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The Renal Excretion of Oxypurines *

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The mechanism whereby oxypurines (xanthine and hypoxanthine) are excreted by the human kidney is relevant to several areas of current investigation. The use of the xanthine oxidase inhibitor allopurinol [1] (4-hydroxypyrazolo (3, 4-d)-pyrimidine) as an adjunct to cancer chemotherapy (1) and in the treatment of hyperuricemia associated with gout (2, 3) results in the accumulation of oxypurines that would normally be degraded to uric acid. The fate of these metabolites is pertinent to the clinical use of such an agent. Study of oxypurine clearance by the normal kidney is also of considerable importance in helping to define the precise derangement of oxypurine metabolism in patients with xanthinuria (4). Dickinson and Smellie have proposed (5) that along with deficient activity of the enzyme xanthine oxidase, these patients may have a defect in the reabsorption of xanthine by the renal tubule to account for the high clearance ratio of oxypurine to creatinine found in their patient. Accordingly, a "combined renal and general metabolic defect" has been postulated to occur. By examining the renal clearance of oxypurines in normal subjects at serum concentrations approximating those of a xanthinuric patient, this hypothesis can be adequately tested.

In the present study, the renal clearance of oxypurines was examined in gouty subjects without renal disease, as well as in one patient with xanthinuria and one normal volunteer. Serum concentrations of oxypurines were augmented by concomitant infusions of oxypurines as well as by allopurinol administration. In addition, the effect of probenecid, sulfinpyrazone, and salicylate on oxypurine excretion was determined by measuring the influence of these agents on the daily urinary excretion of oxypurines in a patient with xanthinuria and in a patient receiving allopurinol.

Methods

All studies were performed while subjects were maintained on diets essentially free of purines at the Clinical Center of the National Institutes of Health. Methylated purines in particular were avoided since they interfere with the method used for the determination of oxypurines in the urine (6). Renal clearance studies were carried out on fasting individuals by standard procedures (7), with concomitant inulin infusions providing an index for the measurement of glomerular filtration rate. Supplementary water was ingested to insure urine volumes of 150 to 400 ml per period. Spontaneous voiding at 30 minutes by the properly trained subject ended each collection period. Mid-period blood specimens were collected in heparinized syringes, placed on ice immediately, and centrifuged within 30 minutes of withdrawal.

Two methods were employed to elevate the normally low serum concentrations of oxypurines when this was desired. One consisted of the prolonged administration of allopurinol in divided doses of 400 to 800 mg daily. The effectiveness of allopurinol in preventing oxypurine degradation to uric acid is presented elsewhere (1–3). The second manner by which oxypurine concentrations were increased was by infusions of xanthine or hypoxanthine. Sodium xanthine [2] and hypoxanthine,[3] both over 98% pure, were dissolved in isotonic saline at a concentration of 2 mg per ml with warming, and sterilized by filtration. The product was tested for sterility and pyrogens. Crystals that formed during storage at room temperature were dissolved by warming the solution before use. A priming dose of 100 mg of xanthine or hypoxanthine in a total volume of 100 ml was followed by infusion of the same oxypurine at a constant rate of 2 mg per minute.

Plasma and urinary inulin concentrations were determined by the method of Walser, Davidson, and Orloff (8). An enzymatic spectrophotometric assay for uric acid was employed (9). Urinary oxypurine concentra-

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[1] Zyloprim, Burroughs, Wellcome & Co., Tuckahoe, N. Y.

[2] Mann Research Laboratories, Inc., New York, N. Y.

[3] Sigma Chemical Co., St. Louis, Mo.

TABLE I

Renal clearance of oxypurine and urate relative to inulin in gouty subjects on allopurinol, 400 to 800 mg per day, as compared to a patient with xanthinuria*

Subject	Plasma oxypurine	Plasma urate	C_{ox}	C_U	C_{In}	C_{ox}/C_{In}	C_U/C_{In}	C_{ox}/C_U
	mg/100 ml	*mg/100 ml*	*ml/min*	*ml/min*	*ml/min*	*ml/min*		
Gout								
E.T.	0.30	2.12	82.9	13.6	112.5	0.74	0.12	6.1
R.L.	0.40	1.88	43.7	4.3	55.0	0.79	0.08	10.2
T.J.†	0.68	2.03	106.5	7.9	87.5	1.22	0.09	13.5
F.J.†	0.71	3.89	93.7	5.6	81.0	1.16	0.07	16.7
					C_{Cr}	C_{ox}/C_{Cr}	C_U/C_{Cr}	C_{ox}/C_U
Xanthinuria (12)								
V.P.	0.5	0.3	45.3	1.8	52.8	0.87	0.03	25.2

* The figures for each clearance study represent an average of at least three 30-minute periods. C_{ox} = oxypurine clearance; C_U = urate clearance; C_{In} = inulin clearance; C_{Cr} = creatinine clearance.
† Known "overproducers" of uric acid.

tions were determined spectrophotometrically by use of a Beckman model DU spectrophotometer. The changes in optical density were measured at 292 mμ after addition of first xanthine oxidase and then uricase to an appropriate sample brought to a volume of 3.0 ml in 0.1 M pyrophosphate buffer pH 8.0 (6). Plasma oxypurines, when sufficiently elevated by the infusion of xanthine, were determined by measuring the change in optical density at 292 mμ after addition of xanthine oxidase. When the plasma oxypurine concentration was too low to be measured accurately by this direct procedure, a modification was devised whereby the dialyzate of plasma was lyophilized and dissolved in a small volume of the pyrophosphate buffer. After being treated with uricase to destroy preformed uric acid, 3.0 ml was added directly to cuvettes for enzymatic assay (6). Final values were expressed in terms of uric acid derived from oxypurines and not fractionated into relative amounts of xanthine and hypoxanthine. Although such fractionation is theoretically possible, as based on the different molar extinction coefficients of xanthine, hypoxanthine, and uric acid, this could not be achieved with sufficient accuracy to warrant use for calculations of their independent renal clearance. During hypoxanthine infusion studies, some conversion to xanthine occurred, for xanthine accounted for 15 to 50% of excreted oxypurines. The calculation of renal clearance in terms of total oxypurine content of plasma and urine (as expressed in terms of uric acid derived from oxypurines) is valid in this situation.

TABLE II

Oxypurine clearance relative to inulin during infusion studies

Subject	Plasma oxypurine concentration	C_{ox}/C_{In}
	*mg/100 ml**	
Endogenous oxypurine concentrations		
W.R.	0.21	0.15
J.H.†	.13	.03
F.J.	.08	.18
R.J.	.12	.28
During xanthine infusion		
W.R.	.48	.43
W.R. on allopurinol	.70	.61
F.J.	.24	1.16
E.T.	.16	1.01
During hypoxanthine infusion		
W.R.	.32	.69
W.R. on allopurinol	.40	.84
J.H.†	.19	1.33
J.H. on allopurinol	.39	1.26
R.J.	.22	1.29
R.J. on allopurinol	.77	1.49

* Milligrams uric acid derived from oxypurines.
† Normal volunteer; remainder of subjects have gout without overt renal disease.

Pharmacological agents known to alter urate excretion were administered to a gouty subject receiving allopurinol and to a xanthinuric patient in order to study their effect on daily oxypurine excretion. Each 24-hour urine collection was stored in the cold with 3.0 ml of added toluene. After an initial control period, divided oral doses of salicylate (1.2 to 4.8 g per day), probenecid (1 to 3 g per day), and sulfinpyrazone (800 mg per day), either alone or in combination, were administered.

Results

Table I shows the simultaneous renal clearance rates of urate, oxypurines, and inulin in four gouty patients receiving allopurinol. The highest plasma oxypurine concentrations were found in two brothers (T.J. and F.J.), who were shown to be overproducers of uric acid by excretion studies (10) and by incorporation of glycine

1-C^{14} into urinary uric acid (11). The rate of clearance of oxypurines was far greater than that of uric acid. In fact, the oxypurine clearance exceeded the inulin clearance in the two patients (T.J. and F.J.) who had the highest plasma oxypurine concentrations.

The renal clearance of endogenously derived oxypurines (Ox), urate (U), and creatinine (Cr) in a xanthinuric patient has been previously reported (12) and is presented in the same Table. If we assume that the endogenous creatinine clearance approximates inulin (In) clearance in the absence of frank renal failure (13), the clearance ratio for oxypurines (C_{Ox}/C_{Cr}) in the xanthinuric patient is in the same range as those found in gouty patients in whom the plasma oxypurine concentrations had been increased to comparable levels by administration of allopurinol.

Table II compares the endogenous clearance of oxypurines with those obtained when infusions of xanthine and hypoxanthine were administered in order to elevate their plasma concentration.

TABLE III

Effect of oxypurine infusion on the clearance of urate relative to inulin

Subject	C_U/C_{In} Control	Infused oxypurine	C_U/C_{In} during oxypurine infusion	Effect of infusion (per cent change)
R.J.	0.099	Hypoxanthine	0.095	−4
J.H.	0.108	Hypoxanthine	0.107	−1
W.R.	0.028	Hypoxanthine	0.036	+29
W.R.	0.028	Xanthine	0.029	+4
J.F.	0.101	Xanthine	0.117	+16
E.T.	0.096	Xanthine	0.116	+21

Infusion of either xanthine or hypoxanthine resulted in much higher clearances than were obtained at endogenous levels. In some cases (E.T., F.J., J.H., and R.J.) C_{Ox}/C_{In} values exceeding 1.0 occurred at lower plasma concentrations with infusion of oxypurine than were observed with administration of allopurinol. However, in three instances when allopurinol was administered during an oxypurine infusion study (W.R. ×2, and R.J.), it resulted in further augmentation of oxy-

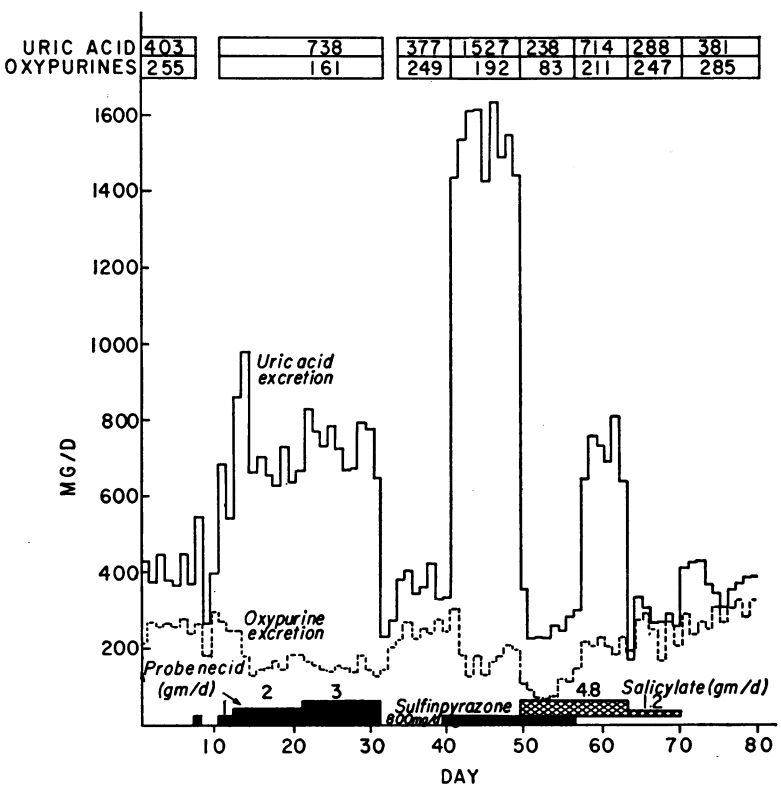

FIG. 1. EFFECT OF URICOSURIC AGENTS ON OXYPURINE EXCRETION IN A PATIENT WITH TOPHACEOUS GOUT RECEIVING ALLOPURINOL, 800 MG PER DAY.

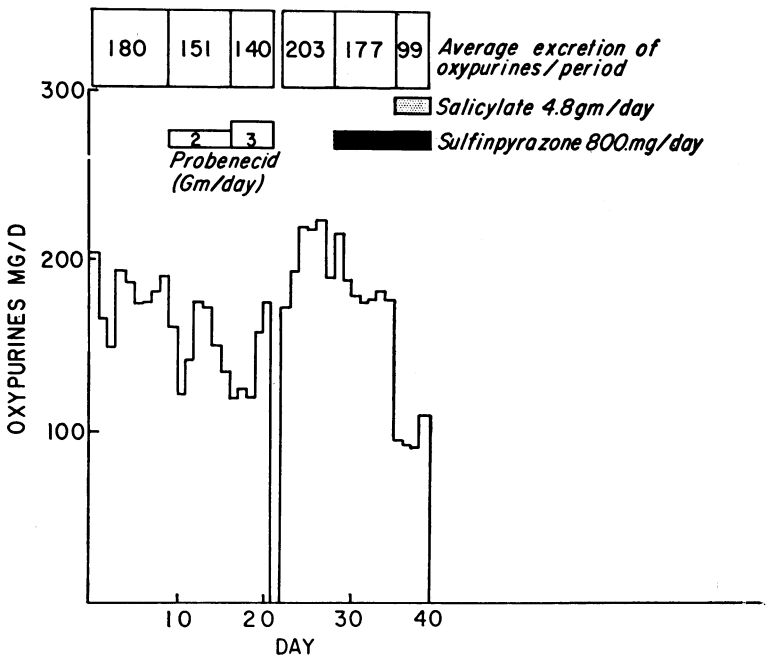

FIG. 2. EFFECT OF URICOSURIC AGENTS ON OXYPURINE EXCRETION IN A PATIENT WITH XANTHINURIA.

purine clearance, probably by promoting further increases in the plasma concentration of the infused material. Of a total of six oxypurine infusion studies, urate clearance increased significantly during three (Table III).

The effect of probenecid, sulfinpyrazone, and salicylate administration on the urate and oxypurine excretion of a gouty subject treated with allopurinol is shown in Figure 1. Probenecid and sulfinpyrazone produced a moderate decrease in oxypurine excretion concomitant with their expected uricosuric action. When salicylate, 4.8 g per day, was added to the sulfinpyrazone regimen, there was significant urate retention, in keeping with previous observations (14). At the same time, oxypurine excretion was significantly reduced to the lowest values encountered during the study. Subsequent withdrawal of sulfinpyrazone unveiled the known uricosuric action of large doses of salicylate, and at this time, oxypurine excretion rose to levels approximating those seen with the other uricosuric drugs. Low doses of salicylate that produced urate retention did not significantly alter oxypurine excretion when compared to the final control period.

The effect of the same drugs on the excretion of oxypurines by a patient with xanthinuria is shown in Figure 2. Probenecid and sulfinpyrazone again produced a depression of oxypurine excretion, but as before, the most striking reduction resulted from the combination of sulfinpyrazone and salicylate. Urate excretion by this patient, which averaged less than 10 mg per 24 hours, was too variable at these low levels to be demonstrably altered by the drugs.

Discussion

Despite the structural similarities of the oxypurines xanthine and hypoxanthine to uric acid, the dynamics of their renal excretion appear to be different. Oxypurine clearance relative to that of inulin greatly exceeded the normal C_U/C_{In} ratio of 0.03–0.12 (10, 15, 16), and in some instances was observed to be greater than 1.0. This would suggest that reabsorption of filtered oxypurines is less efficient and/or that tubular secretion plays a more vigorous role in the excretion of oxypurines as compared to that of uric acid.

The report by Dickinson and Smellie (5) of a xanthinuric patient with a renal clearance of oxypurines that was 85% of creatinine clearance is entirely consistent with our findings (12). Their conclusion that this rapid clearance repre-

sents a defect peculiar to xanthinuria is based on their values for normal subjects of 10 to 20%. Although the data from which this normal range was calculated are not presented in their article, the studies were performed at low endogenous concentrations of plasma oxypurines characteristic of control subjects (17). When we elevated this concentration to the levels found in xanthinuria, a concomitant increase in oxypurine clearance to the same range seen in xanthinuria occurred. Rapid oxypurine clearance was also observed when plasma concentrations were augmented by infusing xanthine or hypoxanthine. This counters the possible objection that clearance values obtained in the presence of a xanthine oxidase inhibitor (allopurinol) are spurious because it may block an analogue of xanthine oxidase postulated to function as a permease for facilitating renal tubular reabsorption (5). Our findings, therefore, do not support the view that xanthinuric patients have a combined renal and general metabolic defect.

When uricosuric drugs were administered in conventional dosage to an allopurinol-treated subject and to one with xanthinuria, oxypurine excretion diminished. The precise manner by which this effect is mediated is unknown. Gutman, Yü, and Berger (18–20) have proposed that in the case of urate excretion, uricosuric agents block the tubular reabsorption of uric acid which, according to their model, normally handles all that is filtered. On the other hand, such agents are considered to interfere primarily with tubular excretion of urate when they "paradoxically" exert a urate-retentive effect when given in low dosage. At present, there is insufficient information available to construct such a bidirectional model for the handling of oxypurines by the renal tubule. Whether uricosuric agents promote renal retention of oxypurines by inhibiting excretion by tubular cells, by providing more reabsorptive sites for oxypurines that had been previously occupied by urate, or via still other mechanisms, cannot be answered on the basis of the evidence presented in this communication. It is noteworthy that probenecid has been shown to exert no influence on oxypurine clearance at endogenous serum oxypurine concentrations (21).

Recent reports have shown that allopurinol is effective in controlling the hyperuricemia of gout (2, 3). Plasma concentrations of urate have decreased by as much as 7 mg per 100 ml; however, the concentration of plasma oxypurines has not exceeded 1 mg per 100 ml. This is doubtless due to the more efficient renal clearance of oxypurines, although a reduction in total purine synthesis has also been suggested (2). In either event, the rapid clearance of oxypurines can be considered to be protective against the danger of their reaching saturation levels in the plasma. Renal lithiasis from xanthine stone formation might be anticipated in patients taking allopurinol chronically since this is a complication of the constitutional lack of xanthine oxidase in xanthinuria. However, no well-documented instances of this complication from allopurinol therapy have been reported to date.

Summary

The renal clearance of oxypurines was measured in humans by standard techniques. Relatively low values were observed at endogenous plasma oxypurine concentrations. When these concentrations were increased, by direct infusion or by allopurinol therapy, oxypurine/inulin clearance ratios increased considerably and at times exceeded 1.0. A patient with xanthinuria cleared oxypurines at a normal rate when allowance was made for her high endogenous plasma oxypurine concentration. Uricosuric agents were found to reduce the renal excretion of oxypurines in a patient with xanthinuria and in a subject who was receiving allopurinol.

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