# The Kinetics of Intramolecular Distribution of $^{15}N$ in Uric Acid after Administration of [$^{15}N$]Glycine

## A REAPPRAISAL OF THE SIGNIFICANCE OF PREFERENTIAL LABELING OF N-(3 + 9) OF URIC ACID IN PRIMARY GOUT

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ABSTRACT The concept of an abnormality of glutamine metabolism in primary gout was first proposed on the basis of isotope data: when [$^{15}N$]glycine was administered to gouty subjects, there was disproportionately great enrichment of N-(3 + 9) of uric acid, which derive from the amide-N of glutamine. An unduly high concentration of $^{15}N$ in glutamine was postulated, and attributed to a hypothetical defect in catabolism of glutamine. Excess glutamine was proposed as the driving force of uric acid overproduction.

We have reexamined this proposition in four gouty subjects: one mild overproducer of uric acid with "idiopathic gout," one marked overproducer with high-grade but "partial" hypoxanthine-guanine phosphoribosyltransferase deficiency, and two extraordinary overproducers with superactive phosphoribosylpyrophosphate synthetases. In the last three, the driving force of excessive purine biosynthesis is a known surplus of α-5-phosphoribosyl-1-pyrophosphate. Disproportionately high labeling of N-(3 + 9) was present in all four gouty subjects, most marked in the most flamboyant overproducers. The precursor glycine pool was sampled by periodic administration of benzoic acid and isolation of urinary hippuric acid. Similarly, the precursor glutamine pool was sampled by periodic administration of phenylacetic acid and isolation of the amide-N of urinary phenylacetylglutamine. The time course of $^{15}N$ enrichment of hippurate differed from that of the amide-N of glutamine. Whereas initial enrichment values of hippurate were very high, those of glutamine-amide-N were low, increasing to a maximum at about 3 h, and then declining less rapidly than those of hippurate. However, enrichment values of hippurate and of phenacetyl glutamine were normal in all of the gouty subjects studied. Thus, preferential enrichment of N-(3 + 9) in gouty overproducers given [$^{15}N$]glycine does not necessarily reflect a specific abnormality of glutamine metabolism, but rather appears to be a kinetic phenomenon associated with accelerated purine biosynthesis per se.

In addition, greater enrichment of N-9 than of N-3 on days 1 and 2 provided suggestive evidence for a second pathway for synthesis of the initial precursor of purine biosynthesis, phosphoribosylamine, perhaps utilizing ammonia rather than the amide-N of glutamine as nitrogen donor. In this limited study, the activity of this potential second pathway did not appear to be selectively increased in gout.

## INTRODUCTION

Patients with primary gout and excessive uric acid excretion fed a test dose of [$^{15}N$]glycine incorporate increased quantities of $^{15}N$ into urinary urate (1–5). Although enrichment of all four nitrogen atoms of uric acid is excessive (5, 6) that of N-(3 + 9)[1] is dispro-

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Some of the experiments of this study were performed in the Department of Medicine, University of Pennsylvania School of Medicine, Philadelphia, Pennsylvania 19104 in 1965–1967 by Drs. Sperling and Wyngaarden.

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[1] In the studies of Gutman and Yu (6), $^{15}N$-9 was calculated according to Eq. 2 or 3 (below), and $^{15}N$-3 assumed to be equal to $^{15}N$-9. As pointed out later in this paper, their assumption does not appear to be valid. But since enrichment of N-(3 + 9), calculated in other ways in this paper, is indeed increased in gouty overproducers, we will retain their terminology except where specified otherwise.

portionately great, especially in flamboyant overexcretors of uric acid (6). Since N-3 and N-9 of uric acid are derived from the amide-N of glutamine (7–9), Gutman and Yu (6, 10) proposed a defect in glutamine metabolism as the basis for excessive purine biosynthesis in primary gout. Since urinary ammonium, which arises principally from glutamine (11–14), is reduced in many gouty subjects (15–17), they further postulated that the defect was a reduction of glutaminase activity (6, 10). The recent finding of hyperglutamatemia in gout has shifted attention toward a possible defect of glutamate metabolism, resulting in diversion of glutamic acid toward glutamine and purine biosynthesis (18–20).

The original studies of $^{15}N$ distribution in uric acid were performed before specific enzymic defects were recognized in gout, e.g., glucose 6-phosphatase deficiency (21, 22), hypoxanthine-guanine phosphoribosyltransferase (PRT)[2] deficiencies of variable severity (23–26), and superactive α-5-phosphoribosyl-1-pyrophosphate (PP-ribose-P) synthetases (27–29). In the last two rare subtypes of gout, the driving force of excessive purine biosynthesis is believed to be the demonstrably raised intracellular level of PP-ribose-P. Accordingly, we decided to determine whether preferential labeling of N-(3 + 9) after administration of labeled glycine also occurred in gouty subjects with accelerated purine production, based on known mechanisms unrelated to a defect of glutamine metabolism. The findings reported in this paper indicate that preferential labeling of N-(3 + 9) occurs in patients with accelerated purine production secondary to PRT deficiency or superactive PP-ribose-P synthetases, as well as in overproducers with idiopathic gout. Thus this labeling pattern appears to be a consequence of kinetic factors common to several different metabolic subtypes of gout, and not necessarily indicative of a defect of glutamine metabolism.

## METHODS

[$^{15}N$]glycine, 95.1 atom percent excess $^{15}N$, used in experiments on subjects R. H., F. L., and S. G., was synthesized according to Schoenheimer and Ratner (30). [$^{15}N$]glycine, 95 atom per cent excess, used in experiments on subjects R. Jo. and O. G., was purchased from Isomet Corp., Oakland, N. J. [1-$^{14}C$]phenylacetic acid was purchased from New England Nuclear, Boston, Mass.

Uric acid was isolated from urine by adsorption on Darco-G60 (Darco Activated Carbon, Atlas Chemical Industries, Inc., Wilmington, Del.) at pH 4.2 overnight at 4°C, eluted with hot 0.1 N NaOH (31), and repeatedly recrystallized from $Li_2CO_3$ solution (32). All uric acid analyses were performed by the differential spectrophotometric method (33) employing purified uricase purchased from Worthington Biochemical Corp., Freehold, N. J.

Uric acid samples were degraded by procedures which yield N-7 as glycine (34) and N-(1 + 3 + 9) (34), N-(1 + 7) (35), N-(1 + 3) (36), and N-(7 + 9) (36) as $NH_3$. After alkalinization, the $NH_3$ was collected in 1 N HCl. Hippuric acid was isolated according to Quick (37, 38). Free urinary glycine was isolated as the dinitrophenyl (DNP) derivative according to Watts and Crawhall (39). Urinary ammonia was recovered from fresh urine after alkalinization with saturated $K_2CO_3$ and collection in 1 N HCl.

Phenylacetylglutamine was isolated from urine after the ingestion of [1-$^{14}C$]phenylacetic acid by consecutive separations on cation and anion exchange resins as follows: An AG-50W × 8 $H^+$ cation exchange resin (200–400 mesh) was converted to its $Na^+$ form, placed in a chromatographic column (40 × 600 mm) and washed with citrate buffer (pH 3.42) according to Stein (40). A urine sample of 50–100 ml was acidified to pH 2.3 and applied to the column, which was washed with successive 100-ml portions of citrate buffers of pH 2.5 and 2.9. Adsorbed compounds were eluted from the resin with citrate buffer of pH 3.42. Two radioactive peaks appeared, one before and one after the urea peak. The first of these represented unchanged [$^{14}C$]-phenylacetic acid, and generally contained about 30% of the counts of the urine sample. The eluates comprising the second peak and containing phenylacetylglutamine (see below) were pooled and concentrated to a small volume by freeze-drying.

An AG-2 X 8 Cl-anion exchange resin (200–400 mesh) was converted to its acetate form according to Hirs, Moore, and Stein (41) and placed in a 25 × 300-mm column. The eluate containing phenylacetylglutamine was reduced to 50 ml vol, brought to pH 7.0, and applied to the resin. The resin was washed with 100 ml of 0.5 M acetic acid and then with successive 50-ml vols of acetic acid of increasing concentrations (1.0 M, 1.5 M, 2.0 M, etc.). The $^{14}C$-labeled material appeared as a single peak is the 2.5 M acetic acid eluate and was collected in one sample of 100 ml. This fraction was taken to dryness by freeze-drying. The solid material was extracted with and recrystallized from hot ethyl acetate.

Both the crystalline material thus obtained, and authentic commercial phenylacetylglutamine, gave an $R_f$ of 0.46 on silica gel thin-layer chromatography, employing butanol: acetic acid: water, 4:1:1, as solvent, and yielded equivalent amounts of ammonia and glutamic acid after hydrolysis in 3 N HCl at 100°C for 3 h (42).

The ammonia derived from the amide nitrogen of glutamine was separated from the hydrolysate, after alkalinization, by aeration into 1 N HCl. Residual glutamic acid was separated from any unhydrolyzed phenylacetylglutamine on Dowex 2- acetate resin (Dow Chemical Co., Midland, Mich.), prepared as described previously, and eluted with 0.5 N acetic acid.

All compounds or fractions not already in the form of ammonia were digested by the micro-Kjeldahl method and steam-distilled to collect the ammonia.

Samples were prepared for $^{15}N$ analysis according to Rittenberg (43). $^{15}N$ analyses were performed in duplicate on a 180° Consolidated Electrodynamic mass spectrometer (Bell & Howell, Electronic Instrumentation Group, Pasadena, Calif.). We wish to thank Mr. William Comstock and Dr. Julius White of the N. I. H. for some of the $^{15}N$ analyses. The majority of the analyses were performed by Dr. L. A.

[2] *Abbreviations used in this paper:* CoA-SH, reduced coenzyme A; formyl-GAM, α-formyl glycinamidineribonucleotide; formyl-GAR, α-formyl-GAR; 5-P, 5-phosphate; GAR, glycinamideribonucleotide; PP-ribose-P, α-5-phosphoribosyl-1-pyrophosphate; PRA, β-5-phosphoribosyl-1-amine; PRT, hypoxanthine-guanine phosphoribosyltransferase.

Pogorski, Chemical Projects Limited, Rexdale, Ontario. $^{15}N$ values on uric acid, on glycine isolated from uric acid and representing N-7, and on $NH_3$ produced by degradation of uric acid and representing N-(1+3+9), N-(1+7), N-(1+3), or N-(7+9) were obtained analytically. All five degradation values were available on most samples of uric acid. From these six analytical values, $^{15}N$ enrichment values were calculated for other N-atoms or pairs of atoms. These calculations, those of various ratios of enrichment values, or of checks of additions of enrichments of individual atoms against analytical values of larger fragments or of total uric acid were performed by a computer program that gave more than 100 calculated values per uric acid sample. The following calculated values were derived using only analytical values for $^{15}N$ enrichments:

$$^{15}N\text{-}1 = 2\left[^{15}N\text{-}\left(\frac{1+7}{2}\right)\right] - [^{15}N\text{-}7], \quad (1)$$

$$^{15}(N\text{-}9)_a = 2\left[^{15}N\text{-}\left(\frac{7+9}{2}\right)\right] - [^{15}N\text{-}7], \quad (2)$$

$$^{15}(N\text{-}9)_b = 3\left[^{15}N\text{-}\left(\frac{1+3+9}{3}\right)\right] - 2\left[^{15}N\text{-}\left(\frac{1+3}{2}\right)\right], \quad (3)$$

$$^{15}(N\text{-}9)_c = 4\left[^{15}N\text{-}\left(\frac{1+3+7+9}{4}\right)\right] - {}^{15}N\text{-}7 - 2\left[^{15}N\text{-}\left(\frac{1+3}{2}\right)\right], \quad (4)$$

$$^{15}N\text{-}(3+9)_a = 4\left[^{15}N\text{-}\left(\frac{1+3+7+9}{4}\right)\right] - 2\left[^{15}N\left(\frac{1+7}{2}\right)\right], \quad (5)$$

$$^{15}N\text{-}(3+9)_b = 3\left[^{15}N\left(\frac{1+3+9}{3}\right)\right] - 2\left[^{15}N\text{-}\left(\frac{1+7}{2}\right)\right] - [^{15}N\text{-}7], \quad (6)$$

$$^{15}N\text{-}3 = {}^{15}N\text{-}(3+9)_a - {}^{15}(N\text{-}9)_a \quad (7)$$

Although agreement of values of $^{15}(N\text{-}9)_a$, $_b$, and $_c$ was generally good, plots of consecutive values of $^{15}(N\text{-}9)_a$ gave the lowest variance, and only these values will be presented. Similarly, consecutive values of $^{15}N\text{-}(3+9)_a$ proved less variable than of $_b$, and will be employed. Of the several methods of calculation of $^{15}N$-3, the least variance was found with $^{15}N\text{-}(3+9)_a - {}^{15}(N\text{-}9)_a$, all of whose terms include analytical values of fragments that contain N-7. Therefore, only these values of N-3 will be used in this paper. Multiple cross-checks disclosed no evidence for systematic over- or underestimation of $^{15}N$ in any of the five degradation fragments of uric acid. A few calculated values for $^{15}N$-3 or $^{15}N$-9 were discarded when greater than 10% variation was found in one of the following comparisons for a given sample of uric acid:

$$4\left[^{15}N\left(\frac{1+3+7+9}{4}\right)\right] = 2\left[^{15}N\left(\frac{1+3}{2}\right)\right] + 2\left[^{15}N\left(\frac{7+9}{2}\right)\right] = 3\left[^{15}N\left(\frac{1+3+9}{3}\right)\right] + {}^{15}N\text{-}7, \quad (8)$$

$$^{15}N\text{-}7 = 4\left[^{15}N\text{-}\left(\frac{1+3+7+9}{4}\right)\right] - 3\left[^{15}N\text{-}\left(\frac{1+3+9}{3}\right)\right]. \quad (9)$$

*Subjects.* Summary data on the subjects of this study are presented in Table I. The data on control subject R. H. were acquired during an earlier study (30). Control subject L. L. was studied at the N. I. H., and [$^{15}N$]uric acid samples were kindly given to us by Dr. J. E. Seegmiller (4).

The four gouty subjects were carefully selected to represent different subtypes of primary gout. S. G. had normal renal function, was overweight, and had a 2-yr history of recurrent acute gouty attacks. He excreted an average of 648 mg uric acid/day on a strict low purine diet during the study (normal 418±70 mg/day on a comparable diet (44)). He is presently classified as a mild overexcretor with "idiopathic gout." R. Jo. was a 17-yr-old boy who had carried a diagnosis of cerebral palsy until an attack of gout supervened at age 15. Erythrocyte PRT activity was 0.22 nmol of IMP formed/mg protein/h, a value of less than 0.25% of normal. This patient has many features of the Lesch-Nyhan syndrome (45), but is not a high grade mental defective and shows no self-destructive tendencies (25). O. G., an Israeli subject, was an extraordinary overproducer with a history of recurrent uric acid lithiasis and acute gout. He was initially selected as a patient with "idiopathic gout" and flamboyant overexcretion. In subsequent studies, both he and a hyperuricemic brother were found to have excessive levels of PP-ribose-P in erythrocytes, and to produce PP-ribose-P at excessive rates (46). O. G. has now been shown to have an erythrocyte PP-ribose-P synthetase with reduced sensitivity to nucleotide inhibitors such that it is more than four times as active as the normal enzyme at concentrations of inorganic phosphate ranging from 0.2 to 0.5 mM but of normal activity above 2 mM (27, 28). T. B. was extensively studied at the N.I.H. and has been the subject of several reports (24, 47–49). At the time of the study reported herein he was suspected of having a mutant PP-ribose-P amidotransferase with altered control features (47). More recently he has been found to have 2–2.5-fold excessive level of PP-ribose-P synthetase activity at all inorganic phosphate concentration values tested up to at least 32 mM (29). Thus the defect in T. B. appears to differ from that of the prototype case of Sperling, Persky-Brosh, Boer, and deVries (28). We are

TABLE I

*Data on Subjects of This Study*

| Subject (males) | Age | Wt | Uric acid | | Incorporation of [$^{15}$N]glycine* | Comment |
|---|---|---|---|---|---|---|
| | | | Serum | Urine | | |
| | *yr* | *kg* | *mg/100 ml* | *mg/24 h* | *% into urinary uric acid/7 days* | |
| Nongouty subjects | | | | | | |
| R. H. | 27 | 84 | 5.4 | 523 | 0.12 | Normal subject. Experimental details in Ref. 38. |
| F. L. | 23 | 75 | 5.1 | 580 | 0.13 | Normal subject. |
| L. L. | 28 | 70 | 5.5 | 388 | 0.17 | Normal subject. (4) |
| Gouty subjects | | | | | | |
| S. G. | 46 | 86 | 8.0 | 648 | 0.17 | Idiopathic gout. |
| R. Jo. | 17 | 44 | 15.3 | 1,100 | 1.02 | Hypoxanthine-guanine phosphoribosyltransferase deficiency. Gout, severe spasticity, choreoathetosis. (25) |
| O. G. | 32 | 90 | 13.5 | 2,400 | 3.32 | Superactive PP-ribose-P synthetase. Gout, uric acid lithiasis. (27, 28, 46) |
| T. B. | 48 | 78 | 10.3 | 1,242 | 1.09 | Superactive PP-ribose-P synthetase. Gout, uric acid lithiasis. (29, 47–49) |

* Values have not been corrected for extrarenal disposal (4).

also indebted to Dr. J. E. Seegmiller for a gift of [$^{15}$N]uric acid samples from this patient.

*Design of experiments.* Each subject was placed on a standard low-purine diet 5 days before the oral administration of 65 mg/kg body wt of [$^{15}$N]glycine, 95 atom per cent excess. Except as noted in the footnotes of Tables II and III, urine was collected in consecutive periods of 18, 4, and 2 h each day throughout the study. At the beginning of the 18th h each day, 1 g of [1-$^{14}$C]phenylacetic acid (specific activity 2 $\mu$Ci/g) was administered orally in 240 ml of water to promote synthesis of phenylacetylglutamine. At the beginning of the 22nd h each day, 400 mg of sodium benzoate was administered orally in 240 ml of water to promote synthesis of hippuric acid. Uric acid (and in one patient, DNP-glycine) was isolated from the 18-h urine samples; phenylacetylglutamine, and ammonia, from the 4-h samples; and hippurate from the final 2-h portions.[3]

A different schedule was employed with patient O. G. in order to obtain data during the first few hours after administration of [$^{15}$N]glycine. The experiment was started by administration of 1 g of phenylacetic acid (2 $\mu$Ci) followed 1 h later by 400 mg of sodium benzoate. [$^{15}$N]glycine was given 1 h after the benzoate and was followed by two urine collections of 1 h each and one collection of 2 h. Second doses of phenylacetic acid and benzoate (combined) were then given, followed by two collection periods of 3 and 4 h, respectively. Third doses of phenylacetic acid and benzoate (combined) were followed by one collection period of 12 h; this terminated the 1st day of the experiment. The 2nd and subsequent days were started by combined administrations of phenylacetic acid and benzoate, after which urine was collected in sequential samples of 6 and

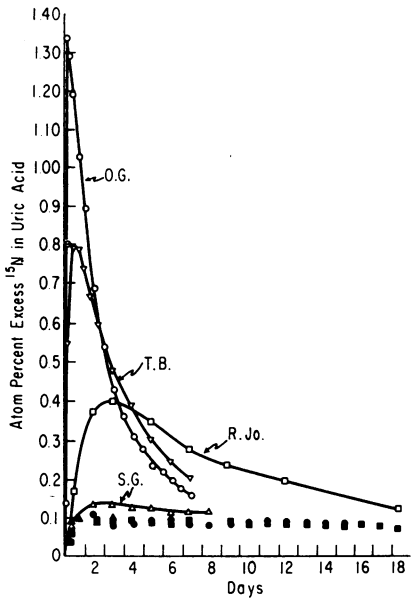


FIGURE 1 $^{15}$N enrichment of urinary uric acid in three control (solid symbols) and four gouty subjects (open symbols).

[3] Subjects L. L. and T. B., patients of Dr. Seegmiller, received 0.1 g/kg of [$^{15}$N]glycine, 60 atom percent excess. They were not given benzoate or phenylacetic acid, and urine was collected in 6, 12, or 24 h periods (4, 24).

TABLE II

*Intramolecular Distribution of Uric Acid $^{15}N$ in Three Nongouty Subjects and Four Gouty Subjects Given [$^{15}N$]Glycine*

| Subject | Day # | Total [$^{15}$N]*Uric Acid* | $^{15}$N-7 | | $^{15}$N-1 | | $^{15}$N-(3 + 9) | |
|---|---|---|---|---|---|---|---|---|
| | | *Atom % excess* | *Atom % excess* | *% of total uric acid-$^{15}N$* | *Atom % excess* | *% of total uric acid-$^{15}N$* | *Atom % excess** | *% of total uric acid-$^{15}N$* |
| Nongouty subjects | | | | | | | | |
| R. H. | 1 (0–10) | 0.0370 | 0.1305 | 88 | 0.0233 | 16 | — | — |
| | 1 (12–22) | 0.0773 | 0.2500 | 80 | 0.0402 | 13 | 0.0055 | 2 |
| | 2 (24–34) | 0.0900 | 0.2624 | 73 | 0.0498 | 14 | 0.0239 | 13 |
| | 2 (36–46) | 0.1002 | 0.2804 | 69 | 0.0522 | 13 | 0.0341 | 17 |
| | 3 | 0.0975 | 0.3031 | 77 | 0.0323 | 8 | 0.0273 | 14 |
| | 4 | 0.0985 | 0.2962 | 75 | 0.0434 | 11 | 0.0272 | 14 |
| | 5 | 0.0909 | 0.2895 | 79 | 0.0277 | 8 | 0.0232 | 13 |
| | 6 | 0.0952 | 0.2780 | 73 | 0.0424 | 11 | 0.0302 | 16 |
| | 7 | 0.0999 | 0.2698 | 67 | 0.0388 | 10 | 0.0480 | 24 |
| | 9 | 0.0922 | 0.2850 | 77 | 0.0208 | 6 | 0.0315 | 17 |
| | 10 | 0.0909 | 0.2642 | 72 | 0.0312 | 9 | 0.0318 | 17 |
| | 11 | 0.1006 | 0.2688 | 66 | 0.0286 | 7 | 0.0525 | 26 |
| | 12 | 0.0970 | 0.2663 | 68 | 0.0437 | 11 | 0.0390 | 20 |
| | 13 | 0.0891 | 0.2635 | 73 | 0.0441 | 12 | 0.0244 | 14 |
| | 14 | 0.0839 | 0.2447 | 72 | 0.0259 | 8 | 0.0325 | 20 |
| | 15 | 0.0837 | 0.2315 | 69 | 0.0541 | 16 | 0.0246 | 15 |
| | 16 | 0.0862 | 0.2171 | 62 | 0.0489 | 14 | 0.0394 | 23 |
| | 17 | 0.0824 | 0.2055 | 62 | 0.0465 | 14 | 0.0388 | 24 |
| | 18 | 0.0730 | 0.2013 | 68 | 0.0389 | 13 | 0.0259 | 17 |
| | 19 | 0.0694 | 0.1832 | 65 | 0.0418 | 15 | 0.0263 | 19 |
| | 20 | 0.0746 | 0.1790 | 59 | 0.0316 | 11 | 0.0439 | 30 |
| | 21 | 0.0656 | 0.1755 | 66 | 0.0353 | 14 | 0.0258 | 20 |
| | 22 | 0.0734 | 0.1763 | 60 | 0.0257 | 9 | 0.0458 | 31 |
| | 23 | 0.0610 | 0.1771 | 72 | 0.0121 | 5 | 0.0274 | 23 |
| | 24 | 0.0599 | 0.1523 | 63 | 0.0345 | 14 | 0.0264 | 22 |
| F. L. | 1 | 0.0789 | 0.2028 | 64 | 0.0678 | 21 | 0.0225 | 15 |
| | 2 | 0.1117 | 0.2963 | 66 | — | — | — | — |
| | 3 | 0.0952 | 0.2268 | 59 | 0.0830 | 22 | 0.0355 | 19 |
| | 4 | 0.0912 | 0.2364 | 65 | 0.0504 | 14 | 0.0390 | 21 |
| | 5 | 0.0916 | 0.2138 | 58 | 0.0754 | 21 | 0.0386 | 21 |
| | 6 | 0.0988 | 0.2489 | 63 | 0.0641 | 16 | 0.0411 | 21 |
| | 7 | 0.0877 | 0.2264 | 65 | 0.0492 | 14 | 0.0376 | 21 |
| | 8 | 0.0841 | 0.2376 | 71 | 0.0306 | 9 | 0.0341 | 20 |
| | 9 | 0.0847 | 0.2219 | 66 | 0.0379 | 11 | 0.0395 | 23 |
| | 10 | 0.0954 | 0.2424 | 64 | 0.0612 | 16 | 0.0390 | 20 |
| | 11 | 0.0996 | 0.2543 | 64 | 0.0553 | 14 | 0.0444 | 22 |
| | 12 | 0.0943 | 0.2365 | 63 | 0.0609 | 16 | 0.0399 | 21 |
| | 13 | 0.0928 | 0.2291 | 62 | 0.0593 | 16 | 0.0414 | 22 |
| | 14 | 0.0847 | 0.2187 | 64 | — | — | — | — |
| | 15 | 0.0857 | 0.2129 | 62 | 0.0529 | 16 | 0.0385 | 20 |
| L. L.‡ | 1 (0–6) | 0.0556 | 0.2372 | 78 | — | — | — | — |
| | (6–12) | 0.0912 | 0.2062 | 70 | 0.0468 | 16 | 0.0211 | 12 |
| | (12–18) | 0.1032 | 0.2379 | 72 | 0.0281 | 9 | 0.0332 | 16 |
| | (18–24) | 0.0872 | 0.2537 | 73 | 0.0443 | 13 | 0.0254 | 15 |
| | 2 (36–48) | 0.0912 | 0.2801 | 77 | — | — | — | — |
| | 3 | 0.1006 | 0.2910 | 72 | 0.0482 | 12 | 0.0317 | 16 |
| | 6 | 0.1006 | 0.2840 | 71 | 0.0696 | 17 | 0.0244 | 12 |

TABLE II—(*Continued*)

| Subject | Day # | Total [$^{15}$N]Uric Acid | $^{15}$N-7 | | $^{15}$N-1 | | $^{15}$N-(3 + 9) | |
|---|---|---|---|---|---|---|---|---|
| | | *Atom % excess* | *Atom % excess* | *% of total uric acid-$^{15}$N* | *Atom % excess* | *% of total uric acid-$^{15}$N* | *Atom % excess** | *% of total uric acid-$^{15}$N* |
| Gouty subjects | | | | | | | | |
| S. G. | 1 | 0.0771 | 0.1708 | 55 | 0.0780 | 25 | 0.0296 | 19 |
| | 2 | 0.1358 | 0.3491 | 64 | 0.0813 | 15 | 0.0563 | 21 |
| | 3 | 0.1343 | 0.3172 | 59 | 0.1080 | 20 | 0.0560 | 21 |
| | 4 | 0.1303 | 0.3340 | 64 | 0.0790 | 15 | 0.0541 | 21 |
| | 5 | 0.1243 | 0.3098 | 62 | 0.0862 | 17 | 0.0506 | 20 |
| | 6 | 0.1147 | 0.2794 | 61 | 0.0678 | 15 | 0.0558 | 24 |
| | 7 | 0.1153 | 0.2857 | 62 | 0.0663 | 14 | 0.0546 | 24 |
| | 8 | 0.1191 | 0.2911 | 61 | 0.0639 | 13 | 0.0607 | 25 |
| R. Jo. | 1 | 0.1679 | 0.5558 | 77 | 0.0412 | 6 | 0.0814 | 22 |
| | 2 | 0.3762 | 1.0445 | 69 | 0.2405 | 16 | 0.1099 | 15 |
| | 3 | 0.4038 | 1.0802 | 67 | 0.1340 | 8 | 0.2303 | 19 |
| | 4 | — | 0.8248 | 62 | — | — | — | — |
| | 5 | 0.3507 | 0.8954 | 64 | 0.1926 | 14 | 0.1744 | 25 |
| | 7 | 0.2828 | 0.7020 | — | 0.1664 | — | 0.1545 | — |
| | 9 | 0.2422 | 0.4750 | 62 | — | 15 | 0.1430 | 27 |
| | 12 | 0.1756 | 0.4125 | 53 | 0.0921 | 12 | 0.1329 | 33 |
| | 18 | 0.1170 | 0.2977 | 58 | 0.0315 | 6 | 0.0894 | 35 |
| T. B.‡ | 1 (0–6) | 0.5561 | 1.2010 | 53 | 0.4254 | 19 | 0.2990 | 27 |
| | (6–12) | 0.7989 | 1.5871 | 49 | 0.7237 | 23 | 0.4424 | 28 |
| | (12–18) | 0.7908 | 1.6937 | 53 | 0.4000 | 13 | 0.5348 | 34 |
| | (18–24) | 0.7420 | 1.5430 | 51 | 0.5580 | 19 | 0.4335 | 30 |
| | 2 (24–36) | 0.6707 | 1.3477 | 50 | 0.5669 | 21 | 0.3841 | 29 |
| | 3 | 0.4820 | 0.9736 | 54 | 0.2636 | 13 | 0.3454 | 33 |
| | 5 | 0.3060 | 0.6047 | 49 | 0.2137 | 17 | 0.2028 | 34 |
| | 6 | 0.2496 | 0.4801 | 48 | 0.1343 | 13 | 0.1920 | 39 |
| O.G. | 1 (0–1) | 0.1384 | 0.4183 | 75 | 0.1143 | 21 | 0.0103 | 4 |
| | (1–2) | 0.8082 | 1.9860 | 62 | — | — | — | — |
| | (2–4) | 1.335 | 3.0821 | 58 | 0.9141 | 17 | 0.6729 | 25 |
| | (4–7) | 1.2979 | 2.9495 | 56 | 0.6500 | 13 | 0.8000 | 31 |
| | (7–12) | 1.1920 | 2.7830 | 58 | 0.4904 | 10 | 0.7988 | 33 |
| | (12–24) | 1.0375 | 2.2110 | 53 | 0.6516 | 16 | 0.6437 | 31 |
| | 2 (24–30) | 0.8963 | 1.8626 | 52 | 0.6685 | 19 | 0.5182 | 29 |
| | 3 (48–54) | 0.5400 | 1.0565 | 49 | 0.4996 | 23 | 0.3057 | 28 |
| | 4 (72–78) | 0.3676 | 0.6786 | 46 | 0.2900 | 20 | 0.2502 | 34 |
| | 5 (96–102) | 0.2819 | 0.4979 | 44 | 0.2485 | 22 | 0.1906 | 34 |
| | 6 (120–126) | 0.2234 | 0.4320 | 48 | 0.2298 | 26 | 0.1159 | 26 |
| | 7 (144–150) | 0.1811 | 0.3473 | 48 | — | — | — | — |

In subjects F. L., S. G., and R. Jo., uric acid was isolated from the 0–18-h urine sample each day. In subject R. H. uric acid was isolated from the 0–22-h sample from day 3 on. In subjects L. L. and T. B. uric acid was isolated from 24-h samples except as noted. Numbers in parentheses for subjects R. H., L. L., T. B., and O. G. represent fractional collections in hours from the time of administration of [$^{15}$N]glycine.

* Values are ½ of those obtained from Eq. 5. If N-(3 + 9) were isolable as a fragment, its analysis would give $^{15}N\left(\frac{3+9}{2}\right)$. The percentage given represents the amount of $^{15}$N in N-3 plus N-9.

‡ Subjects L. L. and T. B. received 0.1 g/kg of [$^{15}$N]glycine, 60 atom percent excess [4,48]. All others received 0.065 g/kg of [$^{15}$N]glycine, 95 atom percent excess (see Methods).

18 h. Uric acid, hippurate, phenylacetylglutamine, and ammonia were isolated from each of the 1-, 2-, 3-, 4-, 6-, and 12-h collections, and uric acid only from the 18-h samples. 2-ml aliquots were taken for collection of ammonia. Uric acid was separated from the rest of the sample by absorption on charcoal. Hippuric acid was precipitated from

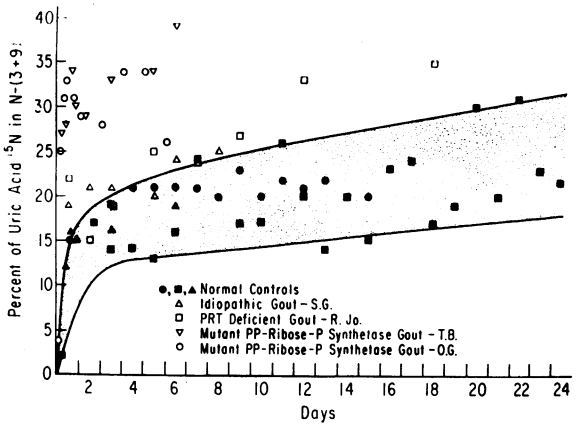


FIGURE 2 Intramolecular distribution of $^{15}$N in uric acid, expressed as percent of total $^{15}$N found in N-(3 + 9). The shaded area includes essentially all values on the three control subjects.

the charcoal filtrate, and phenylacetylglutamine was then isolated from the filtrate of the hippuric acid crystals, by the methods described above.

## RESULTS

*Incorporation of $^{15}$N into urinary uric acid.* Time courses of enrichment of urinary uric acid for the three control and four gouty subjects are shown in Fig. 1. Cumulative incorporation values of $^{15}$N into urinary uric acid in 7 days are shown in Table I. The values on S. G., the patient with idiopathic gout, fall at the upper limits of the normal range in both respects (50), but an enlarged miscible pool and enhanced extrarenal disposal of urate may have obscured more decisive evidence for overproduction in this subject (7). The three other gouty patients present unequivocal evidence for extraordinary overincorporation of $^{15}$N and excessive excretion and flamboyant overproduction of uric acid.

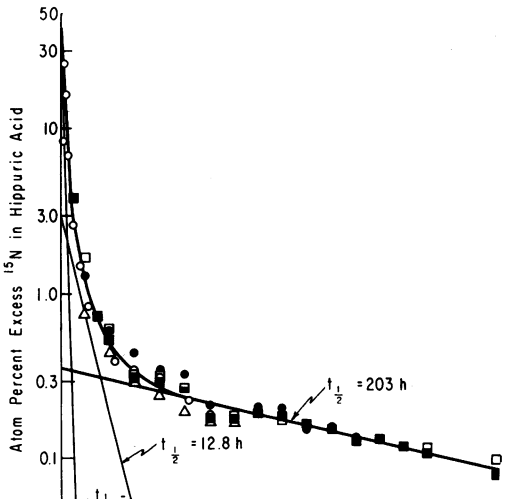


FIGURE 3 $^{15}$N enrichment of urinary hippuric acid in control and gouty subjects (■, R. H.; ●, F. L.; ○, O. G.; □, R. Jo.; △, S. G.). See footnote 5 also.

*Intramolecular distribution of $^{15}$N in uric acid.* Analytical value for $^{15}$N in uric acid and N-7, and calculated values for $^{15}$N in N-1 and N-(3 + 9) are given in Table II, together with the percent of total $^{15}$N in the various N atoms of each sample.

In Fig. 2 the percent of total uric acid-$^{15}$N found in N-(3 + 9) is plotted as a function of time. Within hours an appreciable amount of $^{15}$N has appeared in N-(3 + 9). All of the gouty subjects show elevated percentages of $^{15}$N in N-(3 + 9). Those of S. G. appear slightly above normal on most days, especially on days 1 and 2, but two of seven values fall within the upper range of control values. Those of R. Jo. are slightly high initially (though not without some overlap with normal values) and become more strikingly elevated with time. Those of O. G. and T. B. exceed normal values by a factor of 2 within the 1st day.

These data confirm those of Gutman and Yu (6) in gouty overproducers given [$^{15}$N]glycine. However, the percentages of $^{15}$N found in N-(3 + 9) in their control subjects were very low compared with ours or others in the literature (34). We find that 10 of 14 values of $^{15}$N-(3 + 9) in their three gouty subjects with normal total $^{15}$N incorporation values (0.09, 0.08, and 0.15% of administered $^{15}$N/7 days, uncorrected for extrarenal disposal of urate) fall within the upper segments of our normal range.[4] Thus preferential enrichment of N-(3 + 9) is most convincingly shown in patients with unequivocally accelerated uric acid production de novo; patients with idiopathic gout and more modest degrees of overproduction may show minimally elevated percentage enrichment of N-(3 + 9) with some values which may fall within the normal range.

*$^{15}$N enrichment and turnover of metabolic glycine pools.* Small doses of benzoic acid were administered periodically to two controls (R. H. and F. L.) and three gouty subjects (S. G., R. Jo. and O. G.), in order to sample body glycine (38). Hippurate was isolated from urine collected during the succeeding few hours. The data are plotted in Fig. 3. The values form a typical polyexponential die-away curve, in which enrichment values in gouty and nongouty subjects are indistinguishable.[5]

[4] This finding is not an artifact of the method whereby $^{15}$N(3 + 9) was calculated (see footnote 1), for values of $^{15}$N-9 in their controls (6) are also low compared with those in Table II or reference 34.

[5] The die-away curves were analyzed by an unweighted least squares method. Parameter variances were estimated

We conclude, with others (4, 6), that excessive incorporation of [$^{15}$N]glycine into uric acid in gout is not a reflection of abnormal enrichment or turnover of hepatic glycine, but rather that it is indicative of the utilization of a larger than normal fraction of the glycine turnover for purine biosynthesis per unit time (1). Even in the most extraordinary overincorporators this fraction is less than 1%/day; hence it is not surprising that increased rates of purine biosynthesis do not produce detectable changes in the kinetics of turnover of the glycine pool.

In one subject (R. Jo.) free urinary glycine was isolated as DNP-glycine in order to determine how accurately the $^{15}$N enrichment of hippurate reflects that of glycine itself. The data are plotted in Fig. 4. The $^{15}$N values of hippurate fall about 20% below those of free glycine at any point in time, but the curves are otherwise essentially identical.[6]

*$^{15}$N enrichment and turnover of the metabolic glutamine pool(s).* Gutman and Yu (6) originally proposed that the disproportionately great enrichment of N-(3 + 9) in gouty subjects was attributable to "glutamine amide nitrogen containing an unduly high concentration of $N^{15}$." To evaluate this possibility we have sampled body glutamine by the periodic administration of phenylacetic acid. This approach is analogous to sampling of glycine by administration of benzoic acid (38) or of PP-ribose-P by administration of imidazoleacetic acid (53).

Phenylacetylglutamine is a normal constituent of human urine. Fasting adult subjects excrete 250–500 mg/day, and these quantities represent approximately 50% of conjugated glutamine of urine (40). Excretion of phenylacetylglutamine is increased after ingestion of phenylacetic acid (54) and in patients with phenylketonuria who produce increased quantities of phenylacetic acid (40, 55, 56). The reactions concerned have been defined by Moldave and Meister (57, 58) in human liver mitochondria:

$$\text{Phenylacetic acid} + \text{ATP} \rightleftharpoons \text{Phenylacetyl-AMP} + PP_i, \quad (10)$$

$$\text{Phenylacetyl-AMP} + \text{reduced CoA (CoA-SH)} \rightleftharpoons \text{Phenylacetyl-CoA} + \text{AMP}, \quad (11)$$

$$\text{Phenylacetyl-CoA} + \text{L-glutamine} \rightleftharpoons \text{Phenylacetylglutamine} + \text{CoA-SH}. \quad (12)$$

Preliminary studies were conducted in three normal controls and four normal excretor patients with idiopathic gout, in order to determine the rate of formation and excretion of phenylacetylglutamine. After oral administration of 1 g of [1-$^{14}$C]phenylacetic acid (2-$\mu$Ci $^{14}$C/g), from 66 to 70% of the administered $^{14}$C was excreted in urine in 3 h, and more than 96% in 12 h. About 70% of the $^{14}$C in the 3-h sample was in the form of phenylacetylglutamine. The rates of excretion of [$^{14}$C]phenylacetylglutamine were the same in control and gouty subjects. The periodic oral administration of 1 g of phenylacetic acid was therefore employed to sample what is presumably chiefly the hepatic glutamine pool. Urinary phenylacetylglutamine was isolaated serially from one control (F. L.) and three gouty subjects (S. G., R. J., and O. G.). These four subjects span a four-fold range of urinary uric acid excretion and a 25-fold range of [$^{15}$N]glycine incorporation values (Table I). Immediately after administration of [$^{15}$N]glycine, the enrichment of urinary phenylacetylglutamine was very low. It rose to a maximum at about 3 h (see below) and then declined rapidly. The declining limbs of the enrichment curves are plotted in Fig. 5. As in the case of labeling of urinary hippurate, the enrichment values from a single polyexponential die-away curve, in which enrichment in the four subjects studied appears to be indistinguishable.

We conclude that the excessive incorporation of $^{15}$N into N-(3 + 9) of uric acid in gouty overproducers is not a reflection of abnormal enrichment or turnover of hepatic glutamine, but that (as in the case of glycine)

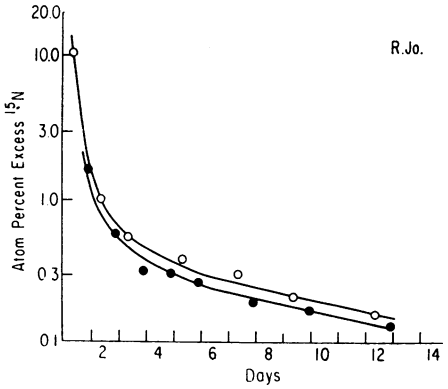


FIGURE 4 Comparison of $^{15}$N enrichment in urinary hippuric acid (○) and urinary free glycine (●) in subject R. Jo.

from the inverse design matrix defined by a Taylor expansion (51) of the nonlinear model and the estimated error variance. Unweighted analysis was selected because measurement errors in the mass spectrometer are constants, approximating ±0.003 atom percent excess (52). A minimum of three exponential functions is required to describe the data presented in Figs. 3 and 5.

[6] Calculation of the size of the first glycine metabolic pool from the $C_o$ value of glycine, assumed to be 20% greater than the $C_o$ value of hippurate (see Fig. 3) gives an average value of 80.8 mg/kg in these five subjects, compared with values of 80 (39) and 83 (3) mg/kg obtained by other techniques in man.

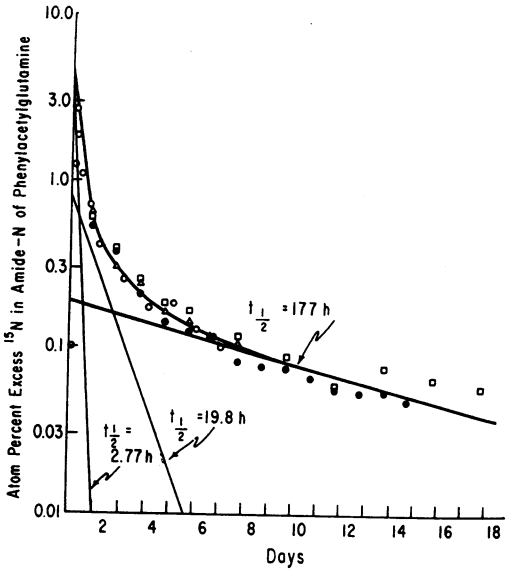


FIGURE 5 $^{15}$N enrichment of amide-N of urinary phenylacetylglutamine in control and gouty subjects. (●, F. L.; ○, O. G.; □, R. Jo.; △, S. G.).

it is indicative of the utilization of a larger than normal fraction of the precursor glutamine pool for purine biosynthesis per unit time.

*Precursor-product relationships.* After the experimental labeling of a precursor pool, the concentration of isotope declines progressively as labeled molecules are replaced by newly synthesized unlabeled compound. The isotope concentration of each successive product of an unbranched pathway will achieve a maximal value when its enrichment equals that of its immediate precursor (59).

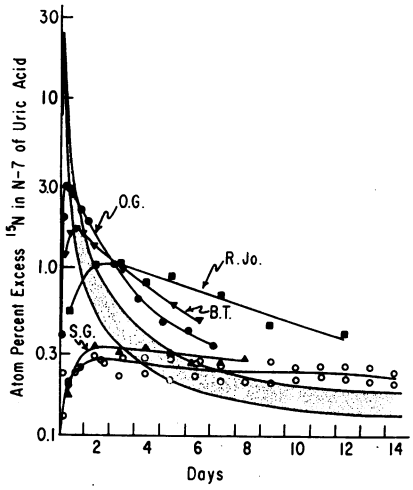


FIGURE 6 Time course of $^{15}$N enrichment of N-7 of uric acid compared with the range of $^{15}$N enrichment values of urinary hippuric acid. (Open circles represent data on control subjects R. H. and F. L.).

Fig. 6 presents the enrichments of N-7 of urinary uric acid in comparison with those of hippurate, as functions of time; Fig. 7 presents the enrichments of N-3 in comparison with those of the amide-N of phenylacetylglutamine.

In spite of several additional reactions between glycinamideribonucleotide (GAR) and α-formyl glycinamidineribonucleotide (formyl-GAM) (the intermediates that first contain the eventual N-7 and N-3 atoms, respectively) and the ultimate product, uric acid, the classic isotopic relationships of precursor and immediate product are closely approximated: each enrichment peak is found in reasonably close proximity to the value of hippurate or amide-N of glutamine at the corresponding time.

In addition, the more rapid the rate of incorporation of $^{15}$N into uric acid (Table I, Fig. 1), the earlier and higher the peak enrichment value is found in both N-7 and N-3. This result requires that the increase in rate of conversion of precursor to product is not balanced by a commensurate expansion of the diluting pool of product, for proportionate expansion of product pool size would lead to an enrichment curve independent of rate of synthesis or turnover of product. This relationship is implicit in the classic treatment of this subject by Zilversmit, Entenman, and Fishler (59). In terms of the purine biosynthetic pathway, the results of Figs. 1, 6, and 7 require that increases in rates of incorporation of $^{15}$N into uric acid are not matched by equivalent expansions of the pools of GAR, formyl-GAM, or the miscible pool of uric acid itself. These conditions in turn require that the biotransformation of late purine intermediates into uric acid be disproportionately augmented when total purine biosynthesis is increased, and that the miscible pool of uric acid expand less than the rate of its biosynthesis is increased. Possible theoretical mecha-

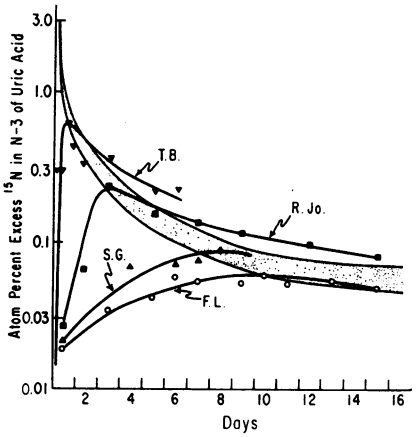


FIGURE 7 Time course of $^{15}$N enrichment of N-3 of uric acid compared with range of $^{15}$N enrichment values of the amide-N of urinary phenylacetylglutamine.

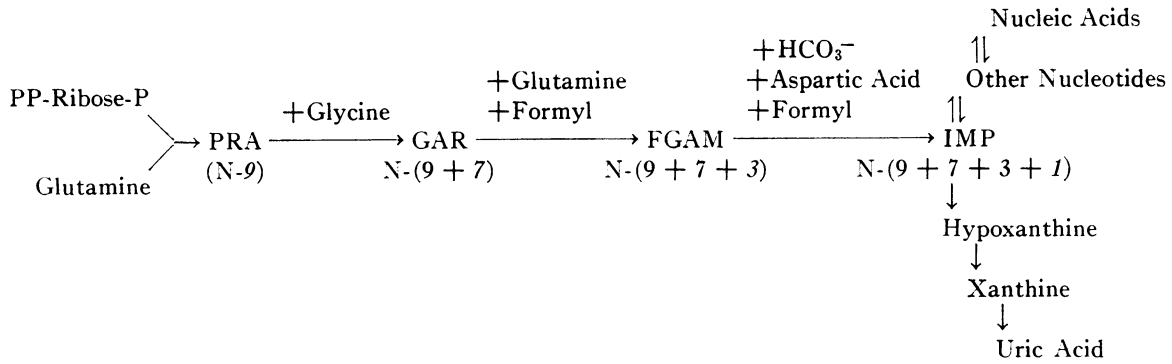


FIGURE 8 Reaction sequence showing order of incorporation of nitrogen atoms into purine ring. F, formyl.

nisms include: first, acceleration of a late reaction by (*a*) concomitant increase in concentration of a collateral substrate, e.g., of aspartate or bicarbonate in Fig. 8, or (*b*) activation of some late enzyme of the biosynthetic pathway by an intermediate produced in excess, i.e., a rate constant, $k = f$ (some precursor); second, feedback controls on the conversion of IMP to AMP (60) or GMP (61, 62) or early saturation of these pathways with shunting of excess IMP into degradative routes; third, reduced recycling of hypoxanthine and guanine into IMP and GMP, respectively, through the inhibition of PRT exerted by these nucleotides (63); fourth, four- to fivefold increases in activity of hepatic xanthine oxidase reported in gouty overproducers (64); fifth, increases in rates of renal (50) and extrarenal (50) disposition of uric acid as plasma urate levels are raised; sixth, deposition of urate in insoluble immiscible form.

*Comparison of labeling of N-3 and N-9.* The results presented thus far favor a kinetic explanation of the observed enhancement of labeling of N-(3 + 9) in gouty overproducers, which is independent of the nature of the specific metabolic abnormality that initiates purine overproduction.[7] However, Reem (65) has suggested an alternative explanation based upon a second pathway for synthesis of phosphoribosylamine that utilizes ammonia and ribose 5-phosphate, rather than glutamine and PP-ribose-P. The ribose 5-phosphate aminotransferase pathway (66) could theoretically lead to excessive incorporation of $^{15}N$ selectively into N-9 of uric acid, if abnormally active in gout.[8] Accordingly, calculations were made of the relative labeling of N-3 and N-9.

In previous studies, the percentage of $^{15}N$ in either N-3 or N-9 was considered to be half the percentage calculated in N(3 + 9) (38), or conversely, the percentage in N-(3 + 9) was assumed to be twice that calculated in N-9 (6). This practice rests upon several assumptions: first, that N-3 and N-9 are equivalently labeled by virtue of an exclusive common origin from the amide-N of glutamine (7–9); second, that both N-3 and N-9 come from the same glutamine pool, or from different pools identically labeled; third, that there are no exchange reactions involving the N-atom of either intermediate; and fourth, that there are no pools of intermediates between PRA (which contains the eventual N-9 atom) and formyl-GAM (which contains the eventual N-3 atom) of sufficient magnitude to dilute N-9 relative to N-3.

We have reexamined the question of equivalence of labeling of N-9 and N-3 in four subjects, including one control (F. L.), one patient with idiopathic gout (S. G.),

[7] A computer model has been constructed simulating the kinetics of the reaction sequence of Fig. 8. The isotopic enrichment of glycine is represented by the equation for the polyexponential die-away curve of hippurate shown in Fig. 3. That of the amide-N of glutamine is represented by a first-order product curve derived from the enrichment of phenylacetylglutamine, which starts at zero, reaches a maximum at 3 h, and thereafter closely approximates the die-away curve of Fig. 5. The model also includes an arbitrary convention which accelerates a late reaction of the sequence (IMP → hypoxanthine) as a higher power function of [PP-ribose-P], in order to achieve a ratio of $> 1$ in the expression (increase in rate of synthesis of $\beta$-phosphoribosylamine)/(increase of product pool size). With this model it is possible to show that an increase in concentration of PP-ribose-P will lead to a substantial increase in the ratio $^{15}N$-3/$^{15}N$-7, or $^{15}N$-(3 + 9)/$^{15}N$-(3 + 9 + 7) within the first few hours after administration of [$^{15}N$]glycine. Similar results are obtained if increased synthesis of uric acid is predicated upon an increase in concentration of glutamine, or an increase in activity of the amidotransferase, provided that compensatory expansion of product pool size is prevented by some convention in each case. This model will be presented in detail in another publication (Starmer, C. F., O. Sperling, and J. B. Wyngaarden. A kinetic model for uric acid labeling in primary gout. In preparation.)

[8] However, in order for an alternative pathway of synthesis of $\beta$-5-phosphoribosyl-1-amine (PRA) to result in greater enrichment of N-9 in patients with excessive rates of purine biosynthesis, and in an increase in the labeling ratio under consideration, the condition that expansion of product pools not cancel out effects of increased rates of synthesis must also be met.

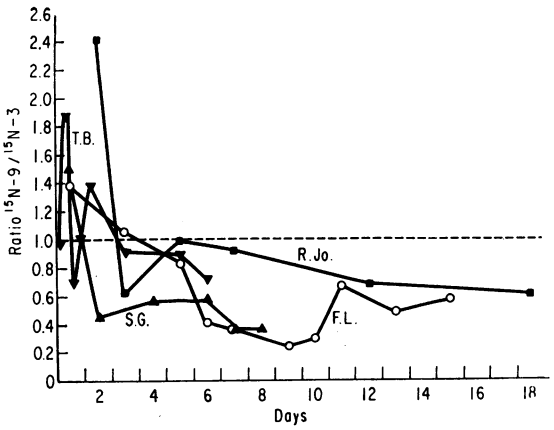


FIGURE 9 Ratio of $^{15}$N enrichment of N-9 to N-3 of uric acid with time.

one patient with gout and partial deficiency of phosphoribosyltransferase (R. Jo.), and one patient with gout and excessive activity of PP-ribose-P synthetase (T. B.). Values for N-9 and N-3 were calculated as described above (Eq. 2 and 7). Fig. 9 shows that, contrary to long-held assumptions, enrichments of N-9 and N-3 are not equivalent. During the first 2 days there is a tendency for N-9 to be more highly enriched than N-3, whereas at later times the reverse is observed. The mean of all values of the ratio $^{15}$N-9/$^{15}$N-3 on day 1 is 1.24, on day 2 is 1.42, on day 3 is 0.86, and on days 4 through 18 is 0.59. Fig. 10 shows that the ratio $^{15}$N-9/$^{15}$N-uric acid is fairly constant in any given subject.[9] By contrast (Fig. 11) the ratio $^{15}$N-3/$^{15}$N-uric acid appears to increase with time in all subjects studied. Note that excessive incorporation of $^{15}$N into uric acid in the overexcretor gouty subjects is not associated with a selective increase of enrichment of N-9 or N-3 relative to the other member of this pair (Fig. 9). Despite the scatter of points, the values of the ratio $^{15}$N-9/$^{15}$N-3 in the four subjects appear to overlap on any given day. In Figs. 10 and 11, T. B., the most dramatic gouty overproducer included in this phase of the study, shows the highest fractional enrichment of both N-3 and N-9. Thus a selectively excessive incorporation of $^{15}$N into N-9 is not the explanation of the elevated $^{15}$N-(3 + 9)/[$^{15}$N]-uric acid ratios observed in gouty overproducers.

*Sequence of enrichment of urinary products.* The study on the remarkable overproducer, O. G., offered an opportunity to observe the sequences of enrichments of various urinary constituents (Fig. 12). The earliest peaks were found in hippurate and ammonia at 1.5 h, in agreement with findings by Wu and Bishop (67) in a normal subject. Maximal enrichment of the amide-N of phenylacetylglutamine occurred at 3 h. Peak enrichment of N-7 was found at 3 h, and N-(3 + 9) at 5.5 h. Glycine, the immediate precursor of hippurate and of N-7, had presumably reached its enrichment maximum somewhat earlier, perhaps at about 1 h (67). Glutamine probably reached its maximal enrichment sometime past 1.5 h, based on the time of peak enrichment of urinary ammonia, and the presumed operation of the glutamine synthetase reaction (see below). The source of the urinary ammonia, highly labeled so early, is probably glycine itself, by deamination in liver and kidney catalyzed by glycine decarboxylase (68) or D-amino acid oxidase (69). In the 1st h, the enrichment of urinary ammonia is nearly 50 times greater than that of the amide-N of phenylacetylglutamine, whereas that of hippurate is less than twice that of ammonia (Fig. 12). Enrichment values of ammonia declined to equal those of the amide-N of phenylacetylglutamine in the 7–12-h sample, and the two values were much the same thereafter throughout the 7 days of the study. The results in control subject F. L. and gouty subject S. G. were similar, but in PRT-deficient subject R. Jo., enrichment values of $NH_3$ averaged 24% greater than those of the amide-N (Table III). Ammonia is thus a plausible candidate for the source of the excessive enrichment of N-9 with respect to N-3 on days 1 and 2 (see Discussion). However, preferential entry of $^{15}$N into N-(3 + 9) is not observed when $^{15}NH_4Cl$ itself is fed to gouty subjects (5). In this circumstance one would anticipate nearly simultaneous, rather than sequential, labeling of glycine and the amide-N of glutamine, and a fixed ratio, $^{15}$N-3/$^{15}$N-7, regardless of rate of purine biosynthesis.

*Ratios of [$^{15}$N]amide to [$^{15}$N]amino in glutamine.* Two known pathways for synthesis of glutamine involve the glutamine synthetase (70) and glutamate synthetase reactions (71).

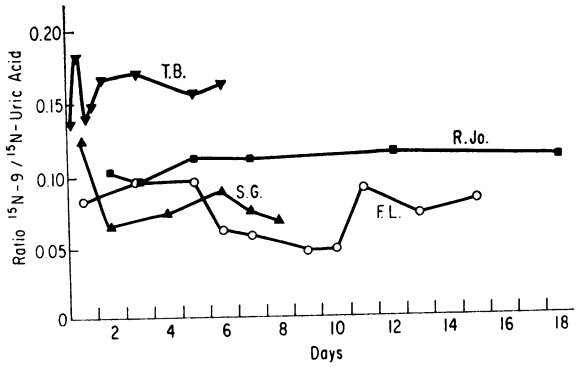


FIGURE 10 Ratio of $^{15}$N enrichment of N-9 to total N of uric acid with time.

[9] This may be a variable feature. Whereas this ratio was also fairly constant with time in five subjects (three control, two gouty) of Gutman and Yu (6) it rose in four others (gouty subjects), as well as in one control studied by Shemin and Rittenberg (34).

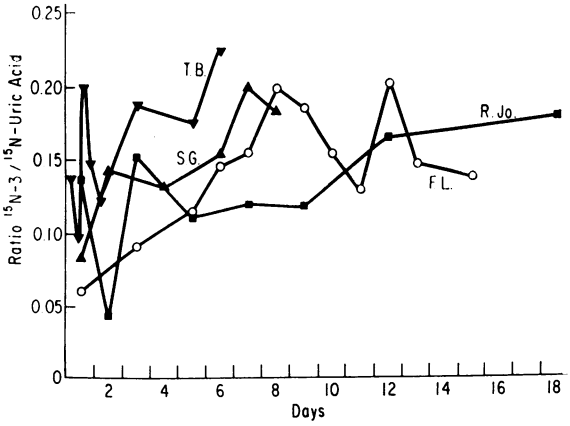


FIGURE 11 Ratio of $^{15}$N enrichment of N-3 to total-N of uric acid with time.

$$\text{Glutamate} + NH_3 + ATP \rightarrow \text{Glutamine} + ADP + P_i, \quad (13)$$

$$2\ \text{Glutamate} + NADP \rightleftharpoons \text{Glutamine} + \alpha\text{-Ketoglutarate} + NADPH. \quad (14)$$

Glutamine synthesized in the former reaction should contain more $^{15}$N in the amide- than the amino-N, whereas glutamine synthesized in the latter reaction should contain equivalently labeled N atoms.

Measurements of $^{15}$N contents of the two nitrogens of glutamine were made on 30 samples from three subjects of the present study (Table III). The $^{15}$N content of the amide N exceeded that of the amino N in all instances but one. The ratio, [$^{15}$N]amide/[$^{15}$N]amino reached 1.5 or greater in each subject, and passed through a maximum on the 3rd–5th day. These results indicate that the glutamine synthetase reaction is the more important reaction for synthesis of glutamine in man, and are consistent with the failure to demonstrate the glutamate synthetase reaction in animal tissues thus far (71).

## DISCUSSION

In this paper we demonstrate the disproportionately increased labeling of N-(3 + 9) of uric acid from [$^{15}$N]-glycine in patients with rare subtypes of primary gout attributable to specific enzymatic defects unrelated to glutamine metabolism. We postulate that the changes in $^{15}$N-(3 + 9)/[$^{15}$N]uric acid ratios observed with accelerated purine production are related to: first, different time courses of enrichment of precursor glycine and glutamine pools, labeled consecutively rather than simultaneously and with different turnover characteristics; and second, kinetic factors which reflect the differences of early enrichment patterns of these precursor pools most strikingly in the most rapid producers, irrespective of the precise molecular cause of purine overproduction.

The first specific step of purine biosynthesis de novo involves the following reaction catalyzed by glutamine phosphoribosylpyrophosphate amidotransferase (72, 73):

$$\alpha\text{-PP-ribose-P} + \text{glutamine} + H_2O \xrightarrow{Mg^{2+}} \beta\text{-phosphoribosylamine} + \text{glutamic acid} + PP_i. \quad (15)$$

This reaction appears to be the slow and rate-controlling step of the purine biosynthetic pathway (74). The amidotransferase is subject to synergistic allosteric feedback inhibition by adenyl and guanyl ribonucleotides (73, 75). The molecular basis of this control mechanism has now been elucidated. Purine ribonucleotides convert the human enzyme, which in its active form has a molecular weight of 133,000, to a larger form that has a molecular weight of 270,000 and is enzymatically inactive. PP-ribose-P reconverts the larger form of the enzyme to the smaller form and stabilizes it in that configuration. Glutamine has no effect upon this association-dissociation reaction (76).

Increases in rates of purine production could in theory result from increased concentrations of the substrates PP-ribose-P or glutamine, increased activity of the amidotransferase, or decreased availability or non-optimal ratios of feedback inhibitors (50, 73).

The $K_m$ value of human amidotransferase for PP-ribose-P is 0.25 mM (75). The intracellular concentrations of PP-ribose-P are 0.005–0.05 mM (erythrocytes and

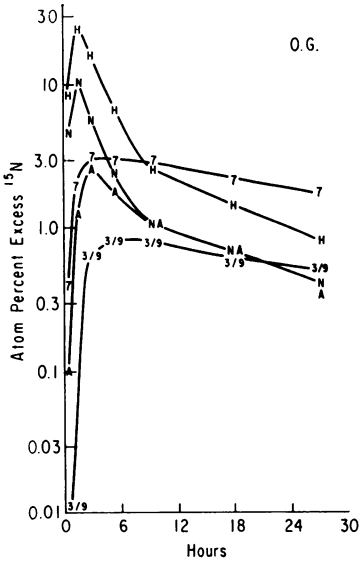


FIGURE 12 Time courses of $^{15}$N enrichment of various nitrogenous products of urine in subject O. G. (H = hippuric acid, N = ammonia, A = amide-N of glutamine, 7 = N-7 of uric acid, 3/9 = N-(3 + 9)/2 of uric acid.

TABLE III

*$^{15}N$ Abundance in Urinary Phenylacetylglutamine* (PAG), *Hippurate, and Ammonia*

| Subject | Day | PAG amide-N | PAG amino-N | Ratio $\frac{\text{Amide } ^{15}N}{\text{Amino } ^{15}N}$ | Hippurate | $NH_3$ | Ratio $\frac{^{15}NH_3}{\text{PAG-Amide-}^{15}N}$ |
|---|---|---|---|---|---|---|---|
| | | *Atom % excess* | *Atom % excess* | | *Atom % excess* | *Atom % excess* | *[$^{15}N$]Amide* |
| Nongouty subject | | | | | | | |
| F. L. | 1 | 0.5415 | 0.3667 | 1.48 | 1.3080 | 0.5039 | 0.93 |
| | 2 | 0.3792 | 0.2368 | 1.60 | 0.6225 | 0.3462 | 0.91 |
| | 3 | 0.2114 | 0.1256 | 1.52 | 0.4459 | 0.2066 | 0.97 |
| | 4 | 0.1416 | 0.0822 | 1.72 | 0.3483 | 0.1438 | 1.01 |
| | 5 | 0.1241 | 0.0720 | 1.72 | 0.3240 | 0.1250 | 1.03 |
| | 6 | 0.1166 | 0.0733 | 1.59 | 0.2194 | 0.1096 | 0.93 |
| | 7 | 0.0817 | 0.0488 | 1.67 | — | 0.0854 | 1.04 |
| | 8 | 0.0765 | 0.0486 | 1.57 | 0.2080 | 0.0798 | 1.04 |
| | 9 | 0.0747 | 0.0477 | 1.56 | 0.2094 | 0.0779 | 1.04 |
| | 10 | 0.0650 | 0.0418 | 1.56 | 0.1467 | 0.0665 | 1.02 |
| | 11 | 0.0554 | 0.0364 | 1.52 | 0.1558 | 0.0602 | 1.08 |
| | 12 | 0.0544 | 0.0357 | 1.52 | 0.1344 | 0.0633 | 1.16 |
| | 13 | 0.0556 | 0.0381 | 1.46 | — | 0.0601 | 1.08 |
| | 14 | 0.0476 | 0.0318 | 1.50 | — | 0.0501 | 1.05 |
| Average ratios | | | | 1.57 | | | 1.02 |
| Gouty subjects | | | | | | | |
| S. G. | 1 | 0.6740 | 0.4461 | 1.51 | 0.7380 | 0.6124 | 0.91 |
| | 2 | 0.3047 | 0.1899 | 1.60 | 0.4441 | 0.3149 | 1.03 |
| | 3 | 0.2406 | 0.1493 | 1.61 | 0.3188 | 0.2492 | 1.03 |
| | 4 | 0.1627 | 0.1065 | 1.56 | 0.2463 | 0.1659 | 1.01 |
| | 5 | 0.1414 | 0.0956 | 1.49 | 0.1908 | 0.1488 | 1.05 |
| | 6 | 0.1176 | 0.0805 | 1.46 | 0.1834 | 0.1189 | 1.01 |
| | 7 | 0.1049 | 0.1101 | 0.96 | 0.1781 | 0.1065 | 1.01 |
| Average ratios | | | | 1.46 | | | 1.01 |
| R. Jo. | 1 | 0.6524 | 0.4999 | 1.31 | 1.6598 | 1.1790 | 1.81 |
| | 2 | 0.3916 | — | — | 0.6189 | 0.4481 | 1.14 |
| | 3 | 0.2456 | 0.1643 | 1.50 | 0.3257 | 0.2982 | 1.21 |
| | 4 | 0.1842 | — | — | 0.3154 | 0.2384 | 1.29 |
| | 5 | 0.1651 | 0.1110 | 1.50 | 0.2812 | 0.2209 | 1.33 |
| | 6 | — | — | — | — | 0.1764 | — |
| | 7 | 0.1158 | 0.0829 | 1.40 | 0.1918 | 0.1510 | 1.30 |
| | 8 | — | — | — | — | 0.1305 | — |
| | 9 | 0.0873 | 0.0656 | 1.34 | 0.1756 | 0.1315 | 1.5 |
| | 10 | — | — | — | — | 0.1013 | — |
| | 11 | 0.0580 | 0.0547 | 1.07 | — | — | — |
| | 12 | — | — | — | 0.1135 | 0.1134 | — |
| | 13 | 0.0789 | — | — | — | 0.0893 | 1.13 |
| | 14 | — | — | — | — | 0.0832 | — |
| | 15 | 0.0635 | 0.0530 | 1.20 | 0.1084 | 0.0705 | 1.11 |
| | 16 | — | — | — | — | 0.0690 | — |
| | 17 | 0.0572 | 0.0480 | 1.19 | — | 0.0681 | 1.19 |
| | 18 | — | — | — | 0.0936 | 0.0747 | — |
| | 19 | — | — | — | — | 0.0584 | — |
| | 20 | — | — | — | — | 0.0565 | — |
| Average Ratios | | | | 1.32 | | | 1.24* |
| O. G. | 1 (0–1) | 0.1001 | — | — | 8.29 | 4.80 | 48.0 |
| | (1–2) | 1.2651 | — | — | 24.5 | 10.56 | 8.34 |
| | (2–4) | 2.7880 | — | — | 16.1 | 5.74 | 2.05 |
| | (4–7) | 1.8797 | — | — | 6.81 | 2.30 | 1.22 |
| | (7–12) | 1.0989 | — | — | 2.60 | 1.0737 | 0.97 |
| | (12–24) | 0.7044 | — | — | 1.50 | 0.7058 | 1.00 |
| | 2 (24–30) | 0.3992 | — | — | 0.853 | 0.4576 | 1.14 |
| | 3 (48–54) | 0.2556 | — | — | 0.397 | 0.2381 | 0.93 |
| | 4 (72–78) | 0.1722 | — | — | 0.320 | 0.1680 | 0.97 |
| | 5 (96–102) | 0.1879 | — | — | 0.301 | — | — |
| | 6 (120–126) | 0.1287 | — | — | 0.229 | 0.1245 | 0.96 |
| | 7 (144–150) | 0.1037 | — | — | 0.182 | 0.1059 | 1.02 |
| Average Ratio | | | | | | | 1.00‡ |

In subjects F. L., S. G., and R. Jo., ammonia and phenylacetylglutamine were isolated from the 18–22 hour sample, and hippurate from the 22–24 hour sample. In subject O. G., ammonia, phenylacetylglutamine and hippurate were isolated from the same urine sample; times of samples are given in hours from the time of administration of $^{15}N$-glycine.

* Day 1 omitted.

‡ Hours 0–7 omitted.

fibroblasts) (77–79). Thus the intracellular concentrations of PP-ribose-P may be in the low ranges of the substrate-velocity curves, and factors that affect their intracellular, perhaps chiefly intrahepatic, concentrations will have a corresponding influence upon purine biosynthesis de novo. The amidotransferase reaction displays sigmoidal kinetics with respect to PP-ribose-P in the presence of nucleotide regulators such as AMP and GMP (75). Small increases in concentrations of PP-ribose-P may therefore affect purine biosynthesis disproportionately. Indeed, in isolated systems such as Ehrlich ascites cells (80) or cultured human fibroblasts (77, 81), manipulations of the concentrations of PP-ribose-P profoundly affect the rate of the first steps of purine biosynthesis.

Concentration values of PP-ribose-P are normal in erythrocytes and cultured fibroblasts of patients with idiopathic gout whose urate excretion values are in the *normal* range (75, 77, 79, 82). These subjects are representative of 70–75% of the gouty population (4, 44). However, PP-ribose-P values are significantly elevated in erythrocytes of patients with partial (77) or virtually complete (77, 78) deficiencies of PRT activity, and in patients with superactive PP-ribose-P synthetases (28, 29), as well as in some gouty patients with idiopathic gout and *excessive* urate excretion values (47, 83, 84).

In an earlier study from this laboratory on nine subjects with idiopathic gout, an increased labeling of the ribose moiety of urinary imidazoleacetic acid ribonucleoside (which is derived from PP-ribose-P) was found in three overproducers after administration of [U-$^{14}$C]glucose, but not in six normal excretors (53). These data strongly suggested overproduction of PP-ribose-P from glucose in the three gouty overproducer subjects. The nature of the metabolic defect in these patients has not been clarified except that one (H. H.) had normal erythrocyte PRT activity.[10]

Taken altogether, available data suggest that increased concentration values of PP-ribose-P may drive purine biosynthesis excessively in some patients with gout. This mechanism is strongly supported by analytical data in two rare subtypes of gout, associated with PRT deficiency or increased PP-ribose-P synthetase deficiency, which together, however, represent only about 2% of the gouty population (26, 27, 85). This mechanism has also been suggested as the basis of purine overproduction in Gierke's disease (22), which is rare, as well as in hyperuricemia associated with variant forms of glutathione reductase characterized by increased enzymatic activity (86), which according to Long (86, 87) are common within the group now classified as having idiopathic gout. This latter purported abnormality has not yet been confirmed or evaluated by others. The kinetics of the glutamine PP-ribose-P amidotransferase reaction are such that small increases of PP-ribose-P concentration could account for the lesser degrees of purine overproduction in patients with idiopathic gout, although at present no data exist in direct support of this hypothesis. The critical values to be determined in patients with all forms of gout are those of PP-ribose-P concentration or production rate in liver, on which no data are as yet available.

Plasma levels of glutamine are 0.5–0.7 mM in both normal and gouty subjects (18, 19, 88). These values are below the glutamine $K_m$ value of 1.6 mM for human placental (75) or lymphoblast (89) amidotransferase. The rate of purine biosynthesis in cultured fibroblasts can be reduced by glutamine starvation (90), for these cells do not have the highly developed enzymatic machinery for synthesis of glutamine present in liver. We have been unable to find data in the literature on concentrations of glutamine in human liver. However, values of free glutamine in the protein-free extracts of liver of fed rats, whether determined after acid (91) or alkaline (92) hydrolysis or by direct enzymatic analysis of extracts of quick-frozen tissue (93), range from 2.15 to 5.03 μmol/g wet weight, or about 3–7 mM in cell water. The $K_m$ for glutamine of PP-ribose-P amidotransferase of rat liver is $< 1$ mM (94). Therefore, in the fed rat this enzyme of the purine pathway may be nearly saturated with respect to glutamine.

If the human enzyme is not saturated with glutamine, variations in glutamine concentrations would be expected to affect the rate of purine biosynthesis, although less exquisitely than variations in concentrations of PP-ribose-P. The kinetics of the amidotransferase reaction are hyperbolic with respect to glutamine at all levels of PP-ribose-P, in the presence or absence of nucleotide inhibitors (75, 94). Purine ribonucleotide inhibition is noncompetitive with respect to glutamine (75, 94) and glutamine does not reverse the association of amidotransferase subunits caused by ribonucleotides (76).

The three elements of the hypothesis of abnormal glutamine metabolism in primary (idiopathic) gout are: first, disproportionate labeling of N-(3 + 9) in urate overproducers after ingestion of [$^{15}$N]glycine (6, 10); second, reduced production of urinary $NH_3$ at a given acid load (15–17); and third, hyperglutamatemia (18, 19) that appears to be independent of dietary protein level (18). The disproportionate labeling of N-(3 + 9) has now been shown to be a general feature of accelerated purine biosynthesis, and therefore this finding does not specifically favor a defect of glutamine metabolism in primary gout. The reduced production of urinary $NH_3$ in gout was initially attributed to a defect in glutaminase activity (6, 10), but this postulated mechanism appears to have been excluded by normal renal glutaminase as-

[10] Wyngaarden, J. B. 1958. Unpublished data.

say values in vitro in four gouty subjects (95). However, Pitts (96) has cited possible disparities between glutaminase activities as assessed in vitro and in vivo, and a "functional deficit" of glutaminase activity in gout has been suggested by Gutman and Yu (15). The hyperglutamatemia (18, 19) has led to the proposal that surplus glutamate may drive purine biosynthesis via glutamine in certain subjects. However, even if there should be a defect of glutamate or of glutamine metabolism, it is not clear how such metabolic errors could account for the isotopic data, for an increase in concentration of free glutamine in liver would result in greater dilution of newly synthesized labeled glutamine and *reduced* rather than increased labeling of N-3 and N-9 relative to N-7 or total uric acid.

The observation that enrichment patterns calculated for N-9 and N-3 followed different time-courses was unanticipated. It had been assumed that N-9 and N-3 were equivalent by virtue of a common origin in the amide-N of glutamine. The nonequivalence cannot be explained by progressive dilution of $^{15}N$ in intermediates along the pathway of purine biosynthesis, for such factors would dilute N-3 with respect to N-9 in all samples.

One theoretical basis for nonequivalence is different glutamine pools as precursors of the two N-atoms. This appears unlikely. All enzymes of purine biosynthesis reside in the cytosol, apparently in a large multienzyme complex capable of total synthesis of IMP from low molecular weight substrates (97). Exchange of $NH_3$ with the amino group of phosphoribosylamine is also unlikely: the amidotransferase reaction is irreversible (72, 73).

A second reaction introducing highly enriched $NH_3$ into N-9 initially and $NH_3$ less highly labeled than the amide-N of glutamine some days later could explain the findings, and is consistent with the observed time-courses of enrichment of $NH_3$ and of the amide-N of glutamine. Both PP-ribose-P amidotransferase (72) and formyl-GAR-amidotransferase (98) of avian liver function with $NH_3$, but the $K_m$ values are very high, and in competition experiments glutamine is utilized preferentially.

In 1961 Nierlich and Magasanik (99) described an alternative first step of purine biosynthesis in which $NH_3$ reacts directly with ribose 5-phosphate (5-P) to form $\beta$-phosphoribosylamine. Because the reaction was later shown to occur nonenzymatically at high ammonium chloride concentrations (100), its potential biological significance has been doubted. In fact, *pur F* mutants of *E. coli*, which lack any activity of glutamine-PP-ribose-P amidotransferase, are strict purine auxotrophs (101), even though they contain normal activity of ribose 5-P aminotransferase.[11] Doubt has also been expressed that this reaction functions in Ehrlich ascites cells (102). Nevertheless, suggestive evidence for this pathway exists in other systems (103–105), and Reem (65, 66) has shown that tissue extract fractions that catalyze this reaction contain no glutamine PP-ribose-P amidotransferase activity. The $^{15}N$-9/$^{15}N$-3 ratios of the present study could perhaps be explained by a direct reaction between ammonia and ribose 5-P, occurring either enzymatically or chemically, giving rise to phosphoribosylamine. The limited data available (Fig. 9) do not suggest that the activity of this putative alternative pathway is selectively increased in gouty subjects.

In summary, the increased fractional labeling of N-3 and N-9 of uric acid in gouty overproducers fed [$^{15}N$]-glycine appears to be chiefly a kinetic phenomenon related to the different time-courses of enrichment of precursor glycine and glutamine pools, and to changes in the patterns of incorporation of $^{15}N$ into intermediary products which contribute to the purine ring, as rates of purine production are increased. The disproportionate labeling of N-(3 + 9) after ingestion of [$^{15}N$]glycine does not selectively favor a specific defect of glutamate or glutamine metabolism in primary idiopathic gout.

[11] Gots, J. May, 1972. Personal communication.

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