Relationship between plasma oxipurinol concentrations and xanthine oxidase activity in volunteers dosed with allopurinol

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1 1-methyl xanthine (1-MX) is metabolized exclusively to 1-methyl uric acid (1-MU) by the enzyme xanthine oxidase.

2 The ratio of 1-MU to 1-MX in the urine, following a dose of 50 mg of 1-MX infused intravenously over 20 min, was used to measure the inhibition of xanthine oxidase induced by different doses of allopurinol.

3 Normal volunteers ($n=8$) were given allopurinol 50, 100, 300 and 600 mg daily for 1 week each, in random order and 1 week separated each treatment. Inhibition of xanthine oxidase was assessed twice, on the last 2 days of each treatment week.

4 Steady-state oxipurinol concentrations increased linearly with increasing dose of allopurinol.

5 There was a hyperbolic relationship between the 1-MU/1-MX ratio and plasma oxipurinol concentrations, with an initial steep decline in the ratio which plateaued when plasma oxipurinol was around 4–6 mg l⁻¹. This reduction in the ratio was quickly reversible upon cessation of allopurinol.

6 The 50% and 90% effective inhibitory oxipurinol concentrations, in relation to the 1-MU/1-MX ratio were 1.4 ± 0.46 and 4.08 ± 2.03 mg l⁻¹ respectively.

7 The concentration of oxipurinol required for almost complete inhibition of the enzyme was substantially less than those often observed in clinical practice.

Keywords oxipurinol allopurinol xanthine oxidase dose-concentration-response methyl xanthine

Introduction

Allopurinol is widely used to treat hyperuricaemia associated with gouty arthritis or renal disease and as prophylaxis against acute hyperuricaemia associated with the treatment of malignant diseases. Allopurinol and its major metabolite, oxipurinol, reduce plasma urate by inhibiting the enzyme xanthine oxidase thereby blocking the conversion of hypoxanthine to xanthine and xanthine to uric acid. The rate-limiting step in purine synthesis is catalysed by the enzyme glutamine phosphoribosylpyrophosphate amidotransferase, which is subject

to feedback inhibition from the purine ribonucleonucleotides, adenylic acid and guanylic acid. These products accumulate during allopurinol therapy, due to the salvage function of the enzyme hypoxanthine-guanine phosphoribosyltransferase, thereby reducing the activity of the purine synthetic pathway (Wyngaarden & Holmes, 1979).

Allopurinol therapy is usually monitored by measuring the reduction in plasma urate along with clinical response. However, an accurate method of quantitating the effect of allopurinol

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would be to measure the degree of inhibition of the target enzyme, xanthine oxidase, as urate concentrations are influenced by factors other than xanthine oxidase activity. These factors include renal function, leaching of urate from tophaceous deposits and intake of exogenous purines. There is a need for a better understanding of the relationship between allopurinol dose, oxipurinol concentrations and the effect of the drug on xanthine oxidase activity and plasma urate. In particular, there is a small group of patients who appear not to respond to allopurinol, despite reasonable doses of the drug (Gibson *et al.*, 1983). This study investigates a method for estimating the activity of xanthine oxidase in humans, *in vivo*. This is based upon the 8-hydroxylation of 1-methyl xanthine (1-MX) to its exclusive metabolite 1-methyl uric acid (1-MU) via the enzyme xanthine oxidase (Birkett *et al.*, 1983) for which 1-MX is a good substrate (Bergmann & Dikstein, 1956; Krenitsky *et al.*, 1972; Lohmann & Miech, 1976, 1976). It has been suggested that the molar ratio of 1-MU to 1-MX in the urine, following either a dose of 1-MX or 1-MX derived from caffeine, might serve as an indicator of xanthine oxidase activity *in vivo* (Grant *et al.*, 1983, 1986). The use of urinary ratios of metabolic product and substrate to reflect the activity of the catalyzing enzyme, or the metabolic clearance of the substrate, has been supported recently (Jackson *et al.*, 1986; Birkett *et al.*, 1983, 1987). This technique was used to examine the relationship between plasma concentrations of oxipurinol and the activity of xanthine oxidase.

Methods

This study was approved by the Ethics and Research Committee of St Vincent's Hospital, Sydney. Informed consent was obtained from eight healthy volunteers, aged between 22 and 30 years, who weighed between 54 and 96 kg. There were six males and two females.

Subjects were placed on a methyl xanthine free diet for 3 days prior to estimating the activity of their xanthine oxidase enzyme. Thus, caffeine, chocolate, cocoa and carbonated soft drinks were prohibited. Compliance with this diet was monitored by assaying a spot urine for 1-MX just prior to the estimation of the baseline 1-MU/1-MX ratio. The activity of xanthine oxidase *in vivo* was determined by infusing 20 ml of a sterile solution containing 50 mg of 1-MX intravenously via an antecubital vein over 20 min using a constant volume infu-

sion rate pump (Terumo STC-521). The solution was prepared under strict aseptic conditions in a laminar flow hood by dissolving 1-MX powder (Fluka, AG lot no. 225868 -118; final material purified by A.B. Draco, lot no. LE 90, Lund, Sweden) in 0.6 % sodium carbonate solution and then filtering this through a Millex 0.2 μ GS filter. The final pH of the solution was 10.3. The purity of 1-MX was verified by elemental analysis and h.p.l.c.. The use of 1-MX was also approved by the Australian Department of Health.

Subjects had pulse and blood pressure monitored at 5 min intervals throughout the infusion and ECG monitoring for at least their first MX infusion. There were no untoward cardiovascular events observed throughout the study. Subject PO did not complete the study because he may have experienced an adverse reaction to the 1-MX. He noted some transient paraesthesiae and sensations of warmth in the abdomen and lower extremities towards the end of two infusions of 1-MX. He was tired and had been feeling stressed prior to these two infusions. Although the nature of these reactions was uncertain, it was considered that continuation of the study in this individual was unwarranted.

Complete 4 h urine collections were made commencing with the 1-MX infusion. Urine samples were preserved prior to analysis with an equal volume of 0.1 M acetic acid. Concentrations of 1-MX and 1-MU were measured in the urine using h.p.l.c. according to the procedure of Birkett *et al.* (1985). The results were expressed as the ratio of 1-MU/1-MX in mg equivalents of MX. The 1-MU/1-MX ratios, measured on consecutive days during the control and each allopurinol dosage period respectively, were averaged (see below). The pairs of ratios for each of these treatments and for each subject were always very similar. For example, the mean difference between duplicate ratios observed with allopurinol 50 mg day⁻¹ was 0.18 ± 0.16 (s.d.). The recovery of administered 1-MX as 1-MU and 1-MX in the urine was 66.8 ± 5.8 (s.d.) % ($n = 82$) and there were no significant contrasts in the recoveries with the different doses of allopurinol.

Subjects had estimations of xanthine oxidase activity made on consecutive mornings at baseline and on the 7th and 8th mornings of 7 day dosing schedules with allopurinol 50, 100, 300 and 600 mg day⁻¹, respectively. Allopurinol was administered in a single dose at the same time each day, which was close to 12.30 h. This was timed to coincide with the end of the 4 h urine collection. The treatment order was randomized according to a 4 by 4 latin square with

two subjects following each of the four treatment orders. At least 1 week separated the four treatment periods. In addition to the methyl xanthine free diet, the subjects fasted overnight prior to each 1-MX infusion and for the duration of the urine collection periods. Alcohol and medications, other than allopurinol, were not permitted throughout the study period. None of the subjects was a smoker. Blood (10 ml) was drawn into Vacutainer™ glass tubes which contained 15 mg of sodium edetate, to coincide with the trough concentrations of oxipurinol just before the morning doses of allopurinol on days 7 and 8.

In order to investigate the time-course of the return of xanthine oxidase activity following allopurinol therapy, 1-MX infusions were administered at 24 h intervals for 4 days following cessation of the 300 mg day⁻¹ dosing with allopurinol (data for subjects KW and SB were not available for day 4). Additionally, plasma oxipurinol concentrations were observed daily during this washout period.

Plasma concentrations of allopurinol and oxipurinol were determined by the h.p.l.c. method of Kramer & Feldman (1979), except that 8-methylxanthine was used as the internal standard instead of paracetamol. Using this internal standard, total chromatography time was reduced from 30 to 15 min. The intra- and inter-assay coefficients of variation of the assay were < 6%. All assays were performed in duplicate.

In order to summarize the data and to present average concentrations of oxipurinol associated with 50 and 90% inhibition of xanthine oxidase respectively, the following expression was employed:

$$\text{xanthine oxidase}_{\text{inhib}} = \frac{\text{xanthine oxidase}_{\text{inhib}}^{\text{max}} \times C^n}{\text{EC}_{50} + C^n}$$

In this expression, xanthine oxidase_{inhib} equals R_O-R_C where R_O is the 1-MU/1-MX ratio at baseline and R_C is the ratio associated with a particular concentration of oxipurinol. *C* is the steady-state concentration of oxipurinol and EC₅₀ is the concentration of oxipurinol at which inhibition of xanthine oxidase is half-maximal (Holford & Sheiner, 1981). *C* is raised to the power *n* in order to achieve an acceptable fit of the data. The goodness of fit of each individual's data was determined iteratively by the method of minimization of least squares using the computer program Funfit (Veng-Pedersen, 1977). Using this method of fitting each individual's data to the expression,

values for the unknown parameters xanthine oxidase_{inhib}^{max}, EC₅₀ and *n* were obtained for each individual.

Results

Oxipurinol concentrations at steady-state increased linearly in the dosage range of allopurinol studied but there was considerable variation between individuals in steady-state plasma oxipurinol concentrations for given doses of allopurinol (Table 1).

Allopurinol 50 mg day⁻¹ was associated with a significant drop in the 1-MU/1-MX ratio from the basal value (Table 2; *P* < 0.05; paired *t*-test). There was a sharp decline in the ratio with the lower doses of allopurinol and this effect flattened off around 200–300 mg of allopurinol day⁻¹ (Table 2).

A scatter plot of the 1-MU/1-MX ratios against steady-state plasma oxipurinol concentrations showed a hyperbolic pattern (Figure 1).

Cessation of allopurinol, after dosing with 300 mg day⁻¹ for 1 week, led to a rapid drop in plasma oxipurinol over 4 days and the accompanying 1-MU/1-MX ratios also changed rapidly (Figure 2) and were superimposable with those expected from the steady-state relationship between plasma oxipurinol concentrations and the 1-MU/1-MX ratio for each individual (Figure 1).

Using the expression presented in the methods, excellent data fits were obtained for plasma oxipurinol vs xanthine oxidase_{inhib} for each individual with correlation coefficients

Table 1 Mean (*n*=2) plasma concentrations of oxipurinol (mg l⁻¹) taken on consecutive days at steady-state in normal subjects given varying doses of allopurinol.

Subject	Allopurinol dose (mg day ⁻¹)			
	50	100	300	600
DQ	0.8	1.2	4.0	6.9
KW	0.8	1.7	5.9	15.5
SB	1.4	1.7	7.5	9.8
FC	0.3	1.7	5.3	13.0
RB	0.8	1.8	4.5	8.8
LC	0.7	2.0	6.6	10.5
PN	1.9	2.9	9	16.3
PO	1.0	*	*	8.9
Mean	0.96	1.86	6.11	11.21
s.d	0.49	0.52	1.75	3.38

*These two doses omitted because of possible adverse effect of 1-methyl xanthine.

Table 2 Mean ($n=2$) of 1-methyl uric acid to 1-methyl xanthine in 4 h urine collection in normal subjects given 1-methyl xanthine 50 mg i.v. on consecutive days and at a steady-state with varying doses of allopurinol.

Subject	Allopurinol dose (mg day ⁻¹)				
	0	50	100	300	600
DQ	2.48	2.28	1.08	0.33	0.30
KW	2.05	1.57	0.82	0.24	0.18
SB	2.19	1.51	0.94	0.25	0.21
FC	2.11	2.04	1.02	0.31	0.12
RB	2.15	2.24	1.59	0.50	0.19
LC	2.21	1.93	1.08	0.24	0.16
PN	2.68	0.63	0.43	0.12	0.07
PO	2.91	2.23	*	*	0.25
Mean	2.35	1.80	0.99	0.28	0.19
s.d	0.31	0.56	0.35	0.12	0.07

*These two doses omitted because of possible adverse effect of 1-methyl xanthine.

Figure 1 Scatterplot of the ratios of 1-MU/1-MX (◆) in the urine vs steady-state plasma oxipurinol concentrations for the eight volunteers each taking allopurinol 50, 100, 300 and 600 mg day⁻¹ on four separate occasions. Plasma oxipurinol concentrations and corresponding 1-MU/1-MX ratios during the washout (◇) of drug following the 300 mg day⁻¹ dosage are included.

greater than 0.9998 (Figure 3). Data for subjects RB and PO were not fitted to this expression: RB had a 1-MU/1-MX ratio in association with allopurinol 50 mg day⁻¹ which was greater than the baseline value; PO had two data points missing, as mentioned in methods. EC₅₀ and EC₉₀ values of plasma oxipurinol concentrations for inhibition of the formation of 1-MU from 1-MX *in vivo* were 1.4 ± 0.46 and 4.08 ± 2.03 mg l⁻¹ respectively.

Figure 2 The relationship between mean plasma oxipurinol concentrations (□) and mean 1-MU/1-MX ratios (◆) for all subjects, except PO (did not take 300 mg day⁻¹ dose of allopurinol), during the washout following dosing with allopurinol 300 mg day⁻¹.

Figure 3 Relationship between plasma oxipurinol and the decrement in the 1-MU/1-MX ratio in subject KW. The data points are fitted to the pharmacodynamic effect model described under methods.

Discussion

Allopurinol dosing in the range 50–600 mg day⁻¹ leads to proportional increases in the steady-state concentrations of oxipurinol in the young, normal volunteers studied. There was moderate variation between the individuals studied with respect to the concentrations of oxipurinol achieved for given doses of allopurinol (Table 1). Some of this variability would be accounted for by expressing the daily dose of allopurinol in mg kg⁻¹ day⁻¹ but this differs from usual clinical practice with this drug. All the volunteers had normal plasma creatinines and creatinine clearances. In a population of elderly people with variable renal function, much greater variability between dose and steady-

state plasma oxipurinol would be expected (Hande *et al.*, 1984).

It was of interest to observe that the relationship between dose and steady-state plasma oxipurinol was linear, given that xanthine oxidase is responsible for the conversion of allopurinol to oxipurinol. Allopurinol clearance was not measured in this study. The clearance of allopurinol is high and, as expected, allopurinol was not detected in plasma at the end of a 24 h dosing interval. However, it might have been expected that with increasing doses of allopurinol there would be a decreasing clearance of allopurinol in association with a less than proportional increase in the concentrations of oxipurinol. The observed result suggests that allopurinol may be metabolized to oxipurinol by alternative pathways to xanthine oxidase. Patients who are genetically deficient in xanthine oxidase have been administered allopurinol (Engelman *et al.*, 1964; Elion *et al.*, 1966; Simmonds, 1969; Simmonds *et al.*, 1974; Chalmers *et al.*, 1969; Auscher *et al.*, 1974). No oxipurinol was produced in two such subjects when allopurinol was administered. However, oxipurinol was measured in plasma in reasonable concentrations in two other subjects and it was suggested that the enzyme, aldehyde oxidase, which is very closely related to xanthine oxidase, was responsible for the metabolism of allopurinol in these individuals (Elion, 1978; Krenitsky *et al.*, 1972). Thus, this enzyme may be responsible for the continued production of oxipurinol in the face of inhibition of xanthine oxidase induced by high doses of allopurinol. Alternatively, the oxidation of allopurinol to oxipurinol may be inhibited only by relatively high concentrations of allopurinol and oxipurinol, when compared with other substrates such as xanthine and hypoxanthine. Thus, administration of allopurinol 50–100 mg kg⁻¹ intravenously was required to inhibit the oxidation of allopurinol to oxipurinol in dogs (Elion, 1978).

The ratio of 1-MU/1-MX in the 4 h urine collections, following an infusion of 1-MX, was sensitive to increasing concentrations of oxipurinol. 1-MU is the only metabolic product of 1-MX and this reaction is catalysed by xanthine oxidase (Birkett *et al.*, 1983). The use of the ratio of product to substrate in urine collections, as an indication of the metabolic clearance of substrate, has been described previously (Jackson *et al.*, 1986; Birkett *et al.*, 1987). A further assumption, made when using this approach, was that allopurinol does not affect the renal clearance of 1-MU or 1-MX.

The relationship between oxipurinol concentrations and the 1-MU/1-MX ratio, as shown

in the scatterplot (Figure 2), is hyperbolic. This general pattern suggested that an 'E_{max}' model should be employed to fit the concentration-effect data (Holford & Sheiner, 1981). However, the concentration term in this expression had to be raised to various powers (*n* ranged from 2 to 6.4) to achieve a satisfactory fit of the data, an approach pioneered by Hill (1910) and related to the 'Sigmoid E_{max} Model' (Holford & Sheiner, 1981). The EC₅₀ and EC₉₀ derived using this approach were remarkably low (1.4 and 4.08 mg l⁻¹ respectively) given that the average plasma oxipurinol concentrations found in populations of hospital patients given allopurinol is around 15 mg l⁻¹ (Emmerson *et al.*, 1987; Day *et al.*, 1988). This might suggest that, in general, patients are being given excessive doses of allopurinol. Alternatively the oxidation of 1-MX to 1-MU may be inhibited by relatively low doses of allopurinol when compared with the dose required to inhibit substantially hypoxanthine and xanthine oxidation to uric acid. Uric acid plasma concentrations are not reported in this paper as these normal volunteers did not have a controlled diet with respect to urate precursors.

The loss of inhibition of xanthine oxidase, as shown by the increase in the 1-MU/1-MX ratio, was rapid upon cessation of allopurinol. In fact, the relationship between plasma concentrations of oxipurinol and the ratio during the allopurinol washout was essentially identical to the steady-state relationship between oxipurinol concentrations and the ratio. This lack of hysteresis in the concentration-effect relationship indicates that the effect compartment is readily accessed by allopurinol and that the inhibition of xanthine oxidase is rapidly reversed. Again, this is somewhat unexpected, given the tight binding and slow association time of allopurinol and, particularly, oxipurinol for xanthine oxidase demonstrated in a series of *in vitro* studies (Spector, 1977).

We propose that the level of inhibition of xanthine oxidase *in vivo* may be indicated by the ratio of 1-MU/1-MX observed in the urine, following administration of 1-MX to humans. This technique may be useful in investigating individuals who are apparent 'non-responders' to allopurinol, particularly if plasma oxipurinol concentrations are also measured. However, further evaluation of this method is required in patient populations and, in particular, in patients with various levels of renal impairment. In addition, further work is required to understand the relationship between allopurinol-induced changes in the urinary ratio of 1-MU/1-MX and inhibition of the synthesis of uric acid.

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References

- Auscher, C., Pasquier, C., Mercier, N. & Delbarre, F. (1974). Urinary excretion of 6-hydroxylated metabolite and oxypurines in a xanthinuric man given allopurinol or thiopurinol. *Adv. exp. Med. Biol.*, **41B**, 663-667.
- Bergmann, F. & Dikstein, S. (1956). Studies on uric acid and related compounds. III. Observations on the specificity of mammalian oxidases. *J. biol. Chem.*, **223**, 765-780.
- Birkett, D. J., Dahlqvist, R., Miners, J. O., Lelo, A. & Billing, B. (1985). Comparison of theophylline and theobromine metabolism in man. *Drug Metab. Dispos.*, **13**, 725-728.
- Birkett, D. J., Miners, J. O. & Attwood, J. (1983). Secondary metabolism of theophylline; bio-transformation products in man-route of formation of 1-methyluric acid. *Br. J. clin. Pharmacol.*, **15**, 117-119.
- Birkett, D. J., Miners, J. O. & Robson, R. O. (1987). *In vivo* assessment of drug metabolism. In *Enzyme induction in man*, eds, Sotaniemi, E. A. & Pelkonen, R. O., pp 71-77. London: Taylor and Francis.
- Chalmers, R. A., Parker, R., Simmonds, J. A., Snedden, W. & Watts, R. W. E. (1969). The conversion of 4-hydroxypyrazolo[3,4-d] pyrimidine (allopurinol) into 4,6-dihydroxypyrazolo [3,4-d] pyrimidine (oxypurinol) *in vivo* in the absence of xanthine-oxygen oxidoreductase. *Biochem. J.*, **112**, 527-532.
- Day, R. O., Miners, J. O., Birkett, D. J., Whitehead, A., Naidoo, D., Hayes, J. & Savdie, E. (1988). Allopurinol dosage selection: relationships between dose and plasma oxipurinol and urate concentrations and urinary urate excretion. *Br. J. clin. Pharmacol.*, **26**, 425-430.
- Elion, G. (1978). Allopurinol and other inhibitors of urate synthesis. In *Uric acid; Handbook of experimental pharmacology*, Vol. 51, eds Kelley, W. N. & Weiner, I. M., pp 485-514. Berlin: Springer Verlag.
- Elion, G. B., Kovensky, A., Hitchings, G. H., Metz, E. & Rundles, R. W. (1966). Metabolic studies of allopurinol, an inhibitor of xanthine oxidase. *Biochem. Pharmacol.*, **15**, 863-880.
- Emmerson, B. T., Gordon, R. B., Cross, M. & Thomson, D. B. (1987). Plasma oxipurinol concentrations during allopurinol therapy. *Br. J. Rheumatol.*, **26**, 445-449.
- Engelman, K., Watts, R. W. E., Klineberg, J. R., Sjoerdsma, A. & Seegmiller, J. E. (1964). Clinical, physiological and biochemical studies of a patient with xanthinuria and pheochromocytoma. *Am. J. Med.*, **37**, 839-861.
- Gibson, T., Simmonds, H. A., Rogers, A. V., Huston, G. J., Webster, D. R., Munro, J. & Emery, P. (1983). Gout with apparent resistance to allopurinol. *Ann. Rheum. Dis.*, **42**, 93-94.
- Grant, D. M., Tang, B. K., Campell, M. E. & Kalow, W. (1986). Effect of allopurinol on caffeine disposition in man. *Br. J. clin. Pharmacol.*, **21**, 454-458.
- Grant, D. M., Tang, B. K. & Kalow, W. (1983). Variability in caffeine metabolism. *Clin. Pharmacol. Ther.*, **33**, 591-603.
- Hande, K. R., Noone, R. M. & Stone, W. J. (1984). Severe allopurinol toxicity. Description and guidelines for prevention in patients with renal insufficiency. *Am. J. Med.*, **76**, 47-56.
- Hill, A. V. (1910). The possible effects of the aggregation of the molecules of haemoglobin on its dissociation curves. *J. Physiol. (Lond.)*, **40**, iv-vii.
- Holford, N. H. G. & Sheiner, L. B. (1981). Understanding the dose-effect relationship: clinical application of pharmacokinetic-pharmacodynamic models. *Clin. Pharmacokin.*, **6**, 429-453.
- Jackson, P. R., Tucker, G. T., Lennard, M. S. & Woods, H. F. (1986). Polymorphic drug oxidation pharmacokinetic basis and comparison of experimental indices. *Br. J. clin. Pharmacol.*, **22**, 541-550.
- Kramer, W. G. & Feldman, S. (1979). High performance liquid chromatographic assay for allopurinol and oxypurinol in human plasma. *J. Chromatogr.*, **162**, 94-97.
- Krenitsky, T. A., Neil, S. M., Elion, G. B. & Hitching, G. H. (1972). A comparison of the specificities of xanthine oxidase and aldehyde oxidase. *Arch. Biochem. Biophys.*, **150**, 585-599.
- Lohmann, S. M. & Miech, R. P. (1976). Theophylline metabolism by the rat liver microsomal system. *J. Pharmacol. exp. Ther.*, **196**, 213-225.
- Simmonds, H. A. (1969). Urinary excretion of purines, pyrimidines, pyrazolopyrimidines in patients treated with allopurinol or oxipurinol. *Clin. Chem. Acta*, **23**, 353-364.
- Simmonds, H. A., Levin, B. & Cameron, J. S. (1974). Variations in allopurinol metabolism by xanthinuric subjects. *Clin. Sci. mol. Med.*, **47**, 173-178.
- Spector, T. (1977). Inhibition of urate production by allopurinol. *Biochem. Pharmacol.*, **26**, 355-358.
- Veng-Pedersen P. (1977). Curve fitting and modelling in pharmacokinetics and some practical experiences with NONLIN and a new program, FUNFIT. *J. Pharmacokin. Biopharm.*, **5**, 513-51.
- Wyngaarden, J. B. & Holmes, E. W. (1979). Clinical gout and the pathogenesis of hyperuricaemia. In *Arthritis and allied conditions*, ed. McCarty, D. J., pp 1193-1288. Philadelphia: Lea & Febiger.

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