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Research Article

THE EFFECT OF PHENYLBUTAZONE ON URIC ACID METABOLISM IN TWO NORMAL SUBJECTS

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Phenylbutazone is a potent analgesic compound widely used in the treatment of gout and other arthritic disorders. Both in gouty and non-gouty subjects the drug sharply reduces the serum urate level (1–8). The mechanism by which this is accomplished has recently received attention in several laboratories, but the results are discrepant. Some investigators report that the drug has a potent uricosuric effect (3, 4, 6, 7), others that the drug produces no important change in urate excretion (2, 5), and still others that it actually causes a diminution of urate output (8). The latter two results, if valid, could have several possible explanations, *viz.*, the rate of urate synthesis has been reduced; uric acid has been disposed of by non-urinary pathways, or catabolized, in increased amounts; uric acid has been redistributed throughout a greater percentage of body water; or, uric acid has been greatly diluted in body fluids because of water retention and expansion of extracellular fluid volume. The present study was designed to evaluate these possible metabolic effects of phenylbutazone.

Two normal subjects were placed on constant low purine diets. Uric acid-N^{15} was infused during control periods and again during phenylbutazone administration, the miscible pool of uric acid and the rate of its turnover were determined, and the results obtained were correlated with changes in excretion values and serum concentrations of uric acid. Also, recoveries of unchanged isotopic uric acid in urine were determined in both periods, in order to evaluate extra-renal disposal or catabolism of urates. Finally, calculations of the volumes of distribution of uric acid were made; the changes observed were correlated with body weight and with hematocrit measurements in one subject. These studies have demonstrated that phenylbutazone is a uricosuric drug without specific effect on extra-renal factors of urate metabolism.

METHODS

Subjects. J. W. was a normal white male, 27 years of age, weighing 75 kg. S. N. was a colored female, 52 years of age, weighing 51 kg. She was convalescing from bronchitis and was considered normal at the time of this study. The composition of the diets and the normal urinary urate excretions and serum urate concentrations for these two subjects are given in Table I. Fluid intake was not restricted in either subject.

Isotopic uric acid. Uric acid-1,3-N^{15} having an isotope content of 28.3 atom per cent excess N^{15} was synthesized according to Cavalieri, Blair, and Brown (9) and Wyngaarden and Stetten (10). This product was used in studies on J. W. and a diluted product in studies on S. N.

Plan of experiments. After four days of dietary preparation, continuous 12 or 24-hour urine collections were begun, and frequent samples of serum were obtained in the fasting state for uric acid analyses. After four to five control days, small quantities of uric acid-N^{15} (200 mg. of uric acid, 28.3 atom per cent excess N^{15} in J. W., 86 mg. of uric acid, 6.5 atom per cent excess N^{15} in S. N.) were infused as the lithium salt in 500 ml. saline over a 30-minute period, and uric acid was then isolated from serial 12-hour urine samples for four to six days. Phenylbutazone was subsequently administered orally in dosage of 800 mg. daily, and after a suitable period of time the infusion of uric acid-N^{15} (100 mg. of uric acid, 28.3 atom per cent excess N^{15} in J. W., 68 mg. of uric acid, 6.5 atom per cent excess N^{15} in S. N.) was repeated, and urinary uric acid again isolated. The temporal relationships of these various procedures are self-evident from inspection of Figures 1 and 2.

Analyses.[1] Uric acid was determined in serum and urine according to Praetorius' (11) modification of the differential spectrophotometric method of Kalckar (12) employing purified hog liver uricase prepared according to Muller and Bauer (13). Uric acid was isolated from urine by adsorption onto and elution from charcoal (14) and was recrystallized from Li_2CO_3 solution (15). The miscible pool of uric acid and the rate of its turnover were calculated according to Benedict, Forsham, and Stetten (16). The intercept and slope of the best straight

[1] The author is indebted to Dr. DeWitt Stetten, Jr., of The Department of Health, Education, and Welfare, National Institutes of Health, Bethesda, Maryland, for the N^{15} analyses on subject J. W., and to Dr. Julius White, of the National Cancer Institute, Bethesda, Maryland, for those on subject S. N.

TABLE I

Pertinent data on subjects of present study

Subject	Sex	Age	Diet			Urinary uric acid	Serum uric acid
			Calories	Protein	Purine N		
		yrs.	*C.*	*gm.*	*mg.*	*mg./d.*	*mg. %*
J. W.	M	27	2,200	50	4	476 ± 7*	5.4 ± 0.1*
S. N.	F	52	2,065	45	4	400 ± 13	4.3 ± 0.1

* Values represent mean ± probable error of the mean.

line of the hemilogarithmic plot of isotope abundance *vs.* time were calculated, together with their probable errors, by the method of least squares. The probable error of the miscible pool was calculated as previously described (10).

RESULTS

Effect of phenylbutazone on miscible pool, turnover rate, serum concentrations and urinary excretion of uric acid

The results of the study on J. W. are presented in Figure 1 and Tables II and III. During the control period a miscible pool of 1272 mg. of uric acid was obtained with a turnover rate of 0.456 pool, or 580 mg. of uric acid per day. This was 104 mg. greater than the urinary excretion of urate which averaged 476 mg. per day. When phenylbutazone was administered, there was a marked uricosuric response, beginning the second day and reaching a maximum of 837 mg. on the fifth day. Concurrently, the serum urate concentration fell from a mean of 5.4 mg. per cent to a low of 1.5 mg. per cent on the eighth day of drug

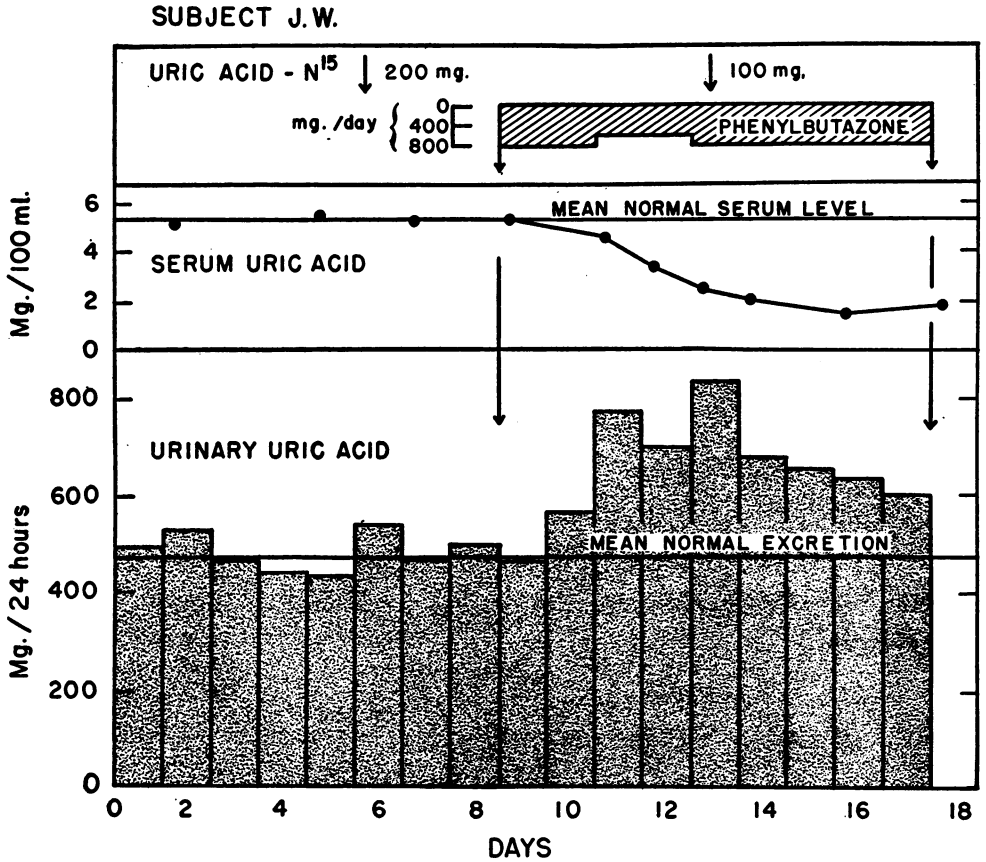

FIG. 1. SERUM URATE CONCENTRATIONS AND URINARY URATE EXCRETION IN SUBJECT J. W. BEFORE AND DURING PHENYLBUTAZONE ADMINISTRATION

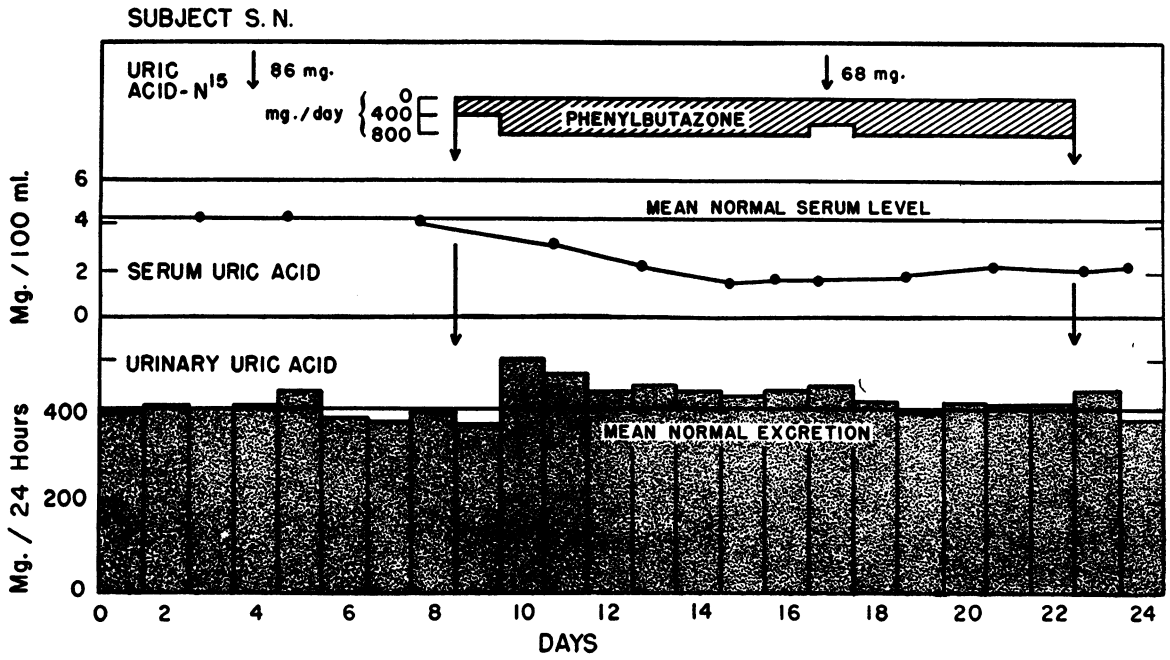

FIG. 2. SERUM URATE CONCENTRATIONS AND URINARY URATE EXCRETION IN SUBJECT S. N. BEFORE AND DURING PHENYLBUTAZONE ADMINISTRATION

administration. On the fifth day the miscible pool of uric acid was determined to be 716 mg., a decline of 556 mg. During this same period the subject had excreted an excess of 598 mg. of uric acid so that the contracture of pool size to this point was adequately explained by the increment in urate excretion. The turnover rate was now 1.084 pools or 776 mg. of uric acid per day. Although this was 196 mg. per day in excess of the control production rate, the difference had only a 69 per cent level of statistical significance (dev./p.e. = 1.5). The total increment of uric acid excretion for the nine days of phenylbutazone administration was 1630 mg., a quantity greater than the original miscible pool itself. Urinary urate at the end of this period was still 600 mg. per day and the serum concentration had reached a plateau at less than 2 mg. per cent (Figure 1). If an average theoretical volume of distribution in which uric acid is present at a concentration equal to that of serum, is taken to be 25.9 liters in this subject (see Table III) and the serum level is estimated to be 1.7 mg. per cent (see Figure 1), one can calculate a miscible pool of about 440 mg. on the ninth day of phenylbutazone ingestion. This would represent a pool contracture of only 832 mg., so that roughly one-half the extra uric acid appearing in urine must have had another origin.

The results of the study on S. N. are presented in Figure 2 and Tables II and III. During the

TABLE II

Magnitude of miscible pool of uric acid and its turnover before and during phenylbutazone ingestion

Subject	Exper. no.	a = dose injected	I_i = isotope injected	I_0 = antiln of intercept	A = miscible pool	K = -slope	KA = turnover	B = urinary excretion	KA − B = surplus
		mg.	*atom per cent excess*	*atom per cent excess*	*mg.*	*day*$^{-1}$	*mg. per day*	*mg. per day*	*mg. per day*
J. W.	1	200	28.3	3.85 ± 0.15*	1272 ± 58	0.456 ± 0.008	580 ± 28	476 ± 7	104 ± 28
	2	100	28.3	3.47 ± 0.46	716 ± 108	1.084 ± 0.050	776 ± 122	644 ± 11	132 ± 123
S. N.	1	86	6.50	0.758 ± 0.035	651 ± 34	0.769 ± 0.012	501 ± 27	400 ± 13	101 ± 30
	2	68	6.50	1.085 ± 0.087	341 ± 32	1.211 ± 0.020	413 ± 39	405 ± 5	8 ± 39

* Values represent the mean ± probable error of the mean.

TABLE III

Volume of distribution of uric acid before and during phenylbutazone ingestion

Subject	Experiment no.	Miscible pool	Serum level	Theoretical volume of distribution	Per cent of body water*
		mg.	*mg. %*	*liters*	*%*
J. W.	1	1272	5.4	23.6	50.8
	2	716	2.5	28.6	
S. N.	1	651	4.3	15.1	56.9
	2	341	1.7	20.0	

* Body water was assumed to be 62 and 52 per cent of body weight for the male and female subjects, respectively (17). Owing to fluid retention during phenylbutazone ingestion, these assumptions could not be applied in the second experiments.

control period a miscible pool of 651 mg. of uric acid was obtained with a turnover rate of 0.769 pool or 501 mg. of uric acid per day. This was 101 mg. greater than her urinary excretion of urate which averaged 400 mg. per day. When phenylbutazone was administered, there was only a meager uricosuric response, with a peak excretion of 511 mg. being recorded on the second day. The daily urinary urate excretion remained slightly above the mean control value for a total of eight days, and during this period the serum urate concentration declined from 4.3 to 1.7 mg. per cent. On the ninth day the miscible pool was found to be 341 mg. of uric acid, a decline of 310 mg. The subject had excreted an excess of 359 mg. during this period. The turnover rate was 1.211 pools or 413 mg. of uric acid per day at this time. This value was 88 mg. per day below the control value, but this difference had only a 78 per cent level of significance (dev./p.e. = 1.8). The urinary urate excretions recorded for the last several days of this study were clearly normal values, so that in this patient the cumulative increment in urinary urate excretion incident to phenylbutazone administration correlated nicely with the decline in size of the miscible urate pool.

Effect of phenylbutazone on extra-renal disposal and catabolism of uric acid

In the control uric acid-N^{15} studies, the total recoveries of isotopic uric acid in urine were 76 per cent in J. W. and 73 per cent in S. N. These values were in good agreement with the portions of the daily turnover of uric acid accounted for by urinary urate excretion in these same subjects, which were 82 and 80 per cent, respectively. The residual 18 to 27 per cent not accounted for by either of these methods were in good agreement with similar determinations in other normal subjects (16, 18) and represented those portions of the daily turnovers disposed of by extra-renal pathways or catabolized to ammonia and urea (10).

Of the uric acid-N^{15} injected during phenylbutazone administration, 93 per cent was recovered unchanged in urine in both subjects. These values are clearly considerably greater than those procured prior to drug administration and they exclude the occurrence of increased extra-renal disposal or catabolism of uric acid in these subjects while on phenylbutazone. A comparison of the rates of synthesis and of excretion of uric acid during phenylbutazone ingestion (Table II) confirms this conclusion. In subject J. W. there was no significant change in the urate fraction escaping renal excretion which was now 17 per cent of the amount synthesized, whereas in subject S. N. there was a decided drop in this value to 2 per cent of that synthesized. In neither instance, however, was there any evidence for increased extra-renal disposal or catabolism of uric acid.

Effect of phenylbutazone on the uric acid space

In Table III are recorded the volumes of distribution of miscible urate for the two experiments in both subjects. The calculated uric acid spaces increased 5.0 liters in J. W. and 4.9 liters in S. N. during the periods from onset of phenylbutazone ingestion to the second administration of labeled uric acid, which were five and nine days, respectively.

The reduction of serum urate concentrations in S. N. and the expansion of urate space may be attributed in part to sodium and fluid retention (19, 20). During phenylbutazone ingestion, S. N.'s weight increased from 51.4 to 53.0 kg., and her hematocrit declined from 41 to 37 per cent. No such observations were made on J. W.

Toxic effects of phenylbutazone

There were no toxic effects of phenylbutazone in S. N. In J. W. gastric irritation and a pruritic maculo-papular eruption of the hands and feet,

together with slight fever, occurred on the third day and persisted throughout the study.

DISCUSSION

In previous studies (16) of the miscible pool of uric acid and the rate of its turnover, it has been shown that good straight lines were obtained if the logarithm of the abundance of N^{15} in urinary uric acid were plotted against time, after intravenous injection of small amounts of uric acid-N^{15}. This finding has been interpreted as supporting the assumptions made in the derivation of the expression

$$Kt = \ln (I_o/I)$$

where I_o is the isotope concentration in body uric acid at the moment of mixing, I the isotope concentration at time, t, thereafter, and K the fraction of the miscible pool replaced by unlabeled uric acid per unit time. On a semilogarithmic plot, $K = -$ slope and $\ln I_o =$ intercept. The antilogarithm of the intercept is used to calculate the size of the miscible pool, A, by the conventional isotope dilution equation (21)

$$A = a(I_1/I_o - 1)$$

where a is the quantity of uric acid injected and I_1 its isotope concentration. The product KA is the quantity of unlabeled uric acid entering the miscible pool per unit time.

In the normal subject on a purine-free diet, it is assumed that dilution of isotope within the miscible pool of uric acid occurs solely as a consequence of the ingress of newly synthesized unlabeled urate molecules. In gouty subjects, however, this decline of isotope abundance also reflects the entrance into the miscible pool of unlabeled molecules derived from solid urate deposits (22) and for this reason, K, obtained from treatment of isotope data in such subjects, no longer can be considered exclusively a rate constant of urate biosynthesis. For this reason, since the rate of synthesis of uric acid was a subject of investigation in the present study, gouty patients could not be employed, and observations were made on normal persons instead.

The results of the urate turnover studies in these two subjects give no support to the hypothesis (8) that phenylbutazone may suppress the rate of urate synthesis. Furthermore, such a postulate was unnecessary in these subjects since the contractures of the miscible pool due to the action of phenylbutazone were entirely attributable to augmented urinary excretion. Nor was any evidence procured favoring increased extra-renal excretion or enhanced catabolism of urate, for the recoveries of labeled uric acid in urine were actually greater during phenylbutazone ingestion than prior to it. Also, the percentage of the urate turnover appearing as urinary uric acid remained unchanged in one subject and was decidedly increased in the other (dev./p.e. = 1.96). It is probable that the rates of extra-renal disposal and catabolism of urate were reduced due to the diminution of serum urate concentration effected by phenylbutazone.

The theoretical uric acid space increased by about 5 liters in each subject during the interims between infusions of labeled urate. This was a 21 per cent expansion in J. W. and a 32 per cent expansion in S. N. In neither case was this change sufficient to explain more than a small portion of the suppression of serum urate concentration observed. The origin of this expansion of urate space is uncertain. In subject S. N. at least part of it would appear to be due to expansion of extracellular fluid volume, judging from the weight gain and drop of hematocrit. The percentage of body water initially occupied by the uric acid space, as calculated for these two subjects, is in very close agreement with the same values as recalculated from Benedict and co-workers (16, 22), which ranged from 46.8 to 55.1 per cent of total body water in four normal male subjects.

One further finding deserves comment. In subject J. W. a total of 1630 mg. of "extra" uric acid was excreted during nine days of phenylbutazone administration, a quantity larger than the original miscible pool which clearly cannot have been its sole source. Bishop, Rand, and Talbott (23) noted the same finding in two hyperuricemic subjects, one of them gouty, who were given Benemid®. In these subjects one might attribute the extra uric acid to mobilization of deposits not included within the miscible pool, but this seems an unlikely explanation in a normal subject. The author (24) has not observed continued excretion of "extra" uric acid in other normouricemic subjects given Benemid® or phenylbutazone. A more

probable explanation for the observation made on J. W. is that the rate of urate biosynthesis increased during the later days of phenylbutazone ingestion, as a consequence of the increased rate of metabolism accompanying fever. Taken in their entirety, the results procured in these subjects favor the view that phenylbutazone has only one important direct action upon uric acid metabolism, *viz.*, the enhancement of urinary urate excretion.

Yü, Sirota, and Gutman (6) have concluded that phenylbutazone is a uricosuric drug which depresses tubular reabsorption of urate. Uricosuria occurred in all of 10 gouty subjects given phenylbutazone intravenously, and the magnitude of this effect appeared to correlate with dosage. C urate/C inulin × 100 rose from a mean control of 6.04 to 19.5 within two hours; Tm_{PAH} was markedly suppressed in two subjects so studied. Seven of eight gouty subjects given phenylbutazone orally in dosages of 800 mg. per day showed increased urinary urate excretion. All exhibited a decrease in plasma urate concentration. In three an apparent inconsistency was observed between the degree of uricosuria and the decline in plasma urate concentration. In two of these there was a distinct fall in plasma urate concentration before the urinary urate excretion was appreciably increased; in one of these two, expansion of extracellular fluid volume consequent upon retention of salt and water was demonstrated. In the third subject, a small uricosuric effect lasted only one day, but the plasma urate concentration declined to 4.6 mg. per cent on the fourth day, the control value being 8.1 mg. per cent. Smyth has also found phenylbutazone a uricosuric drug in gouty subjects (4), as have Bishop and Beecher (7).

Kidd, Boyce, and Freyberg (5) did not find a significant increase in urinary urate excretion in the first 24 or 48 hours after the administration of 400 to 800 mg. of phenylbutazone to nine gouty subjects on uncontrolled diets, even though 12 of 15 patients showed a significant decrease in serum urate level. Johnson and co-workers (8) found a decrease in uric acid output in two gouty subjects on constant diets given 1 gm. of phenylbutazone daily, and failed to find increases in urinary urate in other gouty subjects in whom reductions of serum urate concentrations were observed. The analytic methods employed in both of these studies are not specific for uric acid, and possess inherent limitations which compromise the interpretation of results obtained with them (25, 26). Excretion of urinary chromogens affecting these methods may have been altered in such a manner as to obscure the actual uric acid changes.

It is apparent from Figure 2 that a highly specific and sensitive method for urate determination and rigorous consistency of purine intake are necessary to evaluate changes in urate excretion that may occur during phenylbutazone therapy. Seemingly minor changes may be adequate to explain the serum urate suppression, as this case demonstrates. Also, the sodium and fluid retention caused by phenylbutazone (19, 20) may lead to dilution of plasma urate and a considerable reduction in plasma urate concentration. It seems not improbable that a combination of non-specific analytical methods, inconstancy of dietary purine contribution to urinary urate excretion, and lack of recognition of extracellular fluid volume expansion may account for the observations which failed to demonstrate a correlation between decline of serum urate concentration and appearance of extra urinary urate.

SUMMARY

Uric acid-N^{15} has been administered to two normal subjects on purine-free diets during control periods and again during phenylbutazone ingestion. From a comparison of the magnitudes of contracture of the miscible pools and the cumulative increments in urinary urate excretions, it has been concluded that the uricosuric effect of phenylbutazone adequately explains the observed suppressions of serum urate concentrations. The rates of urate biosynthesis did not change significantly during the periods of study. Also, extra-renal disposal and catabolism of urates as measured by urinary recovery of N^{15} in uric acid were reduced in both subjects, or as measured by the percentage of the urate turnover appearing as urinary uric acid, were unchanged in one and reduced in the other subject. These changes were probably secondary to the marked reduction in serum urate concentration. The uric acid space increased by about 5 L. in both subjects, but this expansion explained only a small portion of the

suppression of serum urate concentration; a part of this increase was attributed in one subject to retention of fluid and expansion of extracellular fluid volume. Taken in their entirety, the results of this study indicate that phenylbutazone has only one important effect on uric acid metabolism in normal subjects, *viz.*, the enhancement of urinary urate excretion.

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