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Paradoxical Retention of Uric Acid by Uricosuric Drugs in Low Dosage.* (22094)

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It has long been known that the renal excretion of uric acid in man is increased by large doses of salicylate and suppressed by small doses(1,2). Recent, more adequately documented studies(3) showed that acetylsalicylic acid in 5.2 g daily oral dosage caused a mean increase of 37.2% in the 24-hour urinary uric acid output of 15 subjects, with a concomitant mean fall of 25.5% in plasma uric acid, whereas 2.0 g daily dosage resulted in a mean decline of 14.4% in the urinary excretion and 7.5% rise in the plasma concentration of uric acid. Renal clearance studies indicate that this reversible effect is related to the concentration of salicylate in blood plasma(4) or, more precisely, in the urine(3); high salicylate concentrations cause an increase in the renal clearance ratio, $C_{\text{uric acid}}/C_{\text{inulin}}$, low salicylate levels depress this ratio.

Our data illustrate these relationships in respect to salicylate and indicate further that the reversibility of effect on uric acid clearance can be demonstrated, by appropriately designed experiments, to occur with all other uricosuric drugs we have examined, namely, phenylbutazone, phenylbutazone analogs, and probenecid. It would appear that the capacity to cause uric acid retention when given in low dosage is, to greater or lesser degree, a property common to uricosuric agents. The observation may have implications of interest in relation to the still obscure nature of renal tubular transport systems for uric acid.

Methods. The experiments were performed in gouty subjects in the intercritical phase of the disorder. Standard renal clearance technics(5) were employed throughout, with minor modifications described elsewhere(6). The general experimental design was to obtain 3 or 4 pre-medication urine collection periods for estimation of the inulin and PAH clearances, then to infuse the drug to be studied at a slow rate yielding low initial plasma drug concentrations, and gradually to increase the rate of drug infusion to attain uricosuric plasma drug levels. The 6-10 urine collection (catheterization) periods obtained during infusion of the drug in each experiment were each of 10-30 minutes duration; urine samples (spontaneous voidings) collected in some instances after termination of the drug infusion represented 2-hour intervals during the day and 4-hour intervals at night. Blood samples were procured at the midpoint of each urine collection period. In the salicylate infusion experiments, 4 g sodium salicylate dissolved in 500 ml isotonic saline solution was injected at a rate of 12-15 mg salicylate/min. for one or more periods, with an increment of 5-10 mg/min. in each urine collection period thereafter. In 2 phenylbutazone experiments, the infusing solution contained 2 g phenylbutazone/250 ml isotonic saline solution, was delivered at a rate of 12 mg/min. for 2 clearance periods and at double the rate thereafter. In the 3 remaining phenylbutazone, the 2 G-25671 and 6 of the 8 phenylbut-

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TABLE I. Representative Experiments Showing Effect of Slow Infusion of Sodium Salicylate, Phenylbutazone and Probenecid on Uric Acid Clearance.

Case	Period (min.)	Rate of drug infusion (mg/min.)	Plasma drug conc. (mg %)	Plasma uric acid (mg %)	$C_{uric\ acid}$ (ml/min.)	$\frac{C_{uric\ acid}}{C_{inulin}}$ (%)
J. S.	Control	0	0	11.5	13.9	10.7
	"	0	0	11.5	14.9	11.7
	"	0	0	11.5	14.4	11.2
		Sodium salicylate infusion (4 g/500 ml saline sol.)				
	15	12	Trace	11.4	13.9	10.7
	13	12	1.5	11.3	7.95	6.36
	11	17	3.1	10.9	7.08	5.66
	11	20	5.8	10.8	9.58	7.92
	12	25	9.0	10.9	13.4	11.4
	12	29	12.0	10.9	18.0	14.4
	12	34	15.0	10.8	21.6	18.6
	10	40	17.1	10.6	27.2	23.6
	11	45	21.0	10.7	31.5	26.5
L. R.	Control	0	0	9.1	8.05	8.47
	"	0	0	9.1	8.09	8.00
	"	0	0	9.2	8.02	8.41
		Phenylbutazone infusion (2 g/250 ml saline sol.)				
	15	12	2.3	9.5	6.15	6.73
	16	12	3.0	9.4	6.04	6.65
	15	24	10.0	9.5	10.4	10.6
	15	24	12.0	9.5	12.7	14.3
	15	24	15.5	9.3	13.9	18.0
	10	24	16.7	9.0	24.4	23.4
R. G.	Control	0	0	7.3	8.73	6.35
	"	0	0	7.2	9.14	5.53
	"	0	0	7.0	8.07	6.52
		Probenecid infusion (0.1 g/500 ml 5% glucose sol.)				
	15	.2		7.4	8.07	5.08
	15	.2		7.6	5.46	4.48
	19	.2		7.2	6.15	4.70
	16	.4		7.0	7.17	5.48
	16	.4		6.9	7.63	5.26
	13	.4		7.0	8.03	5.11
	19	.6		7.1	7.42	5.30
	20	.6		7.0	8.45	6.12
	23	1.2		7.0	10.6	6.80
	17	1.2		7.0	12.0	8.17

azone metabolite 1 experiments, the initial infusion rate was 1.4 mg/min. with an increase of 1.4-2.0 mg/min. in each following collection period. In 2 of the metabolite 1 experiments cited the drug was infused rapidly in a total dosage of 8 mg/kg body weight. The details of the probenecid injections are given in the text. Uric acid was determined by a modification of the method of Buchanan *et al.* (7) incorporating the use of uricase, urea cyanide-carbonate and arseno-phosphotungstic acid, inulin by the Schreiner method(8), creatinine by the method of Bonsnes and Taussky(9), salicylate by the method of Brodie *et al.*(10). For the plasma phenylbutazone, G-25671 and metabolite 1 determinations we are indebted to Dr. J. J. Burns.

Results. Salicylate. Rapid infusion of sodium salicylate at a rate sufficient to maintain plasma total salicylate concentrations at 15-34 mg % effected an increase in $C_{uric\ acid}/C_{inulin}$ from a mean of 5.5% in the control clearance periods to a mean of 20.6% in 6 subjects so studied(3). The experiment included in Table I (Case J.S.) illustrates the effects on $C_{uric\ acid}/C_{inulin}$ of a slow, sustained infusion of sodium salicylate delivered at a rate permitting gradual increase of plasma total salicylate from trace levels to 21 mg %. In the 3 control periods, $C_{uric\ acid}/C_{inulin}$ varied from 10.7 to 11.7%, and remained within this range in the initial period of the infusion. At a

TABLE II. Summary of Slow Infusion Experiments with Uricosuric Drugs, Showing Maximal Suppression of Uric Acid Clearance at Low Plasma Drug Concentrations.

	Case	Drug infused	Plasma drug conc. (mg %)	$C_{uric\ acid}/C_{inulin}$ Control (%)	$C_{uric\ acid}/C_{inulin}$ Minimum (%)	Change (%)
1.	M. L.	Salicylate	2.2	7.22 ± .29	3.38	−53.2
2.	J. S.		2.8	11.2 ± .41	5.66	−49.5
3.	S. K.		4.7	5.14 ± .14	2.80	−45.6
4.	M. M.		1.8	5.48 ± .33	3.10	−43.5
5.	M. S.		4.1	6.40 ± .72	4.11	−35.8
6.	J. B.		8.4	5.16 ± .21	4.38	−15.8
1.	L. O.	Phenylbutazone	2.1	5.73 ± .15	3.48	−39.3
2.	N. P.		4.0	1.87 ± .24	1.22	−34.8
3.	J. S.		2.6	5.24 ± .55	4.20	−19.8
4.	L. R.		3.0	8.30 ± .46	6.65	−18.9
5.	J. B.		1.3	5.15 ± .29	4.72	+ 8.4
1.	M. G.	Metabolite 1	.5	4.97 ± .31	3.34	−32.8
2.	J. Z.		3.6	4.72 ± .25	3.22	−31.8
3.	G. H.		7.0	3.79 ± .13	2.85	−24.8
4.	M. R.		5.9	6.60 ± .17	5.15	−22.0
5.	H. B.		4.3	4.92 ± .35	3.90	−20.7
6.	J. B.		1.3	8.94 ± .14	6.99	−20.7
7.	N. K.		5.0	3.72 ± .33	3.05	−18.0
8.	I. S.		.4	6.98 ± .17	7.67	+10.0

plasma total salicylate concentration of 1.5 mg % $C_{uric\ acid}/C_{inulin}$ was depressed to 6.36%, and persisted well below the premedication ratio until the plasma total salicylate reached 9 mg %, at which point $C_{uric\ acid}/C_{inulin}$ returned to the control limits. At plasma total salicylate levels of 12 mg %, $C_{uric\ acid}/C_{inulin}$ exceeded the premedication ratios and continued to rise with further augmentation of the plasma drug concentration, ultimately reaching 26.5% at a plasma total salicylate level of 21 mg %. In Table II, the pertinent results of 6 such salicylate infusion experiments are recorded. It will be noted that a (transitory) decline in $C_{uric\ acid}/C_{inulin}$ occurred in each instance, the peak decline varying from 53.2-15.8% of the mean premedication figures. In five of these subjects the decline exceeded 30%.

Fig. 1 illustrates an experiment (Case M.L.) in which the plasma total salicylate again was slowly raised by infusion of sodium salicylate, the infusion was then discontinued, and the plasma drug concentration decay curve followed. It will be noted that $C_{uric\ acid}/C_{inulin}$ was depressed at low plasma total salicylate levels in both the initial ascending and terminal descending segments of the curve representing plasma drug concentrations. Distinct uricosuria occurred in the intervening period of plasma total salicylate levels in excess of 10 mg %.

Phenylbutazone. Rapid infusion of phenylbutazone in 10 subjects consistently caused a rise in $C_{uric\ acid}/C_{inulin}$, from a mean control value of 6.0% to a mean peak of 19.5%(11). The effects of a slow, sustained infusion are illustrated in Table I (Case L.R.). $C_{uric\ acid}/C_{inulin}$ fell initially, from a mean control figure of 8.3% to 6.7% at low plasma phenylbutazone concentrations, then rose to uricosuric levels as the plasma drug concentration exceeded 10 mg %. The results of 5 such experiments are summarized in Table II; in 4 a transitory decline in $C_{uric\ acid}/C_{inulin}$ was detected, the peak decline in 2 instances exceeding 30%. Unlike the experience with salicylate, observations for 24-72 hours following termination of infusion of phenylbutazone(11) failed to reveal any post-medication suppression of $C_{uric\ acid}/C_{inulin}$. This is attributed to the slow rate of biotransformation of phenylbutazone (approximately 20%/day)(12) and the consequent persistence of high plasma and urinary drug concentrations(11).

Phenylbutazone analogs. Phenylbutazone metabolite 1 (p-hydroxyphenylbutazone) (13), which exhibits uricosuric activity approximating that of phenylbutazone in equiv-

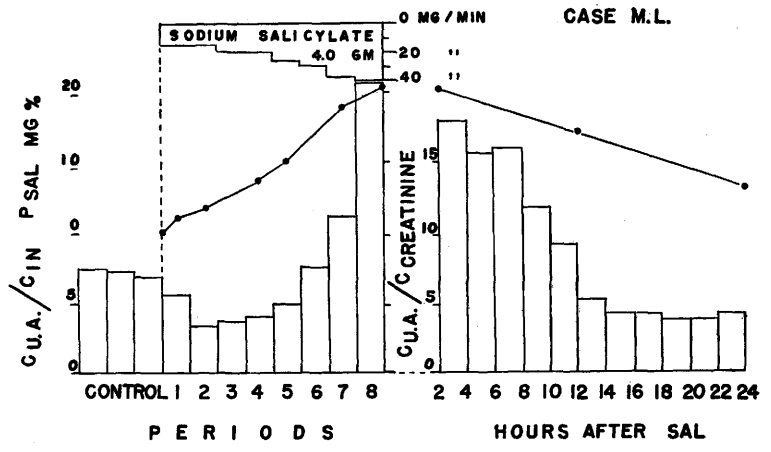

FIG. 1. After 3 control periods, sodium salicylate was infused slowly and plasma total salicylate level gradually raised (linear curve, top). Infusion was discontinued after eight 20-min. periods. Effects of rising and falling plasma drug levels on $C_{uric\ acid}/C_{inulin}$, respectively, $C_{uric\ acid}/C_{creatinine}$ are indicated by the bars (see text).

alent dosage, at low plasma drug levels caused a transitory decline in $C_{uric\ acid}/C_{inulin}$ in 7 of 8 experiments (Table II). In 2 instances the decline exceeded 30%. G-25671, a phenylbutazone derivative in which the butyl side chain is replaced by a phenylthioethyl group, is a more potent uricosuric agent(12). In 7 rapid infusion experiments, G-25671 caused a mean 5-fold increase in $C_{uric\ acid}/C_{inulin}$, from a mean pre-medication value of 6.15% to a mean peak of 30.6%(14). In 2 slow infusion experiments, a transient, moderate decline in $C_{uric\ acid}/C_{inulin}$, reaching −16.1% at its peak, was observed at a plasma G-25671 level of 0.6 mg %; at plasma drug levels of 1.0 mg % uricosuria was already in evidence.

Probenecid. Suppression of $C_{uric\ acid}/C_{inulin}$ could be demonstrated with this potent uricosuric agent(6) only by slow infusion of very small quantities of the drug. Injection at the rate of 0.2 mg probenecid/min. gave a maximum decline in $C_{uric\ acid}/C_{inulin}$ of 27% below the mean ratio of the control period (Case R.G., Table I); at 0.3 mg probenecid/min., the maximum decline was 14.6%; at 0.4 mg/min. suppression of uric acid clearance was no longer demonstrable; and at higher drug infusion rates uricosuria set in promptly.

Discussion. The observations herein recorded may explain, in part, some of the conflicting results reported in the literature, notably in respect to phenylbutazone. This drug has been found by most investigators to increase urinary excretion of uric acid in ordinary therapeutic dosages by mouth, but by others to have no effect or, indeed, to cause retention of uric acid. This confusion is compounded by the fact that phenylbutazone consistently lowers the serum uric acid level even in instances without uricosuria. This discrepancy has been interpreted to imply suppression of uric acid formation by phenylbutazone but direct measurement of the rate of uric acid biosynthesis, using N^{15}-labeled glycine in subjects receiving the drug, fails to support this view(15). The evidence now available indicates that such discrepant declines in serum uric acid are apt to be factitious, reflecting hemodilution due to renal retention of sodium and water caused by phenylbutazone(11,14). Further clarification of this relationship has been achieved by renal clearance studies, including those reported here, which reveal the sequence of renal effects at rising plasma phenylbutazone levels, corresponding to various dosages of phenylbutazone. Appreciable retention of salt and water may begin in association with plasma phenylbutazone levels as low as 1 mg % and continues at higher drug levels(11,14); suppres-

sion of uric acid clearance occurs within an intermediate range of plasma phenylbutazone concentration; and distinct uricosuria begins at plasma phenylbutazone concentrations of about 10 mg %, becoming more marked at higher drug levels(11,14,16). These observations explain how, without suppression of uric acid biosynthesis, administration of phenylbutazone may cause lowering of the serum uric acid level without uricosuria, indeed in the face of uric acid retention.

Discontinuance of salicylate, probenecid and other uricosuric drugs is usually followed by one or 2 days of uric acid retention. This phenomenon, hitherto regarded as "compensatory," would now appear to reflect decline of the plasma drug concentration to the low levels associated with depression of the uric acid clearance. In this connection it should be pointed out that the concentration of drug in the tubular urine is probably more critical than that in plasma, in respect to the reversible action on $C_{\text{uric acid}}/C_{\text{inulin}}$. To be sure, in ordinary circumstances, as in the experiments here recorded, the urinary drug concentration may be presumed to be a function of the plasma drug level but when the two are dissociated—as by concomitant administration of salicylate and sodium bicarbonate—it is found that the determining factor in regulating uric acid clearance is the drug concentration in urine(3,17). The point may be significant in respect to the immediate mechanisms involved.

The uricosuric effects of salicylate, probenecid, phenylbutazone and phenylbutazone derivatives are all ascribable to suppression of tubular reabsorption of uric acid, since clearance studies uniformly show an associated distinct *rise* in $C_{\text{urate}}/C_{\text{inulin}}$ in each instance(4,6,11,14). Conversely, the clearance data of this paper reveal that the uric acid retention produced by these same drugs in low dosage is uniformly referable to a distinct *fall* in $C_{\text{urate}}/C_{\text{inulin}}$. This paradoxical reversibility of effects presumably is related to some characteristic of the tubular transport systems for uric acid, since there is no indication of significant changes in filtered load, *i.e.*, in the filtrability of plasma uric acid(18) or in the glomerular filtration rate. The nature of this characteristic must, for the present, be a matter of speculation. It has already been suggested that a single system for tubular reabsorption of uric acid, presumed to be enzymatic, may be stimulated by low concentrations and inhibited by high concentrations of the same drug(4). Another possibility, not excluded by the evidence at hand, is that 2 more or less distinct systems of tubular transport of uric acid are involved. One of these, of very limited capacity, might be concerned with tubular excretion of uric acid and inhibited by low (and high) drug concentrations; the other, of considerably greater capacity, is clearly responsible for tubular reabsorption of uric acid and evidently is suppressed only by high drug concentrations.

Summary. Salicylate, phenylbutazone, certain phenylbutazone derivatives and probenecid, all of which in high plasma drug concentrations increase the uric acid clearance, are demonstrated by appropriate renal clearance technics to depress the uric acid clearance at low plasma drug concentrations. The significance of this paradoxical reversibility of effects is discussed in relation to controversial aspects of phenylbutazone uricosuria, the phenomenon of "compensatory" uric acid retention after discontinuance of uricosuric agents, and the nature of tubular transport mechanisms for uric acid.

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Hemolytic Mechanism in Sickle Cell-Hgb C Disease.* (22095)

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Splenic overactivity has been implicated in the hemolysis observed in many of the acquired hemolytic syndromes. The role of the spleen in the red cell destruction characteristic of the hereditary hemolytic anemias, however, has not been clearly defined. The available data suggest basically different mechanisms for the pathogenesis of the increased hemolysis in 2 of the hereditary hemolytic syndromes. In sickle cell anemia (homozygous Hgb S disease) the frequent development of an atrophic spleen, the so-called autosplenectomy, implies that the spleen cannot play an active role in red cell destruction. Furthermore, splenectomy in cases of uncomplicated sickle cell anemia neither leads to hematologic improvement nor results in a prolongation of the lifespan of the erythrocyte. Thus, sickle cell anemia erythrocytes transfused into a splenectomized sickle cell anemia patient still showed a markedly reduced lifespan(1). In hereditary spherocytosis, however, the evidence suggests that the increased destruction of the red cells may be mediated through the presence of a normal spleen, since hereditary spherocytosis erythrocytes transfused into a normal individual lacking a spleen have a normal survival time whereas the same cells given to a normal individual with a spleen demonstrate a sharply reduced lifespan(2). This situation exists irrespective of the pre- or post-splenectomy state of the donor with hereditary spherocytosis, or his hematologic status at the time of study. In view of these considerations, it seemed of some interest to perform similar studies on patients with sickle cell-Hgb C disease. Sickle cell-Hgb C disease is characterized by splenomegaly and signs of hemolysis which appear to vary in degree intermittently during the course of the disease. Because splenomegaly often becomes evident at times of hemolytic crises in sickle cell-Hgb C disease, there is reason to suspect that enlargement of the spleen may lead to accelerated rates of red cell destruction. By defining the part played by the spleen in the hemolysis of this disease, it was hoped that an evaluation of the effects of splenectomy and the possible use of this operation as a therapeutic procedure might thereby be made.

Material and methods. Three patients with the diagnosis of sickle cell-Hgb C disease, confirmed by electrophoretic analysis of their hemoglobin types, were used as donors. All were in a relatively asymptomatic state. The spleens of 2 of the donors were palpable just below the costal margin. From one patient, A. F., two 500 ml aliquots of blood were obtained and transfused into a normal individual with a spleen and into a hematologically normal splenectomized recipient who had

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