JCI The Journal of Clinical Investigation

Overproduction of Uric Acid as the Cause of Hyperuricemia in Primary Gout

James B. Wyngaarden

J Clin Invest. 1957;36(10):1508-1515. https://doi.org/10.1172/JCI103547.

Research Article

OVERPRODUCTION OF URIC ACID AS THE CAUSE OF HYPERURICEMIA IN PRIMARY GOUT

By JAMES B. WYNGAARDEN

(From the Department of Medicine, Duke University School of Medicine, Durham, N. C., and the National Institute of Arthritis and Metabolic Diseases, National Institutes of Health, Bethesda, Md.)

(Submitted for publication April 8, 1957; accepted June 13, 1957)

Glycine contributes carbon atoms 4 and 5 and nitrogen atom 7 of the purine ring (1–3). Benedict, Yü, Bien, Gutman and Stetten (4) have described experiments in which the abundance of N^{15} in uric acid was measured for several days following an oral test dose of glycine-N^{15}. In two gouty subjects, three times as much of the administered N^{15} was recovered in urinary uric acid as in normal subjects. In two others, however, normal quantities of N^{15} appeared in uric acid. In these studies, there was a rank order correlation between the daily excretion of uric acid in the urine and the per cent of ingested isotope appearing in urinary uric acid. In subjects who exhibited abnormally high basal uric acid excretions, an abnormally rapid and excessive incorporation of dietary glycine into uric acid occurred. In others whose basal excretion was approximately normal, utilization of glycine for uric acid synthesis was normal. Muller and Bauer (5) and Bishop, Rand, and Talbott (6) have each reported an additional gouty patient excreting normal quantities of uric acid who incorporated normal quantities of glycine-N^{15} into urinary uric acid. The latter authors also described one gouty patient who incorporated excessive quantities of glycine-N^{15} into urinary uric acid on two of three occasions despite a normal urinary urate excretion. These studies, therefore, demonstrated that overproduction of uric acid was present in some gouty subjects, but failed to give a clear indication of the cause of hyperuricemia in another, perhaps predominant group of the gouty population.

Recently, we have administered glycine-1-C^{14} to control and gouty patients in order to label urinary purines, as part of a study of intermediates in urate synthesis (7). The first two gouty patients both exhibited excessive incorporation of C^{14} into urinary urate, one despite a normal urate excretion (7, 8). It was therefore decided to re-investigate the problem of the rate of generation of uric acid in gout, employing tracer doses of glycine-1-C^{14} rather than large doses of glycine-N^{15} as were required in previous studies.

In the present study, glycine-1-C^{14} was administered orally in 2.5 to 25 μc. doses to control and gouty subjects, and the concentration and cumulative incorporation of isotope into urinary uric acid were determined. This paper presents data indicating that overincorporation of glycine-1-C^{14} into uric acid is a consistent finding in primary gout, and that the failure of the glycine-N^{15} technique to disclose overincorporation in some gouty subjects may have been the result of the large dose of glycine employed. These results have been interpreted as showing that the hyperuricemia of primary gout is due to overproduction of uric acid in the majority if not all gouty subjects.

METHODS

Glycine-1-C^{14}, specific activity 1 to 2 mc. per mM, was purchased from Nuclear Instrument and Chemical Company. Uric acid determinations were performed by differential spectrophotometry according to Praetorius (9), employing purified uricase purchased from Worthington Biochemical Corporation. Uric acid was isolated from urine either by adsorption onto charcoal and subsequent elution with alkali (10) or by precipitation with copper (11). It was recrystallized from lithium carbonate solution by addition of acetic acid (12). Uric acid was transferred to stainless steel planchets as an acetone suspension, dried under an infra-red lamp, and counted in a Robinson gas flow counter (13) having a background of four counts per minute. Self-absorption corrections were made by referring all counting values to a standard mass of 3.3 mg. per 1.54 $cm.^2$ planchet area under which condition absolute counting efficiency was 24 per cent. All specific activity values in this paper have been normalized to correspond to a standard dose of 5.0 μc. of glycine-1-C^{14}.

All subjects were maintained on a purine poor diet containing about 55 Gm. of protein for three to five days before administration of glycine and thereafter for the duration of the study. This diet was calculated to contain an average of 30 mg. of purine-N per day. When the urinary uric acid level had reached a constant minimal value, 2.5 to 25 μc. of glycine-1-C^{14} (0.2 to 2.0 mg.)

TABLE I

Data on subjects of study

Name Age, Race Wt. (Kg.)	Diagnosis	Hemogram			Renal status			Uric acid		Glycine-1-C^{14}	Clinical résumé
		Hct. %	Hgb. Gm. %	WBC per mm.³	BUN mg. %	NPN mg. %	Urinalysis	Serum mg. %	Urine mg./day±S.D.	μc.	
C. L. 62, W. 67.9	Osteoarthritis, rt. hip (10 years)	45		9,500	19		Normal with S. G. 1.018	4.1–4.4	355±42	5.0	
W. W. 21, W. 67.3	Duodenal ulcer (9 months)	42	15.2	4,850		34	Normal with S. G. 1.024	4.4	346±50	13.6	No stigmata or family history of gout or of proliferative hematopoietic disorders.
H. B.* 26, C. 62.7	Contact dermatitis		14.0	8,700		27	Normal with S. G. 1.021	3.8	231±66	25.0	
R. A. 32, C. 62.8	Duodenal ulcer, asymptomatic hyperuricemia	42	13.8	6,100		37	Normal with S. G. 1.028	7.6–7.8	343±31	14.1	No family or personal history of articular disease or of renal disease.
O. N.† 61, W. 80.9	Non-tophaceous gout	50		8,600	20		Normal with S. G. 1.018	I 8.3–8.8 II 6.9–7.2	435±42 391±51	5.0 4.3	Thirteen-year history of recurrent gout, average of 2 attacks per year. Mild hepatomegaly. One-year interval between studies.
E. H.‡ 52, W. 89.4	Non-tophaceous gout	45	15.7	9,100	15		Normal with S. G. 1.014 P.S.P. 74% in 2½ hours	8.8→4.5	1,054±196	5.0	Three acute attacks of gout, 5, 2, and ½ years prior to study. Probenecid therapy (see footnote).
D. W.§ 58, W. 93.1	Benign parotid tumor, tophaceous gout, chronic alcoholism	44	14.5	10,500→ 6,700	12		Normal with S. G. 1.022 P.S.P. 71% in 2½ hours	8.0–8.6	317±25	2.5	Eleven-year history of recurrent gout, 5 attacks per year. BSP retention (27 and 19%). Developed tophus 1 month following study.
V. L.† 54, C. 100.0	Early tophaceous gout		14.2	9,300	14		Normal with S. G. 1.014	I 8.4 II 8.2	458±48 404±67	10.0 15.0	Seven-year history recurrent gout. Acute attack during first study. Developed tophus of finger shortly thereafter. BP 160/112. Four-month interval between studies.
W. R. 65, W. 71.8	Non-tophaceous gout, pyelonephritis	42	14.0	14,100→ 7,600		37	Max. urine conc. 1.015 P.S.P. 35% in 2 hours	6.3–6.5	313±61	25.0	Eight-year history of recurrent gout. Admitted with acute pyelonephritis (Proteus vulgaris). Later, I.V.P. = poor excretion in 10 minutes. C^{14} study during convalescence.
H. H.* 62, C. 102.8	Non-tophaceous gout	38	12.2	8,850		37	Normal with S. G. 1.023 P.S.P. 55% in 2 hours	7.7–8.0	685±82	25.0	Attacks of gout 1 year and again 1 month prior to study. Unexplained hyperglobulinemia of 6 Gm. %. BP 178/116.

* Only three and four days of urine were collected on H. B., and H. H., respectively, following C^{14}-glycine. These data are not included in Figures 1 and 2.

† In the second studies on O. N. and V. L., 0.1 Gm. of unlabeled glycine was given per Kg. of body weight, along with glycine-1-C^{14}.

‡ This patient was studied while on probenecid 1.0 Gm. per day. For some months prior to the study, his serum uric acid level was stable at 4.5 to 4.8 mg. per cent, and his average uric acid excretion during the glycine-1-C^{14} study was similar to pre-treatment excretion values (822 and 1,026 mg. per day). He was therefore judged to have mobilized and excreted the excess miscible uric acid and once again to be in a dynamic steady state with respect to uric acid synthesis and excretion.

§ Respiratory $C^{14}O_2$ excretion was measured on this patient, also.

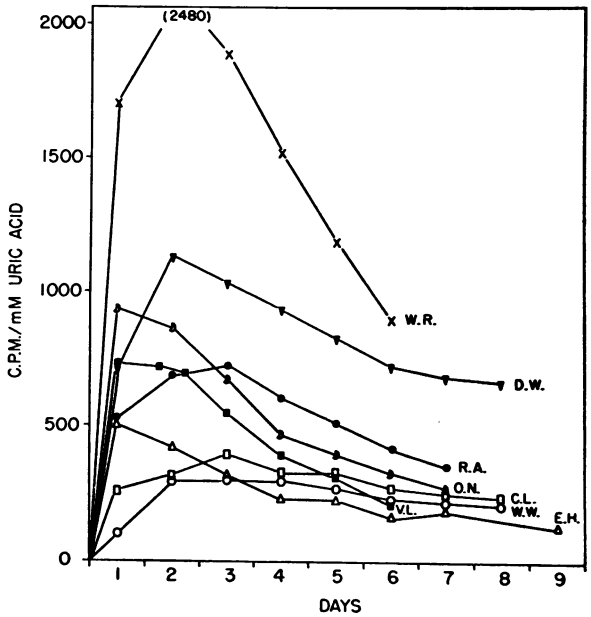

FIG. 1. THE CONCENTRATION OF C^{14} IN DAILY URINARY URIC ACID FOLLOWING ORAL ADMINISTRATION OF GLYCINE-1-C^{14}

The specific activity of uric acid has been plotted against time in days for two control (W. W. and C. L.), one asymptomatic hyperuricemic (R. A.), and five clinically gouty subjects. The specific activity values have been normalized to correspond to a standard dose of 5.0 μc. of glycine-1-C^{14}.

were administered orally in solution with a light breakfast. Daily urine collections were obtained under toluene at room temperature for varying periods after ingestion of labeled glycine. Pertinent data on the subjects of this study are given in Table I.

In one gouty subject (D. W.), the labeling of expiratory CO_2 was determined by methods previously described (14). Uric acid degradations were performed according to Buchanan, Sonne, and Delluva (2). C-6 and C-(2+8) were counted as $BaCO_3$. C-(4+5) were counted as glyoxylic acid semi-carbazone.

RESULTS

The concentrations of C^{14} in urinary uric acid following the ingestion of a single tracer dose of glycine-1-C^{14} are plotted as a function of time in Figure 1. In two control subjects, C. L. and W. W., the specific activities reached maxima on the third day, after which there was a gradual decline in isotope concentration. However, in a third control subject, H. B.,[1] maximal enrichment occurred in the sample excreted 6 to 17 hours following glycine-1-C^{14} administration and was then 510 c.p.m. per mM of uric acid. In the seven hyperuricemic subjects, comprising one patient with asymptomatic hyperuricemia and six with clinical gouty arthritis,[1] there was prompt isotopic enrichment of urinary uric acid, such that the first day specific activity values were up to three and one-half times greater than those found in the control subjects. However, because of differences in quantities of uric acid excreted, concentration data alone do not clearly separate the gouty and control subjects. In the asymptomatic hyperuricemic subject, the specific activity curve was not maximal until the third day but was approximately twice the level of enrichment of control curves throughout. Thus, the day of maximal enrichment also does not clearly separate a gouty from a control subject. In the six clinically gouty subjects, maximal specific activity values were encountered on either the first or second day, and there was a general similarity in the shapes of the enrichment curves of the gouty subjects. There was, however, a five-fold range in the heights of the maximal specific activity values which varied from 510 to 2480 c.p.m. per mM of uric acid.

From knowledge of the quantity of uric acid in each day's urine, and of its C^{14} content, the daily excretion of C^{14} as uric acid has been calculated. This information has been plotted cumulatively and expressed in percentage of administered C^{14} in Figure 2. In control subjects C. L. and W. W., 0.15 and 0.18 per cent of the C^{14} fed as carboxyl-labeled glycine appeared in urinary uric acid in eight days. In H. B., 0.064 per cent appeared in three days, an entirely normal value despite his first day maximal specific activity result. In contrast, the asymptomatic hyperuricemic subject, R. A., incorporated 0.29 per cent of the administered C^{14} into uric acid in seven days, and the patients with clinical gout incorporated from 0.29 to 0.66 per cent in six to seven days. Subject H. H. incorporated 0.18 per cent in four days, again a high value and consistent with results on the other gouty subjects. When the results are expressed in terms of cumulative incorporation of isotope over several days, it is clear that gouty subjects, as a group, differ from controls in the efficiency with which they utilize a tracer dose of glycine-1-C^{14} in the synthesis of uric acid. In the patients

[1] Data on control subject H. B., and gouty subject H. H., are not shown in Figures 1 or 2, since samples were collected for only three and four days, respectively.

studied, there was no overlap between gouty subjects and control subjects. The cumulative C^{14} incorporation into uric acid in the gouty patients exceeded that found in the controls by two- to five-fold.

It will be noted that the cumulative isotope incorporation curve of E. H., the gouty subject excreting 1,054 mg. of uric acid daily, did not differ greatly from those of the other gouty subjects, with the exception that during the first 24 hours following administration of glycine-C^{14} there was a greater incorporation of C^{14} into uric acid than in any other subject (this despite a specific activity value on Day 1 which was identical with that of control H. B.). The significance of this finding is not clear, however, particularly since an even greater rate of incorporation is found on Days 2 and 3 in patient W. R., who excreted only 313 mg. of uric acid daily. In this study, there was no apparent correlation between the basal excretion of uric acid and the per cent of ingested isotope appearing in urinary uric acid (*cf.*, Figure 2 and Table I).

There was also no clear relationship between the results obtained and the advent of an acute gouty attack. Patient W. R. was recuperating from an acute gouty attack which had had its onset four days prior to the administration of isotope, and V. L. developed an acute attack on the second day of the study and was treated with colchicine on Days 3 and 4. Nevertheless, the results procured in these subjects were similar to those of the other gouty subjects.

Labeling of expiratory CO_2

The labeling of expiratory CO_2 was determined following oral administration of glycine-1-C^{14} in gouty subject D. W. The specific activity of expired CO_2 was maximal 10 to 20 minutes following the administration of glycine. CO_2 collected at five hours still had significant C^{14} content, but samples collected at 24 and 48 hours did not measure significantly above background by the counting methods employed.

The peak specific activity of expired $C^{14}O_2$ was 2,605 c.p.m. per milliatom of carbon, calculated under conditions equivalent to those under which uric acid values were expressed. This value was some six-fold greater than the maximal specific activity of uric acid (396 c.p.m. mM), attained on the second day. Since CO_2 is known to be the precursor of C-6 of the purine ring (2, 15), the possibility was considered that C^{14} fed as glycine-1-C^{14} had labeled uric acid not only in C-4 as a consequence of incorporation of intact glycine, but also in C-6 via the bicarbonate pool. For this reason, the

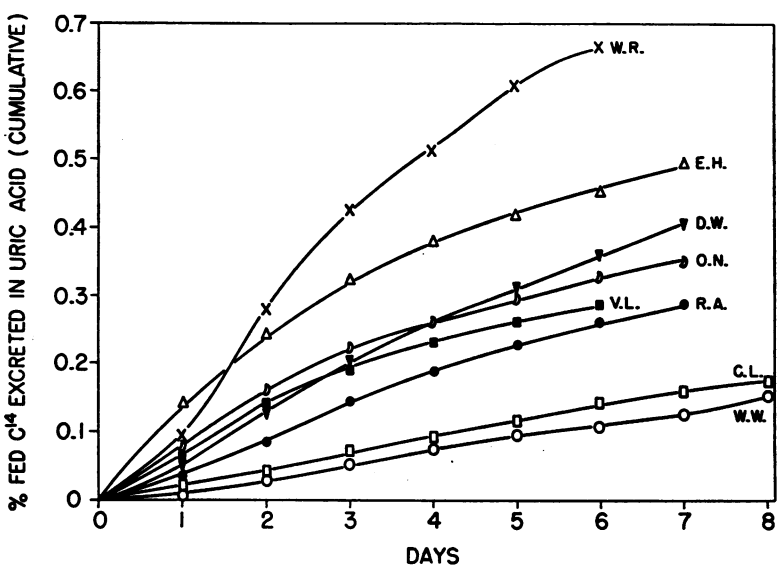

FIG. 2. THE CUMULATIVE EXCRETION OF C^{14} IN URINARY URIC ACID FOLLOWING ORAL ADMINISTRATION OF GLYCINE-1-C^{14}

The per cent of the administered glycine-1-C^{14} which has been excreted as uric acid-C^{14} has been plotted cumulatively.

TABLE II

Degradation of uric acid *

Subject	Diagnosis	Day	C-(4+5)†	Uric acid	C-(4+5) uric acid
			c.p.m./mM‡	*c.p.m./mM*	
W. W.	Control	1	285	297	0.96
		4	808	824	0.98
V. L.	Gout	1	1,330§	1,358	0.98
		5	573	594	0.96
D. W.	Gout	2	386	396	0.98
		5	320	335	0.96
D. D.‖	Myeloid metaplasia	3	866§	908	0.95
		12	802	798	1.00

* C-6 and C-(2+8) were also isolated on each sample and counted as $BaCO_3$.

† C-(4+5) were isolated and counted as glyoxylic acid semi-carbazone.

‡ These are actual counting values. Those plotted in Figure 1 have been normalized to correspond to a standard dose of five μc. of glycine-1-C^{14}.

§ These two samples were further degraded (2) so as to obtain C-4 and C-5 individually as $BaCO_3$. Virtually all the C^{14} was found in the $BaCO_3$ representing C-4 of the purine ring. Since separation of C-5 and C-4 is not complete (2), the small amount of C^{14} found in C-5 was regarded as contamination.

‖ D. D., a 42 year old woman with myeloid metaplasia, received 20 μc. of glycine-1-C^{14}. This study will be reported in detail elsewhere (8).

various carbon atoms of uric acid were isolated from selected samples and analyzed for their specific isotope contents.

Position of labeling of uric acid

The C^{14} concentrations found in the various carbon atoms of selected samples of uric acid are shown in Table II. It is seen that there is no significant labeling of any carbon atoms other than C-(4+5), whether early or late uric acid samples are analyzed. Since C-5 of the purine ring is derived from the α-C of glycine (2), it may be presumed that all the C^{14} is in C-4, the carbon atom specifically donated by the carboxyl-C of glycine (2, 3). If C-5 contained C^{14}, one would not expect C-(2+8) to be devoid of isotope (2). These results are taken to indicate that labeling of uric acid following administration of glycine-1-C^{14} is almost exclusively a consequence of the incorporation of the intact glycine molecule, and that secondary labeling of positions other than C-4 is negligible. These results are in striking contrast to those of Shemin and Rittenberg (1) and of Seegmiller, Laster, and Stetten (16), who found appreciable labeling of positions other than N-7 of uric acid after feeding N^{15}-glycine.

Effect of glycine carrier upon glycine-1-C^{14} utilization

In these studies, seven consecutive gouty subjects showed overincorporation of glycine-1-C^{14} into urinary uric acid, irrespective of the stage of their disease or of the magnitude of urinary uric acid excretion. Had these studies been conducted with glycine-N^{15}, one would have anticipated that perhaps all five subjects showing normal urinary urate excretions would have showed normal isotope incorporation. Indeed, subject O. N. was subsequently given glycine-N^{15} on two occasions and did show normal N^{15} incorporation values (17). The explanation cannot lie in the distribution of isotope in uric acid, for the non-specific labeling of uric acid by N^{15} would tend to introduce more, not less, N^{15} than C^{14}. A potential factor therefore was that of dose, since in studies with glycine-N^{15}, generally 0.1 Gm. per Kg. is given, whereas in the studies reported herein with glycine-1-C^{14}, doses of 0.2 to 2.0 mg. per patient were employed.

To test the influence of the dose factor the studies on O. N. and V. L., gouty subjects excreting normal quantities of urate, were repeated employing 0.1 Gm. of unlabeled glycine per Kg. as carrier for the glycine-1-C^{14}. The specific activity values of urinary uric acid were considerably lower than in the first studies, and the cumulative percentual incorporations of the administered C^{14} over the first five days were depressed by a factor of about 10 in comparison with the initial studies (Table III). The absolute incorporations of glycine in these studies cannot, however, be calculated, since the initial specific activities of the

TABLE III

Effect of glycine carrier upon glycine-1-C^{14} utilization

Subject	Experiment	Glycine fed		Uric acid-C^{14} formed in 5 days
		C^{14}	C^{12}	
		μc.	*Gm./Kg.*	*% administered C^{14}*
O. N.	I	5.0		0.29
	II	4.3	0.1	0.024
V. L.	I	10.0		0.26
	II	15.0	0.1	0.027

glycine pools functioning in purine synthesis (18) are unknown.

DISCUSSION

In an earlier study, Benedict, Yü, Bien, Gutman, and Stetten (4) found that control subjects and gouty subjects excreting normal quantities of uric acid incorporated 0.1 to 0.2 per cent of a test dose of N^{15}, fed as glycine, into uric acid in a nine-day period, whereas two gouty subjects excreting excessive quantities of uric acid incorporated much larger quantities of N^{15}. In the latter patients, 0.45 and 0.56 per cent, respectively, of the N^{15} appeared in urinary uric acid in nine days.

In the present study, the incorporation of C^{14}, fed as glycine-1-C^{14}, into uric acid was also of the order of 0.1 to 0.2 per cent in eight days for the control subjects and was 0.49 per cent in seven days in E. H., a gouty subject known to excrete large quantities of uric acid in urine. However, all five of the gouty subjects who exhibited normal basal uric acid excretions also showed high cumulative isotope incorporation values, which ranged from 0.29 to 0.66 per cent of the fed C^{14} in six to eight days. Some or all of these subjects might be anticipated to show normal incorporation values if studied by the glycine-N^{15} technique. Indeed, as mentioned above, one of them did show normal N^{15} incorporation values on two occasions (17).

The urate degradation studies indicate that glycine has been incorporated into the purine ring intact, and that virtually all the C^{14} is in C-4 of uric acid. These results suggest that overincorporation of glycine-1-C^{14} into uric acid is a consistent metabolic defect in primary gout, both in those with the gouty trait and those who have, or have had, clinical gouty arthritis. They further suggest that overproduction of uric acid is the fundamental cause of hyperuricemia in primary gout, regardless of the stage or severity of the disease or of the magnitude of the urinary urate excretion.

All of the reasons for the differences in results obtained with glycine-N^{15} and glycine-1-C^{14} are not clear. At least two factors do appear to have roles. First, the introduction of N^{15} into positions other than N-7 of the purine ring, because of transamination and entry of N^{15} into aspartic acid and the amide-N of glutamine, the precursors of N-1 and of N-3 and N-9, respectively (19–21), would tend to introduce a greater percentage of a given dose of glycine-N^{15} into uric acid than of glycine-1-C^{14}, which appears to label C-4 quite specifically. Second, the large dose of glycine required for the N^{15} studies appears to result in a decided reduction of the percentual incorporation of C^{14} into uric acid. These are opposing influences, and the apparent good agreement of results obtained with glycine-N^{15} and glycine-1-C^{14} in control subjects and in gouty subjects excreting large quantities of uric acid may therefore be fortuitous. The influence of the dose factor has not as yet been evaluated in control subjects or in gouty subjects excreting large quantities of urate, so a more definitive explanation of this problem is not now possible.

The curves of prompt and excessive enrichment of urinary uric acid found in the gouty subjects fed glycine-1-C^{14} resemble those published by Stetten's group for their gouty subjects who incorporated N^{15} from glycine excessively into uric acid. In explanation of these N^{15} curves, the arguments advanced by Benedict and associates (22) and by Stetten (23) for a shunt mechanism of urate synthesis, whereby dietary glycine can promptly enter urinary urate without the obligatory intervention of nucleic acid purines, apply equally well to the C^{14} enrichment curves shown in Figure 1 The configurations of the curves obtained in the gouty subjects indicate that labeled uric acid is being formed excessively and is turning over more promptly than normal. And since the turnover time of ribonucleic acids is about 8 to 10 days, and of desoxyribonucleic acids even longer (24), the prompt enrichment of urinary uric acid could not have proceeded via nucleic acids. Some of the mechanisms permitting this prompt direct entry of isotope into urinary uric acid, which obviously occurs to a limited extent also in normal subjects, are currently under study (8, 25).

If normally the direct pathways contribute only a relatively small fraction of the total uric acid that is produced per unit time (the larger portion arising by traditional reactions involving nucleic acid catabolism), it then becomes possible to offer an explanation as to why a two- to five-fold increment in utilization of a tracer dose of glycine-

1-C^{14} for urate synthesis in primary gout is not of necessity indicative of a two- to five-fold increment in total uric acid synthesis. Suppose, for example, that of a given quantity of purine nucleotides formed from glycine, 25 per cent is normally rapidly cleaved and converted to uric acid. A doubling of the rate of nucleotide synthesis, would then, in the absence of any change in the quantity of nucleotides utilized in nucleic acid production, result in a five-fold increase in the appearance of labeled glycine in uric acid while representing only a two-fold increment in total uric acid synthesis. Such a mechanism, operating variably in different patients, might explain the lack of a high urinary urate output in W. R. despite a very great C^{14} incorporation into uric acid, as contrasted with the very high urinary urate outputs in E. H. and H. H. associated, more logically, with high C^{14} incorporation. According to this concept, primary gout might involve a defect in the regulation of nucleotide synthesis and degradation, purine nucleotide generation in excess of tissue needs resulting in excessive uric acid production by direct routes of synthesis.

These studies do not, however, explain why gouty subjects who are overproducers of uric acid do not all excrete excessive quantities of uric acid in urine. Several factors may be of importance. It has been shown that the fraction of filtered urate reabsorbed by gouty persons is not significantly different from normal. This suggests that absence of overexcretion of urate in the presence of an elevated serum level of urate is a reflection of some degree of impairment of glomerular filtration, and several studies indicate that this may be the most common explanation (26–28). Impairment of renal function was demonstrated in W. R., and may have been present in mild degree in other subjects.

The uric acid not excreted via the kidney has three quantitatively important fates available to it: deposition in tophi, excretion by way of bile and gastrointestinal tract (29), and destruction in tissues (30, 31). Two of our patients (D. W. and V. L.) were observed to develop tophi about the time the studies were performed. Excretion by way of bile may be quite variable in magnitude (29), and recently it has been stated that in some patients whose body uric acid is increased, a greater *percentage* of uric acid is oxidized than in normals (32). Thus, compensating dispositions of urate may exist, and may condition the severity of the hyperuricemia. These factors require better definition, but the evidence available at this time suggests that overproduction of uric acid in primary gout is the fundamental defect responsible for the hyperuricemia of the disease.

SUMMARY

1. The rate of generation of uric acid in gouty subjects has been re-investigated in studies wherein tracer doses of glycine-1-C^{14} were fed and the excretion of C^{14} in urinary uric acid was determined.

2. The gouty subjects comprised six patients with histories of acute gouty arthritis, and one with the trait of asymptomatic hyperuricemia. Two gouty subjects excreted excessive quantities of urate in urine, whereas the other four, and the asymptomatic hyperuricemic subject, excreted quantities of urate well within the range of normal.

3. All seven gouty subjects exhibited quantitatively excessive incorporation of C^{14} into urinary urate. Degradation of the labeled urate suggested that glycine had been incorporated intact and indicated that non-specific labeling of the purine nucleus by secondary reactions was negligible.

4. These results constitute strong evidence that overproduction of uric acid from glycine and other small molecules is the fundamental defect responsible for the hyperuricemia of primary gout.

5. The characteristics of the curves of C^{14} incorporation into uric acid strongly suggest, as did earlier studies with glycine-N^{15}, that nucleic acids are not involved in the overproduction of uric acid in primary gout.

ACKNOWLEDGMENTS

The author wishes to express his indebtedness to Dr. DeWitt Stetten, Jr. for helpful discussions during the course of this work, and to Mrs. Alberta Blair and Mrs. Linnelle Hilley for able technical assistance.

REFERENCES

1. Shemin, D., and Rittenberg, D., On the utilization of glycine for uric acid synthesis in man. J. Biol. Chem., 1947, 167, 875.
2. Buchanan, J. M., Sonne, J. C., and Delluva, A. M., Biological precursors of uric acid. II. The role of lactate, glycine, and carbon dioxide as precursors of the carbon chain and nitrogen atom 7 of uric acid. J. Biol. Chem., 1948, 173, 81.

3. Karlson, J. L., and Barker, H. A., Biosynthesis of uric acid labeled with radioactive carbon. J. Biol. Chem., 1949, 177, 597.
4. Benedict, J. D., Yü, T. F., Bien, E. J., Gutman, A. B., and Stetten, D., Jr., A further study of the utilization of dietary glycine nitrogen for uric acid synthesis in gout. J. Clin. Invest., 1953, 32, 775.
5. Muller, A. F., and Bauer, W., Uric acid production in normal and gouty subjects, determined by N^{15} labeled glycine. Proc. Soc. Exper. Biol. & Med., 1953, 82, 47.
6. Bishop, C., Rand, R., and Talbott, J. H., Rate of conversion of isotopic glycine to uric acid in the normal and gouty human and how this is affected by vitamin E and folic acid. Metabolism, 1955, 4, 174.
7. Wyngaarden, J. B., and Blair, A., Pathways of urate synthesis in gout. Ann. Rheumat. Dis., 1955, 14, 433.
8. Wyngaarden, J. B., Intermediary purine metabolism and the metabolic defects of gout. Metabolism, 1957, 6, 244.
9. Praetorius, E., An enzymatic method for the determination of uric acid by ultraviolet spectrophotometry. Scandinav. J. Clin. & Lab. Invest., 1949, 1, 222.
10. Geren, W., Bendich, A., Bodansky, O., and Brown, G. B., The fate of uric acid in man. J. Biol. Chem., 1950, 183, 21.
11. Hawk, P. B., Oser, B. L., and Summerson, W. H., Practical Physiological Chemistry, 13th Ed. New York, McGraw-Hill Book Co., 1954, p. 911.
12. Folin, O., Laboratory Manual of Biological Chemistry, 3rd ed. New York, D. Appleton & Co., 1922, p. 299.
13. Robinson, C. V., Windowless, flow type, proportional counter for counting C^{14}. Science, 1950, 112, 198.
14. Wyngaarden, J. B., Segal, S., and Foley, J., Physiological disposition and metabolic fate of infused pentoses in man. J. Clin. Invest., 1957, 36, 1395.
15. Heinrich, M. R., and Wilson, D. W., The biosynthesis of nucleic acid components studied with C^{14}. I. Purines and pyrimidines in the rat. J. Biol. Chem., 1950, 186, 447.
16. Seegmiller, J. E., Laster, L., and Stetten, D., Jr., Incorporation of 4-amino-5-imidazolecarboxamide-4-C^{13} into uric acid in the normal human. J. Biol. Chem., 1955, 216, 653.
17. Seegmiller, J. E., Laster, L., and Wyngaarden, J. B., Unpublished results.
18. Benedict, J. D., Kalinsky, H. J., Scarrone, L. A., Wertheim, A. R., and Stetten, D., Jr., The origin of urinary creatine in progressive muscular dystrophy. J. Clin. Invest., 1955, 34, 141.
19. Lukens, L. N., and Buchanan, J. M., A new intermediate in purine biosynthesis. Federation Proc., 1956, 15, 305.
20. Goldthwait, D. A., Peabody, R. A., and Greenberg, G. R., Glycine ribotide intermediates in the *de novo* synthesis of inosinic acid. J. Am. Chem. Soc., 1954, 76, 5258.
21. Levenberg, B., and Melnick, I., Formylglycinamidine ribotide and 5-aminoimidazole ribotide—Intermediates in purine biosynthesis. Federation Proc., 1956, 15, 117.
22. Benedict, J. D., Roche, M., Yü, T. F., Bien, E. J., Gutman, A. B., and Stetten, D., Jr., Incorporation of glycine nitrogen into uric acid in normal and gouty man. Metabolism, 1952, 1, 3.
23. Stetten, D., Jr., Recent contributions to the understanding of the metabolic defect in gout. Geriatrics, 1954, 9, 163.
24. Furst, S. S., Roll, P. M., and Brown, G. B., On the renewal of the purines of the desoxypentose and pentose nucleic acids. J. Biol. Chem., 1950, 183, 251.
25. Wyngaarden, J. B., Blair, A., and Schuck, J., On the mechanism of the "shunt" pathway of uric acid synthesis. Clin. Research Proc., 1957, 5, 108.
26. Sirota, J. H., Yü, T. F., and Gutman, A. B., Effect of benemid (p-[di-n-propylsulfamyl]-benzoic acid) on urate clearance and other discrete renal functions in gouty subjects. J. Clin. Invest., 1952, 31, 692.
27. Yü, T. F., Sirota, J. H., and Gutman, A. B., Effect of phenylbutazone (3,5 dioxo-1,2-diphenyl-4-N-butylpyrazolidine) on renal clearance of urate and other discrete renal functions in gouty subjects. J. Clin. Invest., 1953, 32, 1121.
28. Friedman, M., and Byers, S. O., Increased renal excretion of urate in young patients with gout. Am. J. Med., 1950, 9, 31.
29. Buzard, J., Bishop, C., and Talbott, J. H., The fate of uric acid in the normal and gouty human. J. Chronic Dis., 1955, 2, 42.
30. Wyngaarden, J. B., and Stetten, D., Jr., Uricolysis in normal man. J. Biol. Chem., 1953, 203, 9.
31. Bien, E. J., and Zucker, M., Uricolysis in normal and gouty individuals. Ann. Rheumat. Dis., 1955, 14, 409.
32. Pollycove, M., Tolbert, B. M., Lawrence, J. H., and Harman, D., Uric acid metabolism; the oxidation of uric acid in normal subjects and patients with gout, polycythemia and leukemia. Clin. Research Proc., 1957, 5, 38.