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Leif B. Sorensen

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Research Article

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Mechanism of Excessive Purine Biosynthesis in Hypoxanthine-Guanine Phosphoribosyltransferase Deficiency

LEIF B. SORENSEN

From the Department of Medicine, the Pritzker School of Medicine, University of Chicago, and the Argonne Cancer Research Hospital, Chicago, Illinois 60637

ABSTRACT Certain gouty subjects with excessive *de novo* purine synthesis are deficient in hypoxanthine-guanine phosphoribosyltransferase (HG-PRTase [EC 2.4.2.8]). The mechanism of accelerated uric acid formation in these patients was explored by measuring the incorporation of glycine-^{14}C into various urinary purine bases of normal and enzyme-deficient subjects during treatment with the xanthine oxidase inhibitor, allopurinol.

In the presence of normal HG-PRTase activity, allopurinol reduced purine biosynthesis as demonstrated by diminished excretion of total urinary purine or by reduction of glycine-^{14}C incorporation into hypoxanthine, xanthine, and uric acid to less than one-half of control values. A boy with the Lesch-Nyhan syndrome was resistant to this effect of allopurinol while a patient with 12.5% of normal enzyme activity had an equivocal response. Three patients with normal HG-PRTase activity had a mean molar ratio of hypoxanthine to xanthine in the urine of 0.28, whereas two subjects who were deficient in HG-PRTase had reversal of this ratio (1.01 and 1.04). The patterns of ^{14}C-labeling observed in HG-PRTase deficiency reflected the role of hypoxanthine as precursor of xanthine. The data indicate that excessive uric acid in HG-PRTase deficiency is derived from hypoxanthine which is insufficiently reutilized and, as a consequence thereof, catabolized inordinately to uric acid. The data provide evidence for cyclic interconversion of adenine and hypoxanthine derivatives. Cleavage of inosinic acid to hypoxanthine via inosine does not contribute significantly to the formation of uric acid in either normal man or in patients with HG-PRTase deficiency.

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HG-PRTase was not completely absent in red blood cells from a boy with the Lesch-Nyhan syndrome; with hypoxanthine as substrate, the activity in erythrocyte hemolysates was 0.64% of normal values.

INTRODUCTION

A substantial proportion of patients with primary gout have a metabolic defect characterized by overproduction of uric acid which is the result of an excessive rate of *de novo* purine biosynthesis (1, 2). Recently it has been shown that a deficiency of the enzyme hypoxanthine-guanine phosphoribosyltransferase (HG-PRTase) exists in some, though probably in a minority, of these patients (3, 4). A virtually complete deficiency of this enzyme (3) has been demonstrated in children with an X-linked neurological disorder associated with overproduction of uric acid first described by Lesch and Nyhan (5). In addition, a partial deficiency of HG-PRTase (4) has been observed in hyperuricemic subjects presenting with uric acid lithiasis, monoarticular arthritis, or, rarely, in cases associated with a very low enzyme activity, neurologic dysfunction. HG-PRTase activity was the same in affected members of the same family, but differed from family to family, suggesting that the gouty population with this enzyme deficiency comprises a heterogeneous group of genetic mutations.

HG-PRTase (EC 2.4.2.8) converts the free purine bases, hypoxanthine and guanine, to their respective ribonucleotides by reaction with 5-phosphoribosyl-1-pyrophosphate (PRPP). HG-PRTase is presumably necessary for the normal control of purine biosynthesis, although its exact role remains undefined.

To further elucidate the mechanisms involved, I have studied the incorporation of glycine-^{14}C into hypoxanthine, xanthine, and uric acid during treatment with allopurinol (4-hydroxypyrazolo[3,4-*d*]pyrimidine) in subjects with normal and deficient HG-PRTase. The data obtained provide evidence that excessive purine

synthesis in HG-PRTase deficiency is related to inadequate reutilization and, hence, to increased catabolism of hypoxanthine.

METHODS

Included in this study were one normal subject; two brothers with gout characterized by the synthesis of abnormally large quantities of uric acid, but with normal HG-PRTase activity; one gouty patient with a partial deficiency of HG-PRTase; and one patient with the Lesch-Nyhan syndrome. All subjects were maintained on a purine-free diet for 5 days before as well as during the study periods.

Plasma and urinary uric acids were determined by differential spectrophotometry employing purified uricase (6). Urines were collected at room temperature using toluene as preservative. Urinary hypoxanthine and xanthine were determined by an enzymatic spectrophotometric method.[1] The principle in this method is based on the conversion of hypoxanthine and xanthine to allantoin by xanthine oxidase and uricase. Since allantoin does not absorb ultraviolet light above 260 mμ, hypoxanthine and xanthine can be accurately measured by following the decrease in optical density at the wavelengths of maximum absorption by these compounds (at pH 9.3, for hypoxanthine, $A_{max} = 257.5$ mμ; for xanthine, $A_{max} = 280$ mμ). Briefly, hypoxanthine and xanthine in urine were determined as follows: (*a*) 5 ml of urine was acidified to pH 2 with hydrochloric acid and passed through a 0.5 × 15 cm column of Dowex 50X8 50-100 mesh in the hydrogen form; (*b*) the column was rinsed with distilled water and the purines were eluted with 100 ml of 1 M ammonium hydroxide; (*c*) the eluate was flash evaporated to dryness, and the residue was dissolved in a few drops of 1 N sodium hydroxide and diluted to 25 times the original urine volume with 0.02 M 2-amino-2-methyl-1-propanol buffer at pH 9.3; and (*d*) four cuvettes were prepared, each containing 3 ml of the purine solution. 1 U of uricase (Leo) was added to the second and third cuvettes. Xanthine oxidase was then added to each of these two cuvettes and the absorbance changes read at 257.5 and 280 mμ, respectively. Sufficient enzyme was added to complete the reactions in 30 min. The percentages of hypoxanthine and xanthine in a mixture of these purines can be calculated from the relative decreases in optical absorption at these two wavelengths. Knowing the relative contributions of hypoxanthine and xanthine to the absorbance at 257.5 and 280 mμ, one can calculate the concentration of hypoxanthine and xanthine from the decrease in absolute absorbance of either wavelength. The sum of hypoxanthine and xanthine was determined by converting the purines in the fourth cuvette to uric acid with xanthine oxidase, and measuring the uric acid formed with uricase. The values obtained by the direct and indirect methods agreed to within 5%.

The incorporation of glycine in to hypoxanthine, xanthine, and uric acid was measured while the patients were maintained on allopurinol (Zyloprim) in doses ranging from 5.3 to 14 mg/kg per day. Glycine-1-^{14}C (SA: 25.8 mCi/mmole) was injected intravenously in a dose of 1 μCi/kg body weight on the fourth day of allopurinol treatment except in the case of patient A.D. who had been maintained on allopurinol for the previous 6 months. Urine was collected in 12-hr samples for the first 3 or 4 days and thereafter in 24-hr samples for a total of 7 days.

[1] Sorensen, L. B., F. Kawahara, and D. Chow. Enzymatic spectrophotometric determination of hypoxanthine and xanthine. In preparation.

Urinary purines were separated by two-stage ion exchange chromatography using Dowex 50 in the hydrogen form. The method was similar in principle to those used by Weissmann, Bromberg, and Gutman (7), Wyngaarden, Blair, and Hilley (8), and Ayvazian and Skupp (9). The first column, which utilized 8% cross-linked resin of 50-100 mesh, served to isolate urinary purines. An aliquot of urine which contained approximately 25 mg of hypoxanthine plus xanthine was acidified to pH 2 with hydrochloric acid and passed through a 1.8 × 40 cm column of resin. Uric acid, allopurinol, and its oxidation product alloxanthine, were not retained on this column. The column was washed with water and eluted with 500 ml of 1 M ammonium hydroxide. The preparation of the first eluate for the second-stage ion exchange chromatography involved flash evaporation to dryness of the ammonium hydroxide eluate, solubilization of the residue in 100 ml of hot water, acidification of the mixture to pH 2 with 1 N sulfuric acid, precipitation of the purines with 20 ml of 1 M silver nitrate, and storage at 4°C in the dark for 2 or 3 days, to effect complete precipitation. The precipitate was centrifuged and washed 3 times by suspension in 20-ml portions of water. The purines were regenerated by suspending the precipitate in 20 ml of hot 0.05 M hydrochloric acid, and separated from the silver chloride precipitate by centrifugation. The silver chloride was washed twice with 10 ml of hot 0.05 M hydrochloric acid. The combined solutions were passed through a filter directly onto a second column, which served to isolate and separate the individual purines by gradient elution. This 2.5 × 30 cm column contained 90 g of 12% cross-linked resin, 200-400 mesh, in the hydrogen form. The sample was washed into the column with 50 ml of water. The gradient elution system used a reservoir flask that contained 3 liters of 2 M hydrochloric acid, and a mixing flask that contained 1750 ml of water. Mixing within the lower chamber was accomplished by a magnetic stirrer. The column eluate was collected in 15-ml fractions. The ultraviolet absorption of the effluent fractions was measured at 260 mμ in a Beckman DU spectrophotometer. After collection of 2.5 liters of eluate, the upper flask was replaced by a flask containing 6 M hydrochloric acid. The elution pattern is shown in Fig. 1. The identity of

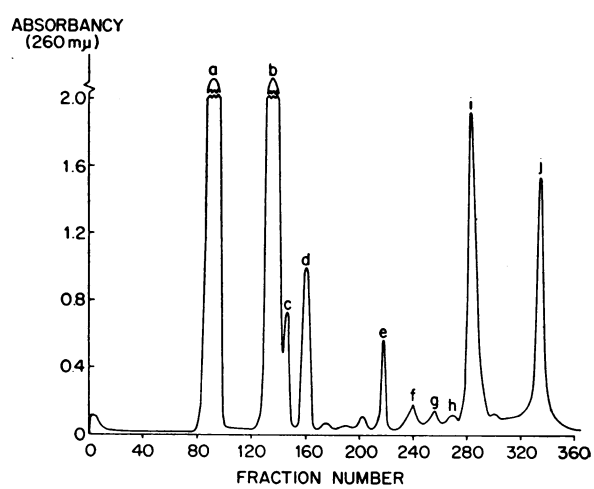

FIGURE 1 Elution pattern of urinary purines. a, xanthine; b, hypoxanthine; c, 7-methylxanthine; d, 1-methylxanthine; e, 1,7-dimethylxanthine; f, guanine; g, N^2-methylguanine; h, 1-methylguanine; i, 7-methylguanine; and j, adenine.

TABLE I

Clinical Data, Uric Acid Pool and Turnover Values,

Subject	Age	Weight	Gouty arthritis	Tophi	Urate pool size	Turnover
	yr	*kg*			*mg*	*mg/day*
Normal values (26 subjects)*					1200–1300	600–700
J.G., normal	27	57	—	—	—	—
D.K., gout	33	106	+	+++	3681	1508
E.K., gout	30	118	+	0	3247	1693
A.D., gout	54	75	+	+	2397	1185
D.T., Lesch–Nyhan	8	16	0	0	—	—

* Healthy male hospital employees.

‡ Mean ±SD.

the compounds comprising the various peaks was established by comparison with the elution sequence of known compounds, by differential spectrophotometry at pH 2 and 9, by enzyme analysis, and by migration of individual purines on paper chromatograms (7). Despite avoidance of the major dietary sources of methylated purines, i.e. coffee, tea, and chocolate, 1-methylxanthine, 7-methylxanthine, and 1,7-dimethylxanthine were frequently present in small amounts. This finding undoubtedly reflects the widespread occurrence of methylated xanthines in plants (7), and the fact that the subjects were receiving allopurinol, which inhibits the enzymatic oxidation of several methylated purines, including 1-methylxanthine (10) and 7-methylxanthine.[1]

The fractions representing the separate peaks of hypoxanthine and xanthine were pooled, evaporated to dryness, and redissolved in 2.5 ml of dilute sodium hydroxide. Determination of the specific activity of hypoxanthine and xanthine involved measurement of the concentration of purine by enzymatic conversion to uric acid, reevaporation to dryness of 2 ml of the samples in a desiccator (vacuum, anhydrous calcium chloride), wet combustion of the purine in a vacuum line with collection of evolved carbon dioxide in an ionization chamber (11), and radioassay of ^{14}C in a vibrating reed electrometer. Specific activities of various purines, notably adenine and 7-methylguanine, were also measured in selected urine samples. Uric acid was isolated from urine and purified as described elsewhere (11). Radioassay of uric acid-^{14}C was done in a vibrating reed electrometer.

Using methods that have been described previously (11, 12), uric acid pool, uric acid turnover, and incorporation of glycine-1-^{14}C into uric acid were measured in several of the patients during a control period when no drugs were given.

HG-PRTase and adenine phosphoribosyltransferase activities were determined by a radiochemical method.[2] The incubation mixture contained 50 mM Tris buffer, pH 7.4, 5 mM $MgCl_2$, 1 mM 5-phosphoribosyl-1-pyrophosphate, 0.48 mM purine-8-^{14}C (Schwarz Bio Research Inc., Orangeburg, N. Y.), and 1.0–1.5 mg of protein from dialyzed erythrocyte hemolysate in a final volume of 250 μl. 0.05 μCi of ^{14}C was used for each assay except in patients with the Lesch-Nyhan syndrome for whom, because of the markedly decreased HG-PRTase activity, 5.0–7.5 mg of protein and 1 μCi of hypoxanthine-^{14}C or guanine-^{14}C were used. After incubation for 10 min at 37°C, the reactions were terminated by adding 4 ml of ice-cooled water containing 1 μmole of the corresponding nucleotide. The reaction mixture was passed through a 0.4 × 1.5 cm column of copper-loaded chelating resin (Chelex 100, 200-400 mesh, Bio-Rad Laboratories, Richmond, Calif.). Nucleotides are not retained and can be washed from the column with a small amount of water. In contrast, the purine bases are strongly bound to the chelating resin. Nucleotide formation was determined by counting ^{14}C contained in the eluate in a liquid scintillation counting system using Bray's solution (13).

[2] Chow, D., F. Kawahara, T. Saunders, and L. B. Sorensen. Determination of hypoxanthine-guanine phosphoribosyltransferase. In preparation.

RESULTS

A summary of clinical data, pool and turnover values, and purine phosphoribosyltransferase activities appears in Table I. All four patients with hyperuricemia had overproduction of uric acid. Patients D.K. and E.K., who were brothers, had normal HG-PRTase activity in red blood cells. Laboratory tests showed that they also had elevated prebeta lipoproteins, increased serum triglyceride levels, and a diabetic glucose tolerance curve.

A.D. was a 54 yr old man who had his first attack of gouty arthritis at age 22. He had a partial deficiency of HG-PRTase equivalent to 12.5% of normal activity. Three maternal uncles had suffered from gout and all four of his brothers had hyperuricemia, three of them having experienced acute gouty arthritis.

D.T. was an 8 yr old boy with all the clinical features of the Lesch-Nyhan syndrome, including spastic cerebral palsy, choreoathetosis, mental retardation, self-mutilation, and recurrent uric acid lithiasis. Hemolysates from this patient contained small but definite amounts of HG-PRTase, clearly evident in time course studies. The respective activities with hypoxanthine and guanine as substrates were about 0.64 and 0.05% of normal.

and Activities of Purine Phosphoribosyltransferases

Urine uric acid	Plasma urate	Phosphoribosyltransferase activity		
		Hypoxanthine	Guanine	Adenine
mg	*mg/100 ml*	*mμmole/mg of protein per min*		
400 ±40	<7	2.14 ±0.15‡	2.49 ±0.24‡	0.431 ±0.049‡
387	4.0	—	—	—
461	10.6	1.75	2.24	0.487
714	8.4	2.22	2.38	0.444
790	11.5	0.260	0.320	0.661
840	7.7	0.0137	0.0013	0.839

Similar results have been obtained for other patients with the Lesch-Nyhan syndrome studied in this hospital. These values are distinctly higher than those reported by Kelley, Greene, Rosenbloom, Henderson, and Seegmiller who used high voltage electrophoresis for separation of nucleotides and purines (4).

In accordance with the observations by Seegmiller, Rosenbloom, and Kelley (3), we found the activity of adenine phosphoribosyltransferase, an enzyme that catalyzes the formation of adenylic acid from adenine and PRPP, to be increased in red blood cells from patients with HG-PRTase deficiency.

The effect of allopurinol upon the excretion of the major purines is shown in Fig. 2 and Table II. This compound is a potent inhibitor of xanthine oxidase, the enzyme that catalyzes the conversion of hypoxanthine to xanthine and of xanthine to uric acid. Allopurinol reduced the amounts of uric acid in the urine in all subjects except for one patient with gout who had extensive tophaceous involvement. This patient had, however, an 8-fold increase in the excretion of uric acid precursors, indicating that xanthine oxidase was being effectively inhibited. The fact that the specific activity values of ^{14}C in uric acid were never more than half of those in the precursors in this patient suggests that during the period of allopurinol treatment approximately one-half of urinary uric acid originated from tophi (Fig. 4).

Allopurinol reduced total urinary oxypurines (defined as the sum of hypoxanthine, xanthine, and uric acid) in the normal subject from 2.39 mmoles/day in the control period to 1.83 mmoles/day during the treatment period. Similarly in the gouty subjects E. K., who had normal HG-PRTase activity, the pretreatment value of 4.13 mmoles of oxypurine per day fell to 3.22 mmoles/day while he was on allopurinol in a dose of 10 mg/kg per day. On the other hand, patients who were deficient in HG-PRTase showed an increase in the precursors of uric acid commensurate with the reduction in urinary uric acid, so that the total excretion of urinary oxypurine remained constant or even increased slightly. The modest rise in total purines in patient A.D. (Fig. 2, D) is probably related to the more efficient clearing by the kidney of hypoxanthine and xanthine as compared to uric acid leading to excretion in the urine of purine that would normally be excreted into the gastrointestinal tract.

During allopurinol treatment the molar ratios of hypoxanthine to xanthine in subjects with a deficiency of HG-PRTase differed strikingly from those ratios in subjects with normal enzyme activity. Xanthine predominated over hypoxanthine in the urine from the normal subject and from the two patients with gout who had normal HG-PRTase activity. The molar ratios of xanthine to hypoxanthine were 2.61, 4.90, and 3.11, respectively (Table II). These data suggest that the principal pathway of uric acid production in these individuals is via xanthine that has been formed from guanine rather than from hypoxanthine.

Patients with HG-PRTase deficiency excreted more hypoxanthine than xanthine. The molar ratio of hypoxanthine to xanthine was 1.01 for the adult with partial HG-PRTase deficiency and 1.04 for the boy with the Lesch-Nyhan syndrome (Table II). When correction was made for the differences in body weight, the patient with the Lesch-Nyhan syndrome had urinary hypoxanthine values that were 40 times higher than the mean values for three patients with normal HG-PRTase activity. These observations imply that hypoxanthine is the major source of the excessive uric acid production in HG-PRTase deficiency.

The patterns of labeling of hypoxanthine, xanthine, and uric acid are shown in Figs. 3–6. In each instance

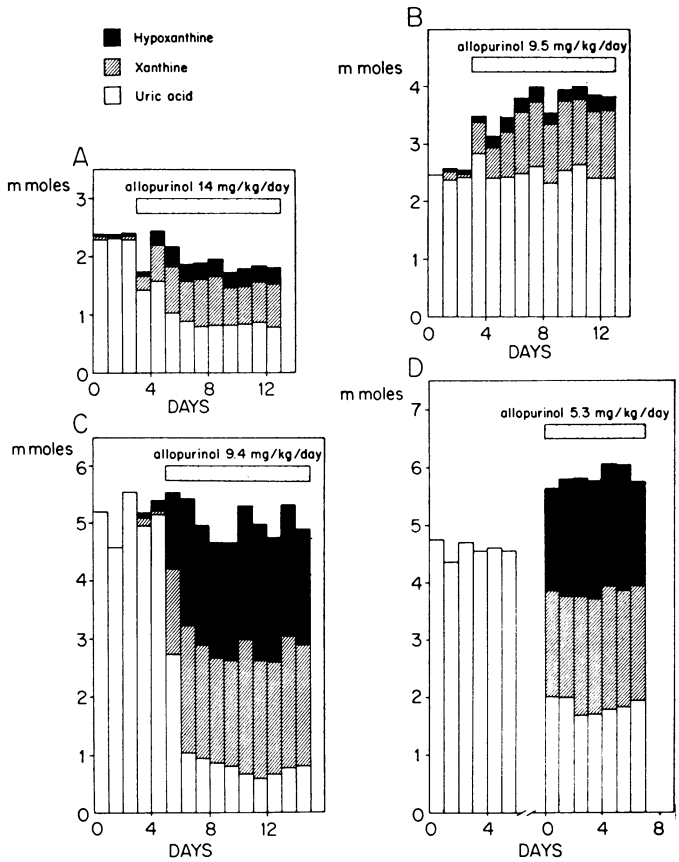

FIGURE 2 Excretion in urine of oxypurines before and during treatment with allopurinol. A, normal subject (J.G.); B, gout, normal HG-PRTase (D.K.); C, Lesch-Nyhan syndrome (D.T.); and D, gout, 12.5% of normal activity of HG-PRTase (A.D.).

the specific activity is expressed as disintegrations per minute per millimole of purine adjusted to a glycine-^{14}C dose of 1 μCi/kg body weight.

For hypoxanthine the isotope concentration reached a maximum within the first 6 hr in all four patients, then fell rapidly over the next 20 hr. In patients with HG-PRTase deficiency the initial labeling of hypoxanthine was 5 and 2½ times higher than normal. The shape of the specific activity curves for hypoxanthine between days 1 and 7 was different in deficiency states of HG-PRTase and in states of normal enzyme activity. In the latter case the isotope concentration was almost station-

TABLE II

*Mean Urinary Oxypurine Excretion in a Control Period and During Treatment with Allopurinol**

Subject	Hypoxanthine		Xanthine		Uric acid		Total oxypurine	
	Control	Allopurinol	Control	Allopurinol	Control	Allopurinol	Control	Allopurinol
				μmoles/day				
J.G.	45	278	46	727	2301	825	2392	1830
D.K.	51	232	120	1133	2384	2476	2555	3841
E.K.	42	357	116	1131	3967	1730	4125	3218
A.D.	87	2003	133	1976	4701	1855	4921	5834
D.T.	184	2126	68	2051	5000	732	5252	4909

* Mean values of seven determinations beginning at least 3 days after institution of allopurinol therapy.

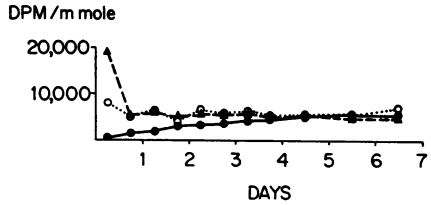

FIGURE 3 Specific activities of urinary purines after injection of 1 μCi/kg body weight of glycine-^{14}C into a normal subject (J.G.). Glycine-^{14}C was injected on the 4th day of allopurinol administration. ▲---▲, hypoxanthine; ○···○, xanthine; ●———●, uric acid.

ary between day 1 and day 7. In enzyme deficiency states there was evidence of a small second peak which appeared between 24 and 48 hr; ^{14}C concentration then gradually decreased to less than half the value of the second peak over the next 5 days.

Xanthine was also maximally labeled within the first 6 hr in all patients studied. The xanthine specific activity values were at first considerably less than those of hypoxanthine but later tended to approximate and parallel them.

The patterns of incorporation of ^{14}C into uric acid were similar in all patients but the one with the Lesch-Nyhan syndrome. Initially there was only slight labeling of uric acid. Specific activities than gradually rose to maximal values after 3 to 6 days. During the latter part of the studies uric acid specific activity values approached those of its precursors in two cases while in a third case (D.K.) the concentration of isotope in uric acid remained lower, presumably because non-isotopic uric acid in tophi contributed to the dilution of labeled uric acid in the soluble pool. The boy with the Lesch-Nyhan syndrome showed maximal labeling of uric acid within the first 24 hr. Subsequently the concentration of ^{14}C in uric acid fell gradually, but more slowly than in hypoxanthine and xanthine, so that the specific activity in uric acid became slightly higher. This latter observation may be related to the fact that

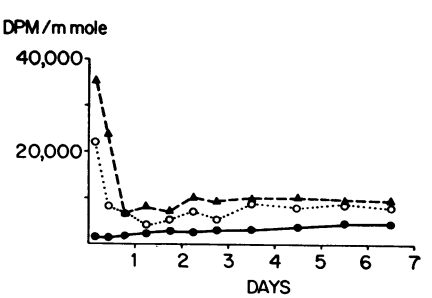

FIGURE 4 Specific activities of urinary purines after injection of 1 μCi/kg body weight of glycine-^{14}C into a gouty subject with normal HG-PRTase activity (D.K.). Glycine-^{14}C was injected on the 4th day of allopurinol administration. Symbols as in Fig. 3.

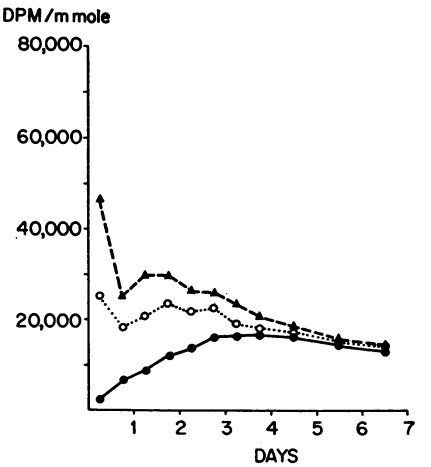

FIGURE 5 Specific activities of urinary purines after injection of 1 μCi/kg body weight of glycine-^{14}C into a gouty subject with 12.5% of normal activity of HG-PRTase (A.D.). The patient was maintained on allopurinol for 6 months before injection of glycine-^{14}C. Symbols as in Fig. 3.

the kidney clears hypoxanthine and xanthine more rapidly than uric acid.

The concentration of ^{14}C in urinary hypoxanthine from the same four subjects has been plotted logarithmically vs. time in Fig. 7. Within the period of study each curve appears to be biphasic, consisting of a rapid and a slow component. The major process contributing to the initial, rapid component is assumed to be the conversion of labeled glycine to hypoxanthine via inosinic acid and

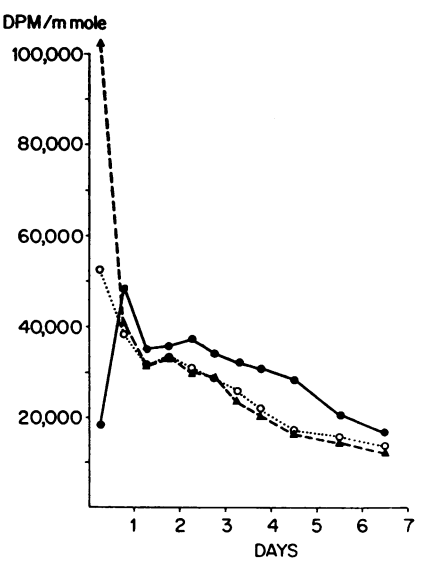

FIGURE 6 Specific activities of urinary purines after injection of 1 μCi/kg body weight of glycine-^{14}C into a patient with the Lesch-Nyhan syndrome (D.T.). Glycine-^{14}C was injected on the 4th day of allopurinol administration. Symbols as in Fig. 3.

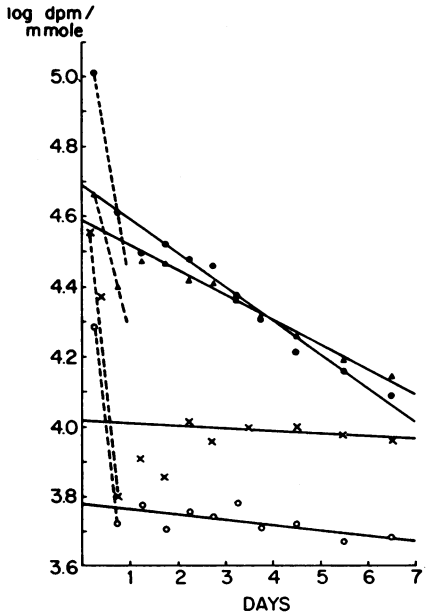

FIGURE 7 Logarithmic plots of the isotope concentration in hypoxanthine vs. time. ●———●, the Lesch-Nyhan syndrome; ▲———▲, gout, 12.5% of normal HG-PRTase activity; ×———×, gout, normal HG-PRTase activity; ○———○, normal subject.

adenine nucleotides. The slope of this component is determined by the turnover of free nucleotides and the admixture of nonlabeled hypoxanthine arising from the breakdown of tissue nucleic acids. The slower component presumably represents the turnover of the adenine moiety of nucleic acids. The most significant difference between subjects with deficient and normal HG-PRTase is in the slopes of isotope decline in the second component. The rate constants of this process were calculated from observations between 42 and 156 hr. These data fitted a first order kinetic system quite well, but the probability that the slower component comprises a multicompartmental system should be strongly entertained. A standard formula for comparison of the slopes of two regression lines was used to evaluate the significance of the difference between the slopes (14). The rate constants and the results of statistical analysis are given in Table III.

The very slow decline of the second slope observed in patients with normal HG-PRTase activity indicates that under normal conditions hypoxanthine is continually reutilized. This finding corroborates similar evidence obtained in studies of xanthinuric patients (15, 16). On the other hand, a deficiency of HG-PRTase prevents adequate recycling of hypoxanthine and thereby leads to increased catabolism of hypoxanthine.

In order to determine the extent to which hypoxanthine is derived by cleavage of newly formed inosine monophosphate (IMP), the isotope concentration in adenine was determined in the first urine sample after injection of glycine-^{14}C, and was then determined again on the 5th day. Comparison of the specific activities of hypoxanthine and adenine (Table IV) suggests that adenine derivatives are the principal source of hypoxanthine and that IMP cleavage plays a minor role in the genesis of hypoxanthine. Cleavage of even a minute fraction of newly formed IMP-^{14}C would be expected to yield higher initial concentration of ^{14}C in hypoxanthine compared to adenine because ^{14}C in the adenine pool is diluted at an early stage by unlabeled adenine arising from tissue nucleic acids.

Table IV also gives specific activity values of 7-methylguanine. Early labeling of this compound was observed in all patients studied. Similar findings have been reported by Wyngaarden et al. (8).

^{14}C was not incorporated into scattered samples of methylated xanthines indicating the exogenous origin of these compounds.

The effect of allopurinol on the rate of *de novo* purine biosynthesis was measured by following the incorporation of isotopic glycine into urinary hypoxanthine, xanthine, and uric acid (Table V). The 7 day incorporation value for ^{14}C in the normal subject was 0.0581% of the administered isotope. Comparative control values in normal subjects ranged from 0.15 to 0.20%. Similarly, 0.12% of injected glycine-^{14}C was recovered in oxypurines from the gouty subject D.K. during the allopurinol study, while in a 7 day period when no drug was given, his brother E. K. who had a similar degree of overproduction of uric acid, excreted 0.250% of injected isotope in uric acid. Thus in two subjects with normal

TABLE III
Slopes of Regression Lines for Isotope Decline in Hypoxanthine and Significance Levels of the Differences Between the Slopes

Subjects	K*	SE‡
J.G.	−0.0363	0.0151
D.K.	−0.0173	0.0140
A.D.	−0.1634	0.0069
D.T.	−0.2222	0.0151
Slopes compared	**P§**	**t**
D.T. vs. J.G.	<0.001	8.98
A.D. vs. J.G.	<0.001	7.59
D.T. vs. A.D.	<0.005	3.53
D.K. vs. J.G.	>0.4	0.855

* Δ specific activity of hypoxanthine/day.
‡ Standard error.
§ P value from t test for paired samples. Significance values based on two-tailed distribution.

TABLE IV

Specific Activities of Urinary Purines from Four Subjects After Intravenous Injection of Glycine-1-^{14}C in a Dose of 1 μCi/kg Body Weight

Subject	Time	Hypoxanthine	Adenine	Xanthine	7-Methylguanine
	day		*dpm/mmole*		
J.G.	0.25	19,300	14,300	7,900	5,200
	4.5	5,200	5,600	5,400	2,600
D.K.	0.15	35,700	31,200	22,100	10,200
	4.5	10,100	10,900	7,700	6,200
A.D.	0.25	46,400	42,900	25,000	11,800
	4.5	18,300	17,100	16,900	7,400
D.T.	0.25	102,000	71,800	52,500	13,300
	4.5	16,200	17,200	17,300	5,400

HG-PRTase activity allopurinol reduced the *de novo* purine synthesis to less than half the pretreatment rate.

The boy with the Lesch-Nyhan syndrome had comparable incorporation values in the control and allopurinol studies, indicating failure of allopurinol to suppress purine biosynthesis in this conditon. The response to allopurinol was equivocal in the patient who had 12.5% of normal HG-PRTase activity in that allopurinol decreased the incorporation of glycine into the major purines by 38%, while the excretion of total oxypurine rose slightly.

DISCUSSION

Possible modes by which HG-PRTase deficiency leads to accelerated purine synthesis have been discussed by Kelley et al. (4). These include (*a*) decreased concentration of inosinic and guanylic acids, leading to reduced feedback inhibition of PRPP amidotransferase, the rate-limiting enzyme of *de novo* synthesis; (*b*) decreased utilization of PRPP for direct synthesis of guanine monophosphate (GMP) and IMP, making this substrate potentially available for purine biosynthesis *de novo;* and (*c*) activation of PRPP amidotransferase by increased concentration of hypoxanthine or guanine.

Balis has postulated that HG-PRTase completes the sequence of reactions in a cyclic interconversion of adenine and hypoxanthine derivatives and that deficiency of this enzyme causes depletion of intracellular nucleotides due to increased catabolism of inosinic acid to inosine and hypoxanthine (17). The biochemical bases of accelerated purine biosynthesis have been investigated in cultured skin fibroblasts obtained from patients with HG-PRTase deficiency (18, 19). These studies have yielded the following information: (*a*) enzyme-deficient cells had greatly increased concentration of PRPP although the rate of synthesis of this compound was not increased; (*b*) there were no significant differences between normal and deficient fibroblasts with respect to the concentrations of guanosine triphosphate or of total adenine ribonucleotides; and (*c*) an excess of hypoxanthine or guanine caused a stimulation of the rate of the early reactions of purine synthesis *de novo.*

The major objective of the present investigation was to acquire information from appropriate in vivo studies with respect to the source of excessive uric acid formation in HG-PRTase deficiency. Such information was obtained by comparing the effect of allopurinol on urinary oxypurines in subjects with normal and deficient HG-PRTase and by following the incorporation of glycine-1-^{14}C into the major purines. In the presence of normal enzyme activity allopurinol treatment resulted in proportionately greater increases in the excretion of xanthine compared to hypoxanthine. The mean molar ratio of xanthine to hypoxanthine for three subjects with normal HG-PRTase was 3.36:1. A similar preponderance of xanthine over hypoxanthine has been observed in previous studies on the effect of allopurinol on purine excretion (20, 21) and in patients with xanthinuria, a congenital absence of xanthine oxidase (9, 16). These data suggest that the principal pathway of uric acid production is normally through xanthine that has been formed from guanine rather than from hypoxanthine. Patients with HG-PRTase deficiency excreted more hypoxanthine than xanthine. When correction was made for the difference in body weight, the boy with the Lesch-Nyhan syndrome had urinary hypoxanthine values that were 40 times higher than the mean value for three subjects with normal HG-PRTase activity, while an adult with a partial deficiency of the enzyme equivalent to 12.5% of normal activity had a 10-fold increase in urinary hypoxanthine compared to normal. The patterns of ^{14}C-labeling of the oxypurines in HG-PRTase deficiency reflect the role of hypoxanthine as precursor of xanthine. These findings imply that hypoxanthine is the major source of the excessive uric acid production in HG-PRTase deficiency.

TABLE V

Incorporation of Glycine-^{14}C into Urinary Purines (7 Days)

Subject	Control study Uric acid*	Allopurinol study Hypoxanthine	Allopurinol study Xanthine	Allopurinol study Uric acid*	Allopurinol study Total oxypurine
	% of dose	*% of dose*			
Normal value	0.150–0.200				
J.G.		0.009	0.024	0.025	0.058
E.K.	0.250				
D.K.		0.007	0.027	0.087	0.121
A.D.	0.781	0.191	0.153	0.143	0.487
D.T.	2.770	1.172	1.101	0.535	2.718

* Corrected for extrarenal elimination of uric acid.

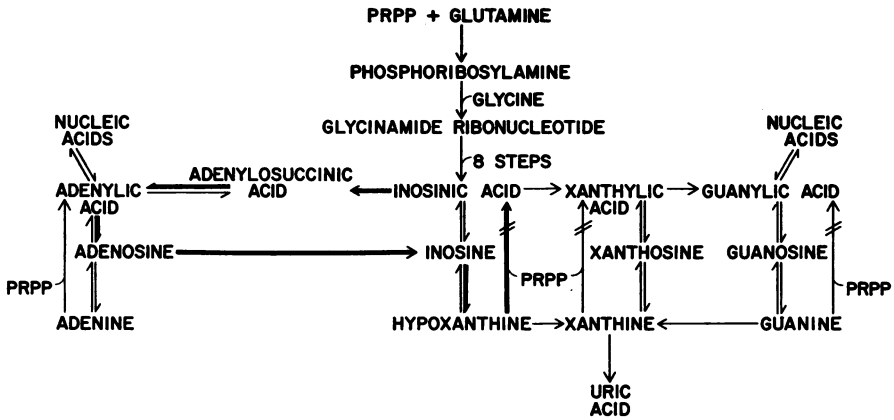

FIGURE 8 Simplified scheme of *de novo* purine biosynthesis, reutilization pathways, and purine interconversions. The heavy arrows indicate the cyclic interconversion of adenine and hypoxanthine derivatives. The blocked reactions in HG-PRTase deficiency are shown by the parallel lines intersecting the arrows.

The slow dilution in specific activity of hypoxanthine after the first 24 hr in subjects with normal HG-PRTase activity is evidence for continual reutilization of hypoxanthine in normal purine metabolism. Similar conclusions have been reached from studies of purine metabolism in patients with xanthinuria. A xanthinuric patient had a daily turnover of xanthine of 276 mg, 79% of which was excreted in the urine. In the same patient the turnover of hypoxanthine was 960 mg daily, of which only 5.7% appeared in the urine (16). Moreover, hypoxanthine and adenine were utilized to a much greater extent than were xanthine and guanine. Only 1% of intravenously injected adenine-^{14}C was recovered in the urine after 24 hr (9) and 28% of hypoxanthine-^{14}C after 21 days (16). The recoveries of guanine and xanthine were 74% in 2 wk and 75% in 3 wk, respectively (9, 16). These data indicate quite clearly that hypoxanthine is an active intermediary metabolite that is being continually recycled, presumably through synthesis to inosinic acid which is in turn converted to 5′-adenine and guanine nucleotides. On the other hand, xanthine and guanine are only slightly reutilized. Thus, the principal role of HG-PRTase appears to be the conservation of hypoxanthine rather than guanine.

The incorporation of glycine into hypoxanthine in patients with HG-PRTase deficiency was quite different from that observed in subjects with normal enzyme activity. After a small second peak of isotope enrichment on day 2, there was a fairly rapid decline in ^{14}C concentration. The rate constants for this diluting process were 0.22 day^{-1} for a boy with the Lesch-Nyhan syndrome and 0.16 day^{-1} for an adult with partial deficiency. This finding indicates a greater than normal loss of ^{14}C from the hypoxanthine pool and dilution of isotope by newly formed unlabeled hypoxanthine arising from the turnover of the complex pools of adenine and hypoxanthine derivatives.

The deficiency of HG-PRTase provides a logical biochemical rationale for the loss of hypoxanthine. Failure of normal reutilization of hypoxanthine for the direct synthesis of IMP leaves less of this nucleotide available for conversion to adenosine monophosphate (AMP) and GMP. These purine nucleotides are natural feedback inhibitors of PRPP amidotransferase, the enzyme responsible for the rate-controlling step of purine synthesis. A relaxation of the cooperative feedback inhibition serves as a stimulus for the increased *de novo* synthesis. It would appear that an insufficient concentration of AMP is as important as any shortage of GMP in slackening a regulatory feedback mechanism in HG-PRTase deficiency. Consistent with this proposition is the finding by Seegmiller, Klinenberg, and Watts (22) that adenine decreased purine biosynthesis *de novo* by 45% without significantly altering the turnover of uric acid in a patient who had only 1% of the normal activity of HG-PRTase. Adenine is readily converted to its nucleotide by adenine PRTase in both normal subjects and patients with HG-PRTase deficiency. An increased formation of AMP through the reutilization pathway results in a concurrent depression in its generation from IMP formed *de novo*.

In subjects with normal HG-PRTase *de novo* purine biosynthesis is depressed in situations where the reutilization of hypoxanthine is augmented as a consequence of increased concentration of this purine, e.g., in xanthinuria and in patients treated with allopurinol. The present data can be synthesized into a scheme of cyclic interconversions of adenine and hypoxanthine derivatives proposed by Balis (17). This scheme involves the following sequence of reactions: AMP to

adenosine, to inosine, to hypoxanthine, to IMP, and back to AMP (Fig. 8). Lack of HG-PRTase prevents this cycle from functioning.

A decreased reutilization of hypoxanthine could conceivably result from a genetically determined increase in xanthine oxidase which would favor the oxidative catabolism of hypoxanthine to uric acid. Recently Carcassi, Marcolongo, Marinello, Riario-Sforza, and Boggiano (23) have reported increased activity of xanthine oxidase in liver biopsy material from patients with primary gout. Since xanthine oxidase is an inducible enzyme (24), such an increase could also be a secondary phenomenon.

The rapid labeling of urinary adenine observed in the present studies provides evidence that a portion of glycine-^{14}C is quickly converted into AMP. A comparison of the specific activities of adenine and hypoxanthine 5 days after the injection of glycine-^{14}C suggests that hypoxanthine is derived principally from adenine derivatives and that cleavage of IMP to hypoxanthine via inosine does not contribute in any great measure to the formation of uric acid in either normal man or in patients with HG-PRTase deficiency. The labeling patterns of adenine and hypoxanthine are quite similar to those observed in several of the patients studied by Wyngaarden et al. (8), although the interpretations of the data vary in some respects.

Early labeling of 7-methylguanine was seen in all subjects studied, corroborating similar evidence presented by Wyngaarden et al. (8). 7-Methylguanine is known to be a constituent of transfer RNA and ribosomal RNA (25). It is also known that methylation of nucleic acid occurs at the macromolecular level. The early labeling of 7-methylguanine is an indication that certain species of RNA turn over at a rapid rate.

The early and accelerated appearance of glycine-^{14}C into uric acid in some patients with primary gout has in the past been interpreted to mean the existence of a "shunt" pathway whereby newly synthesized purine bases were converted to uric acid without prior incorporation into nucleic acids. The present data do not lend support to such a proposition.

Allopurinol produced a significant decrease in the rate of purine biosynthesis *de novo* in subjects with normal HG-PRTase activity. This effect was demonstrated by measuring the cumulative incorporation of glycine-^{14}C into hypoxanthine, xanthine, and uric acid during allopurinol treatment and by summating the daily excretion of the three oxypurines before and during allopurinol therapy. The latter parameter was not valid for a patient with extensive tophaceous involvement, who actually increased the excretion of oxypurine while on allopurinol. Nevertheless, the drug substantially reduced biosynthesis *de novo* in this patient as evidenced by decreased incorporation of glycine-^{14}C into uric acid and its precursors.

Allopurinol did not suppress the incorporation of glycine-^{14}C into the major purines, nor did it reduce total oxypurine excretion in the patient with the Lesch-Nyhan syndrome. The patient who had 12.5% of the normal activity of HG-PRTase had a modest reduction of glycine-^{14}C incorporation into oxypurines during the period of treatment with allopurinol. Whether this difference is a valid measure of a reduction of purine biosynthesis *de novo* or whether it reflects variations in the pool size and turnover of glycine is open to question. In this connection it is of interest that azathioprine (Imuran) effectively inhibited *de novo* purine synthesis in the same patient (12). Since this inhibition is effectuated by nucleotide derivatives acting at the rate-limiting step catalyzed by PRPP amidotransferase (26), it follows that azathioprine or one of its intermediary metabolites, e.g. 6-mercaptopurine, has been converted to the nucleotide form. In patients with the Lesch-Nyhan syndrome, azathioprine has no discernible effect on purine biosynthesis (27).

Possible mechanisms by which allopurinol inhibits *de novo* purine synthesis have been discussed by Kelley, Rosenbloom, Miller, and Seegmiller (28). The inhibitory effect requires the presence of HG-PRTase activity. The finding that allopurinol increased the incorporation of labeled hypoxanthine into soluble nucleotides and nucleic acids in mice (29) suggests that the effect of allopurinol on *de novo* purine synthesis is related to higher concentration of hypoxanthine, which in turn, in the presence of adequate HG-PRTase activity, increases the formation of IMP via the reutilization pathway.

The activity of HG-PRTase in hemolysates from the boy with the Lesch-Nyhan syndrome was at least 10 times higher than the values reported by Kelley et al. (4). Whether this discrepancy reflects differences in the assay technique or whether it means that some patients with the Lesch-Nyhan syndrome have small, but detectable HG-PRTase activity remains to be resolved. In this connection it is of interest that Fujimoto and Seegmiller (30) found skin fibroblasts from patients with the Lesch-Nyhan syndrome to contain HG-PRTase activity that was around 3% of normal, although the activity in erythrocyte hemolysates was less than 0.04% of normal values.

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