7, except perhaps in H.G. Nevertheless, in both nongouty and gouty subjects N-7 was labeled, presumably by recycling and incorporation of glycine, reconstituted chiefly from serine to which N^{15} was donated by transamination (19). These processes seemed to be unusually proficient in H.G. after administration of ammonium-N^{15} chloride, just as was apparent after the dose of glycine-N^{15}.

C. Distribution of N^{15}-(1 + 3 + 9) among N-9, N-3 and N-1. N^{15}-9 was obtained by difference from N^{15}-(1 + 3 + 9) and N^{15}-(1 + 3), also by difference from N^{15}-(7 + 9) and N^{15}-7. N^{15}-3 was assumed to approximate N^{15}-9 (20) since both derive from glutamine amide-N^{15}, and on this assumption N^{15}-1 could be calculated from N^{15}-(1 + 3 + 9) and N^{15}-(1 + 3).

In normal subject N.J. virtually all of N^{15}-(1 + 3 + 9) was localized at N-9 and N-3 in the uric acid samples obtained on days 1 and 2; N^{15}-1 was too low to estimate. (This distribution corresponds to the results on day 0.5 in the pigeon fed ammonium-N^{15} cited in Reference 21). On day 3, N^{15}-9 and N^{15}-3 began to decline and N^{15}-1 to increase, presumably as a consequence of randomization of N^{15} by transamination. By day 7 the N^{15} abundance of all four uric acid nitrogens was approximately the same (Figure 4). In normal subject R.W. the intramolecular abundance and distribution of uric acid-N^{15} was similar except that randomization of N^{15} occurred earlier, on day 1; consequently the initial figures for N^{15}-9 and N^{15}-3 were lower and those for N^{15}-1 higher than in N.J.

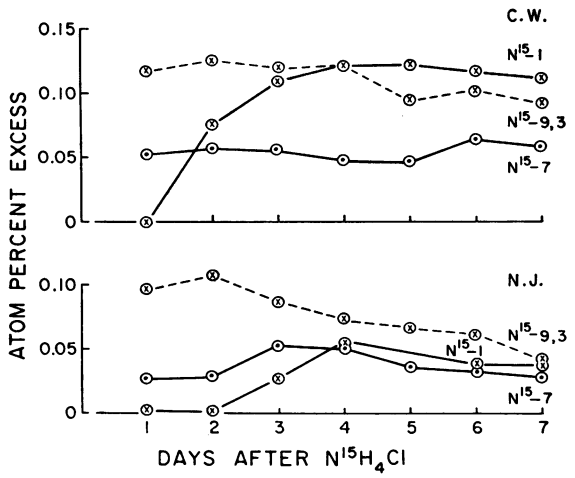

FIG. 4. DISTRIBUTION OF URIC ACID-N^{15} AMONG N-9, 3, N-1 AND N-7 AFTER $N^{15}H_4Cl$ ADMINISTRATION TO A GOUTY OVEREXCRETOR (TOP) AND A NORMAL SUBJECT (BOTTOM).

The corresponding data in gouty overexcretor C.W. are shown in Figure 4. The distribution of uric acid-N^{15} follows the same general sequence as in normal subject N.J. but at higher levels of isotope enrichment. Gouty overexcretor H.G. was anomalous, as already noted, in failing to show the early enrichment of N^{15}-9 and N^{15}-3 exhibited by the others. In gouty normal excretor A.R., N^{15} distribution curves were very similar to those of normal subject N.J. except at slightly but fairly consistently higher levels of isotope abundance.

3. *Urinary excretion of ammonium-N^{15} and urea-N^{15} in gouty subjects*

Excretion of ammonium in the urine was not perceptibly increased after a dose of 6.5 g of (isotope-labeled) ammonium chloride, but a moderate rise occurred in the one experiment in which 13 g was administered. Only 0.5 per cent of the ammonium-N^{15} given appeared in the urine on day 1 as ammonium-N^{15}; this had an isotope abundance of 1.5 atom per cent excess. The cumulative ammonium-N^{15} excreted over the first week was but 1 per cent of the dose. When glycine-N^{15} in equivalent isotope dosage was administered (3), 2 per cent of the dose appeared in the urine on day 1 as ammonium-N^{15}, which contained 3.75 atom per cent excess N^{15}. The cumulative ammonium-N^{15} excreted over the first week was 3 per cent of the dose. [Glycine is well known to be a rapidly available source of urinary ammonia (22)].

More isotope was excreted as urea-N^{15} when ammonium-N^{15} was given than after administration of glycine-N^{15}. On day 1 a mean of 42 per cent of the ammonium-N^{15} dose was eliminated as urea-N^{15} (4.0 atom per cent excess N^{15}); on day 2 an additional 13 per cent appeared, thereafter much less; at the end of the first week the cumulative excretion of urea-N^{15} averaged 66 per cent of the dose. After giving the equivalent amount of N^{15} as glycine-N^{15}, the corresponding figures were 21 per cent (about 2.0 atom per cent excess N^{15}), 10, and 40 per cent, respectively (3). These results after ammonium-N^{15} and glycine-N^{15} administration conform, in general, to the findings of Sprinson and Rittenberg on the ensuing urinary excretion of ammonium-N^{15} and urea-N^{15} (23).

Compared with the flood of urea-N^{15} in the urine on days 1 and 2, the output of uric acid-N^{15}, even in gouty overexcretors, was infinitesimal indeed. After glycine-N^{15} administration, the per cent dose excreted as uric acid-N^{15} was about 1 : 1,000 of that excreted as urea-N^{15} in normal subjects, and about 3 to 5 : 1,000 in gouty overexcretors. After ammonium-N^{15} administration the proportion was even smaller—about 1.5 : 10,000 in normal subjects and of the order of 5 : 10,000 in gouty overexcretors. Because the elimination of urea-N^{15} dropped off sharply after day 3, whereas that of uric acid-N^{15} continued for longer periods, the differences just cited were slightly less pronounced when calculated on the basis of cumulative output over the first week. Thus, after glycine-N^{15} administration the ratios of per cent dose excreted as uric acid-N^{15} : urea-N^{15} were about 3 : 1,000 for normal subjects and about 10 to 15 : 1,000 for gouty overexcretors. After ammonium-N^{15} administration the corresponding figures were 1 : 1,000 and 2 to 4 : 1,000, respectively. It is obvious that any deflection of urinary nitrogen from urea to uric acid would be apparent in at least some cases of gout when uric acid nitrogen excretion is measured but would be imperceptible in measurements of urea nitrogen.

DISCUSSION

Despite the minute proportion of administered glycine-N^{15} or ammonium-N^{15} incorporated as

uric acid-N^{15} in man, and the number of intermediates and pools of intermediates intervening in biosynthesis, meaningful patterns of incorporation evidently can be obtained not only for total uric acid-N^{15} but also for the intramolecular distribution of the isotope. As indicated under Results, analysis of these patterns makes it possible to infer the metabolic pathways by which these precursors are utilized for uric acid biosynthesis in normal and gouty subjects, and to discern additional differences between the gouty and nongouty.

The most striking and consistent deviations from the normal were found in gouty overexcretors of uric acid. Whether given glycine-N^{15} or ammonium-N^{15}, the abundance of uric acid-N^{15} in these subjects invariably was in substantial excess of the normal. After glycine-N^{15} administration more of the excess uric acid-N^{15} was found in N-7 than in any other one nitrogen atom, due in part to direct incorporation of more glycine-N^{15} from the comparatively small diluent glycine pool and in part to recycling of reconstituted glycine relatively rich in N^{15}. However, almost half of the total uric acid-N^{15} appeared at N-(1 + 3 + 9) from day 1 on—significantly more than initial N^{15}-(1 + 3 + 9)/uric acid-N^{15} ratios in the normal; this indicates rapid transfer and abnormally large utilization at these sites of nitrogen derived from degraded glycine. After ammonium-N^{15} was given, the bulk of the excess uric acid-N^{15} in gouty overexcretors appeared initially at N-9 and N-3. With subsequent randomization of N^{15} by transamination, the isotope was more uniformly distributed among the four nitrogens of the uric acid molecule, for the first week still in greater than normal abundance.

In the gouty normal excretors given glycine-N^{15}, the total uric acid-N^{15} appeared to be within the limits of normal variation (before correction for exchange with their larger uric acid pools) but, like the gouty overexcretors, N^{15}-(1 + 3 + 9) was disproportionately high on day 1 as compared with the normal. After administration of ammonium-N^{15} the uric acid-N^{15} abundance in the several cases in this category varied: greater than normal, within normal limits, and apparently even less than normal, before correction for exchange with their larger uric acid pools and for possible differences in metabolic nitrogen pools. As in the other subjects studied, the N^{15} abundance of N-9 and N-3 initially was greater than at N-7 or N-1, but the isotope subsequently was more uniformly distributed among the four uric acid nitrogen atoms.

In the course of the study isolated metabolic variations were noted in certain individuals; for example, in the utilization of intact glycine-N^{15} in Patient H.G. and in the duration of sequestration of ammonium-N^{15} as glutamine-N^{15} in others. In any one subject, however, the patterns of isotope incorporation into uric acid showed rather remarkable regularity. The broad implications of these patterns merit comment.

When the abnormally high uric acid-N^{15} of gouty overexcretors given glycine-N^{15} was first discovered, it was suggested that "an unusually large uricotelic component" might be responsible for the implied overproduction of uric acid (3); that the inborn error of (primary) gout constitutes a metabolic derangement in which "a disproportionate quantity of simple nitrogen and carbon precursors of uric acid is diverted from the main metabolic channels culminating in urea and carbon dioxide formation to pathways leading to urate formation" (24). The present report offers additional data in support of a diversion of precursor amino acid nitrogen (and by inference also carbon precursors) to *de novo* uric acid biosynthesis and implicates some of the specific reactions involved in increased uric acid formation, which is obvious in some cases but only inferential in others. In secondary gout, such diversion of purine precursors and overproduction of uric acid clearly is a consequence of the aggressive demands of exaggerated hemopoiesis for nucleic acid formation (25, 26). In primary gout, however, the basic causes are still obscure. It may be assumed that the diversion of precursors to uric acid biosynthesis is not due to genetic transmittal of a supranormal complement of enzymes for purine biosynthesis but, as in other inborn errors of metabolism, is related to deficiency of one or another enzyme. As pointed out elsewhere (27) there can be no intrinsic block in the sequential reactions of uric acid biosynthesis per se in the inborn error of gout, since uric acid is the end product of cellular purine biosynthesis in man, and no indications of such a block have been forthcoming. On the contrary,

the only derangements in uric acid biosynthesis per se thus far disclosed in gout seem to involve fuller utilization of precursors by the normal metabolic pathways of purine biosynthesis, and this appears to be the result of one or more as yet undefined and apparently relatively minor errors in the disposition of amino acid nitrogen.[4]

The crux of the problem of overproduction of uric acid in primary gout, it is suggested, centers in the reasons why, with equal loads of precursor amino acids—whether free or combined as protein (28)—the gouty subject tends to deliver excessive quantities of nitrogenous substrate to the enzymes which initiate and presumably set the pace for *de novo* uric acid biosynthesis. This is indicated by the initial, disproportionate N^{15} abundance of N–(1 + 3 + 9), i.e., N–9, particularly when glycine-N^{15} is administered, and by what appears to be a parallel increase in turnover of 5-phosphoribosylpyrophosphate in gouty subjects recently reported by Wyngaarden, Jones and Ashton (29). The initial reactions in question are the irreversible transfer of the amide nitrogen of glutamine to 5-phosphoribosylpyrophosphate by the action of phosphoribosylpyrophosphate amidotransferase, and the ensuing union of 5-phosphoribosylamine so formed with glycine to give glycinamide ribonucleotide (11). Also participating may be an alternative initial reaction recently described by Nierlich and Magasanik (30), in which ribose-5-phosphate, ammonia, and adenosine triphosphate combine directly to form 5-phosphoribosylamine and, when glycine is present, glycinamide ribonucleotide.

More than the normal quantity of precursor amino acid nitrogen may be made available to the initiating mechanisms of *de novo* uric acid biosynthesis in primary gout as a consequence of default of other pathways competing for utilization of these amino acids (27). In this view the essential causes of overproduction of uric acid should be sought in deficiencies of one or more enzymes involved in the metabolism of certain amino acids and the broad spectrum of urea and other nitrogen excretion mechanisms, or both, recently reviewed by Cohen and Brown (31) and discussed in relation to glycine and serine by Neuberger (19). Wyngaarden and co-workers (29) hypothesize a specific deficiency in a postulated feedback regulatory system for control of *de novo* purine biosynthesis; it is suggested that this may involve adenosine triphosphate, one of several purine nucleotides which inhibit the activity of phosphoribosylpyrophosphate amidotransferase (32).

[4] It is not intended to exclude the possibility of dislocations at other metabolic sites, such as those recently listed (27), since what is at present included in the designation "primary gout" may well prove to encompass several discrete metabolic anomalies. These possibilities, however, have not yet been sufficiently explored.

SUMMARY

Four nongouty and six subjects with primary gout were given glycine-N^{15}, and two nongouty and six subjects with primary gout were given the equivalent amount of N^{15} as ammonium-N^{15} chloride. The abundance and intramolecular distribution of uric acid-N^{15} were then determined and compared in the gouty and nongouty.

Gouty overexcretors of uric acid consistently incorporated grossly excessive N^{15} into uric acid, as compared with the nongouty, whether given glycine-N^{15} or ammonium-N^{15}. The gouty normal excretors did not have unequivocally greater than normal uric acid-N^{15} abundance after glycine-N^{15} administration unless a correction was made for their expanded uric acid pools. After ammonium-N^{15} administration, the uric acid-N^{15} abundance was somewhat greater than normal in two gouty normal excretors even without such correction.

After administration of glycine-N^{15}, N-7 was enriched more than any other one uric acid nitrogen in both gouty and nongouty subjects. However, more of the total uric acid-N^{15} appeared initially at N–(1 + 3 + 9) in the gouty (normal as well as overexcretors) than in the nongouty. Thus the gouty subjects uniformly utilized more glycine nitrogen for uric acid biosynthesis, not only directly as intact glycine and after recycling of reconstituted glycine (N-7), but also after transfer of glycine nitrogen to the amide nitrogen of glutamine (N-9,3) and the amino nitrogen of aspartic acid (N-1).

After administration of ammonium-N^{15}, most of the uric acid-N^{15} appeared initially at N–(9 + 3) in both gouty and nongouty subjects—in gross excess in the gouty overexcretors, however, and in somewhat greater than normal abun-

dance (even before correction for expanded uric acid pools) in the one gouty normal excretor so studied. With subsequent randomization of N^{15}, presumably by transamination, the distribution of isotope among the four uric acid nitrogens became more uniform.

There appear to be differences between gouty and normal man in respect to the utilization of precursor amino acids, nitrogen being diverted in the gouty from competing pathways to the sequential reactions of *de novo* uric acid biosynthesis. Given the same load of amino acid precursors, whether free or combined as dietary protein, the gouty subject apparently makes available to the enzymes initiating *de novo* uric acid biosynthesis more substrate than does normal man; this results in pronounced overproduction of uric acid in some instances, less pronounced or inferential overproduction in others. It is suggested that the underlying inborn metabolic error in primary gout, at least in some cases, may be an innate deficiency of one or more enzymes concerned with the disposition of amino acid nitrogen by metabolic pathways in competition with *de novo* uric acid biosynthesis.

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