SUPPRESSION OF THE SHUNT PATHWAY IN PRIMARY GOUT 
BY AZATHIOPRINE

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An increasing volume of studies1–3 lends support for a dual etiology of hyperuricemia in primary gout. In this disorder there may be either normal or excessive production of uric acid. Patients with primary gout related to overproduction of uric acid show a pattern of incorporation of a labeled precursor (e.g., glycine) into urinary uric acid which consists of a high initial value followed by a fairly rapid decline in isotope concentration during the succeeding days (Fig. 1). This pattern has been taken as evidence of a shunt pathway whereby precursor is incorporated into uric acid more promptly than in normal man by bypassing nucleic acid purines. This shunt pathway is generally considered to be responsible for overproduction of uric acid in primary gout.

When azathioprine (Imuran) was given to patients who displayed excessive excretion of uric acid in the urine, we found a significant reduction in both plasma and urinary uric acid. To evaluate this finding more precisely, the incorporation of glycine into uric acid during treatment with azathioprine was studied in three patients who had previously been shown to possess the shunt pathway.

The administration of azathioprine to patients with primary gout was an indirect result of a joint study with Drs. N. Bricker and R. Rieselbach at Washington University, St. Louis, on the nature of primary gout in a patient who had developed progressive renal failure due to gouty nephropathy which necessitated renal homotransplantation in December 1964.4 The patient has, since then, been maintained on azathioprine in order to suppress a homograft reaction.
Experimental.—Three gouty subjects previously shown to be overproducers of uric acid were studied in detail. In addition, plasma and urinary uric acid were determined daily in one patient with primary gout not related to overproduction of uric acid, and in two healthy individuals. Each subject received azathioprine for a period of 7–10 days in a daily dose of 300 or 400 mg (3.4–4.6 mg per kg body weight). No depressive effect on the bone marrow nor any other untoward reaction was observed as a consequence of this treatment.

For 3 days before as well as during the study, the patients were maintained on a diet essentially free of purines, but with sufficient protein to maintain nitrogen balance. Plasma and urinary uric acid were determined by an enzymatic spectrophotometric method. Glycine-1-C\textsuperscript{14} was injected intravenously in a dose of approximately 100 \( \mu \)c. In studies on the effect of azathioprine upon uric acid metabolism, glycine-C\textsuperscript{14} was given 48 or 72 hr after initiation of drug treatment. Uric acid was isolated from urine and purified as described elsewhere, except that the first step in the isolation procedure involved either the addition of 200 mg of uric acid in solution as carrier to 200 ml of urine, or the adsorption of urinary uric acid to charcoal (Darco G-60) and its subsequent elution with hot 0.1 \( N \) NaOH.

Radioassay of C\textsuperscript{14} was done either in a vibrating reed electrometer after wet oxidation of uric acid in a vacuum combustion line, or in a liquid scintillation spectrometer (Packard Tricarb, model 3324) using the following technique: preweighed amounts of uric acid (usually less than 50 mg) were dissolved in 2 cc of Hyamine hydroxide, then mixed with 18 cc of a mixture of PPO (2,5-diphenyloxazole), 3 gm; POPOP (1,4-bis-(5-phenyloxazolyl)benzene), 100 mg; and toluene, 1000 cc. In this system toluene-C\textsuperscript{14} counted with an efficiency of about 70\%. In counting uric acid a modest quenching was observed which was proportional to the quantity of uric acid counted (a sample weighing 50 mg reduced the counting efficiency to 85\% of the efficiency at zero weight). All samples were corrected for quenching either by including internal standards, or by applying a factor derived from a curve relating degree of quenching to sample weight.

The miscible pool and turnover of uric acid were determined as described elsewhere, except that radioassay of C\textsuperscript{14} was done in a vibrating reed electrometer.

Results.—The effect of azathioprine on plasma and urinary uric acid in one of the gouty subjects with overproduction of uric acid is graphically presented in Figure 2. The patient had been maintained on a uricosuric drug (sulfinpyrazone 600 mg daily) for 2 yr and this treatment was continued throughout the study. With addition of azathioprine, plasma uric acid fell from a pretreatment level of between 4.42 and 4.80 mg per cent to a low of 2.13 mg per cent on the 10th day, while the 24-hr urinary uric acid excretion fell from a high of 848 mg to a low of 367 mg. Seven days after discontinuation of the drug, plasma and urinary uric acid had returned to pre-

![Fig. 1.](image1.png)  
**Fig. 1.**—Concentration of C\textsuperscript{14} in urinary uric acid following injection of glycine-1-C\textsuperscript{14} into a normal subject and a gouty subject with overproduction of uric acid.

![Fig. 2.](image2.png)  
**Fig. 2.**—Effect of azathioprine (Imuran) on plasma and urinary uric acid in a patient with primary gout. Throughout the study he was also on a uricosuric drug.
Fig. 3.—Concentration of C\(^{14}\) in urinary uric acid following intravenous injection of glycine-1-C\(^{14}\) at zero time into three patients with primary gout associated with overproduction of uric acid. All data are adjusted to a dose of 100 μc of glycine-C\(^{14}\). The lower curves represent data obtained after pretreatment with azathioprine for 48-72 hr. Patient J. L. was reinjected with glycine-C\(^{14}\) on the 10th day, 72 hr after azathioprine had been discontinued.

Treatment levels. No significant changes in plasma and urinary uric acid were observed when azathioprine was given to two normal individuals and to a gouty patient whose hyperuricemia was related to a dysfunction in the enzymatic renal tubular transport mechanism of uric acid.

Figure 3 shows the incorporation of labeled glycine into urinary uric acid in three gouty subjects with excessive urinary uric acid excretion. The data of the upper curves were obtained when the patients were untreated, and clearly demonstrate the presence of the shunt pathway. The lower curves represent the data obtained when the patients had been pretreated with azathioprine for 48–72 hr before injection of glycine-C\(^{14}\). With the exception of an abortive early peak in one of the patients, there was a striking conversion of the incorporation pattern to normal in all three patients. Patient J. L. was restudied 72 hr after azathioprine had been discontinued: The glycine incorporation curve at this time indicated a fractional return of the direct pathway of uric acid synthesis.

Table 1 summarizes all data on the cumulative incorporation of glycine into uric acid.

$$\text{glutamine} + \alpha-5\text{-phosphoribosyl}-1\text{-pyrophosphate} + H_2O$$

$$\beta-5\text{-phosphoribosyl}-1\text{-amine} + \text{glutamic acid} + PP$$

$$+ \text{glycine} + \text{ATP}$$

$$\beta\text{-glycinamide} \text{ribonucleotide} + \text{ADP} + \text{Pi}$$

$$\text{IMP}$$

$$\text{AMP} \rightarrow \text{GMP}$$

nucleic acids, etc.

shunt pathway

degradative reactions

uric acid

Fig. 4.—Simplified scheme of the formation of uric acid.
The results in the preceding section indicate that when azathioprine is given to gouty subjects with overproduction of uric acid, urinary uric acid falls to within the normal range. Furthermore, the incorporation of precursor glycine into urinary uric acid reverts to a normal pattern, and the cumulative recovery of glycine-C14 in urinary uric acid assumes values comparable to those obtained in normal individuals. The exact site of action of azathioprine upon purine biosynthesis is not yet known, but work is currently under way in this laboratory to elucidate the precise locus of interaction.

There is growing evidence for the view that the most important mechanism for the precise control of a biosynthetic pathway is feedback inhibition. Figure 4 depicts a simplified scheme of the formation of uric acid. The first step in purine biosynthesis is the enzymatic reaction between glutamine and 5-phosphoribosyl-1-pyrophosphate to yield 5-phosphoribosylamine. The reaction generating phosphoribosylamine is irreversible and therefore becomes the rate-limiting reaction in de novo purine biosynthesis. Once formed, phosphoribosylamine will undergo a series of reactions to yield inosine-5'-phosphate (IMP), the parent purine compound. Most of the IMP is converted to adenylic acid (AMP) and guanylic acid (GMP), which are then incorporated into more complex polynucleotides. Even in the nongouty subject a small part of inosinic acid appears to be degraded by a more direct pathway to uric acid. It is an increase of this latter pathway which characterizes primary gout with overproduction of uric acid.

The exact biochemical defect responsible for the exaggerated shunt pathway in primary gout is unknown. Factors governing the rate of synthesis of 5-phosphoribosylamine include the concentrations of the substrates glutamine and 5-phosphoribosyl-1-pyrophosphate, and the activity of the synthesizing enzyme. Wyngaarden and associates8 have been primarily interested in the regulation of the first enzyme of purine biosynthesis. They found that this enzyme had at least two separate regulatory sites, one for adenyl and one for guanyl ri-
bonucleotides. The conversion of IMP to AMP and IMP to GMP is also controlled by feedback inhibition. Recently, Gutman and Yu9 have suggested that the increased purine synthesis of primary gout may represent a consequence of a defect in utilization of glutamine for renal production of ammonia, and as a result, extra glutamine is diverted into the first and rate-determining reaction of de novo purine biosynthesis.

In studies with azathioprine the specific activity of C14 in urinary uric acid (DPM of C14 per mg uric acid) after glycine-C14 administration was similar to that observed in normal subjects, which suggests that azathioprine does not suppress the synthesis of adenylic and guanylic acids and their incorporation into nucleic acids. Additional evidence in support of this view is provided by the finding that azathioprine had no effect upon uric acid metabolism in normal subjects.

The possibility cannot be excluded that the functional activity of the direct pathway of uric acid synthesis from IMP is affected by regulatory factors, and furthermore, that the biochemical error in primary gout with overproduction of uric acid may be related to a defect in the rate control of the shunt pathway which, in turn, becomes the driving force on the rate of synthesis of 5-phosphoribosylamine.

The potential usage of azathioprine in the treatment of primary gout related to overproduction of uric acid must await further experiments designed to establish the dose range that will effectively lower the production of uric acid.

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9 Operated by the University of Chicago for the U.S. Atomic Energy Commission.
5 Kindly supplied by Dr. Peter Mawdsley, Burroughs Wellcome and Co., Tuckahoe, New York.