Biochemical Studies on the Treatment of Primary Hyperoxaluria

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The increased urinary oxalate excretion which is characteristic of primary hyperoxaluria causes recurrent urinary calculi and nephrocalcinosis, with death at an early age (Hall, Scowen, and Watts, 1960; Hockaday, Clayton, Frederick, and Smith, 1964), and no effective treatment for the disease has been reported. Glyoxylate is the main immediate metabolic precursor of oxalate, and if the enzyme or enzymes which catalyse the oxidation

could be inhibited *in vivo* the urinary oxalate excretion should be reduced and renal damage averted. The enzymes which catalyse this oxidation of glyoxylate to oxalate *in vivo* have not been identified, but xanthine oxidase (xanthine oxygen: oxidoreductase (Enzyme Classification 1.2.3.2.)), aldehyde dehydrogenase (aldehyde: NAD oxidoreductase (E.C. 1.2.1.3.)), and aldehyde oxidase (aldehyde: oxygen oxidoreductase (E.C. 1.2.3.1.)) catalyse the oxidation of a wide range of aldehydes to carboxylic acids. Allopurinol (Gibbs and Watts, 1966), disulfiram (Graham, 1951), and acetomenaphthone (Rajagopalan, Fridovich and Handler, 1962) inhibit aldehyde oxidations by xanthine oxidase, aldehyde dehydrogenase, and aldehyde oxidase respectively *in vitro*.

Experimental pyridoxine deficiency produces calcium oxalate nephrocalcinosis and urinary calculi in the cat and rat (Gershoff, Faragalla, Nelson, and Andrus, 1959a; Andrus, Gershoff, and Faragalla, 1959). Faber, Feitler, Bleiler, Ohlson, and Hodges (1963) demonstrated hyperoxaluria in human subjects given pyridoxine-deficient diets, but hyperoxaluria was not observed in an infant with dietary

pyridoxine deficiency (Scriver and Hutchison, 1963). Gershoff, Mayer, and Kulczycki (1959b) reported that the urinary excretion of oxalate by normo-oxaluric human subjects in whom there was no reason to suspect dietary pyridoxine deficiency was reduced by giving pyridoxine, and that this effect was enhanced if folic acid was administered concurrently. Ludwig (1963) reported a patient with urinary stones and hyperoxaluria whose urinary oxalate excretion decreased when pyridoxine was given. Elder and Wyngaarden (1960) also studied a patient whose hyperoxaluria disappeared after she was admitted to hospital, and they have recently suggested that an unsuspected dietary deficiency may have been corrected in this case (Wyngaarden and Elder, 1966). Mayer, Al-Waidh, Karp, and Zinsser (1961) reported that most oxalate stone formers showed biochemical evidence of pyridoxine deficiency.

This paper reports a study of the effects of giving allopurinol, disulfiram, acetomenaphthone, pyridoxine, and pyridoxine with folic acid, on the urinary oxalate excretion in primary hyperoxaluria. The oxalate excretion was also studied at two levels of protein intake.

Patients and Methods

The patients were in hospital but ambulant during the studies.

Patient 1. She was an asymptomatic 7-year-old girl, height 119 cm., weight 22.8 kg., with bilateral renal calculi, blood urea 62 mg./100 ml., and grossly raised urinary oxalate excretion (Table I). Her clinical state remained unchanged during these studies. The parents are unrelated, and one sib has died from uraemia associated with renal calculi, systemic oxalate deposits (oxalosis) being demonstrated at necropsy. The patient's condition deteriorated about three months after the completion of the present studies, and she died from uraemia, widespread oxalosis being observed. During the present investigations she took a diet which provided

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TABLE I

Effects of Allopurinol, Disulfiram, and Acetomenaphthone on Urinary Oxalate Excretion in Primary Hyperoxaluria

Period		Drug Régime	Oxalate Excretion					
			(mg. (COOH) ₂ · 2H ₂ O/24 hr.)			(mg. (COOH) ₂ · 2H ₂ O/mg. Creatinine)		
			Range	Mean	Standard Deviation	Range	Mean	Standard Deviation
<i>Patient No. 1</i>								
1	9	None	133-207	172	26	0·215-0·451	0·302	0·068
2	11	Allopurinol 100 mg. 12-hourly	136-178	157	13	0·231-0·298	0·267	0·023
3	4	Allopurinol 100 mg. 8-hourly	151-162	—	—	0·255-0·270	—	—
4	16	Allopurinol 100 mg. 6-hourly	121-217	159	25	0·209-0·388	0·284	0·052
5	12	None	137-172	160	15	0·226-0·405	0·284	0·049
6	3	Disulfiram 125 mg. daily	151-158	—	—	0·232-0·266	—	—
7	21	Disulfiram 125 mg. 12-hourly	137-168	151	10	0·200-0·345	0·253	0·035
8	5	{ Allopurinol 100 mg. 12-hourly + disulfiram 125 mg. 12-hourly }	138-187	154	21	0·209-0·302	0·247	0·035
9	11	{ Allopurinol 100 mg. 6-hourly + disulfiram 125 mg. 12-hourly }	115-155	138	17	0·198-0·272	0·235	0·027
10	21	None	114-229	168	28	0·147-0·389	0·263	0·063
11	13	Acetomenaphthone 5 mg. 6-hourly	182-224	197	13	0·225-0·356	0·283	0·032
12	10	{ Allopurinol 100 mg. 6-hourly + acetomenaphthone 5 mg. 6-hourly }	199-214	204	6	0·260-0·318	0·283	0·024
13	9	Allopurinol 100 mg. 6-hourly	156-211	189	15	0·239-0·346	0·286	0·038
14	47	{ Allopurinol 100 mg. 6-hourly + disulfiram 125 mg. 12-hourly }	114-221	173	27	0·143-0·396	0·254	0·050
15	10	None	155-206	175	14	0·284-0·445	0·357	0·046
<i>Patient No. 2</i>								
1	12	None	162-319	232	40	0·264-0·400	0·339	0·040
2	16	Allopurinol 100 mg. 6-hourly	171-276	225	27	0·280-0·506	0·377	0·073
3	12	None	129-379	263	69	0·303-0·439	0·359	0·038

a calculated intake of 155 g. carbohydrate, 50 g. protein, 65 g. fat, 22 mg. ascorbic acid, and 1400 calories/24 hr. Foods which are especially rich in oxalate and ascorbic acid (rhubarb, strawberries, beets, spinach, chocolate, tea, cocoa, and citrus fruits) were excluded from the diet.

Patient 2. He was an asymptomatic 12-year-old boy, height 133 cm., weight 30·7 kg., with a right renal calculus, blood urea 40 mg./100 ml., and grossly raised urinary oxalate excretion. (He was reported as 'Case 4' in 'Family 4' of Hall *et al.* (1960).) His diet was unrestricted except that the high oxalate foods and citrus fruits were excluded.

Patient 3. She was an asymptomatic 14-year-old girl, height 183 cm., weight 46·5 kg., referred to as 'Subject 3' in a previous study (Crawhall and Watts, 1962). She had bilateral renal calculi, blood urea 34 mg./100 ml., and grossly increased urinary oxalate excretion.

Urine collections. The urine was filtered quickly through a coarse filter paper (Greens no. 904½), while still warm, the filtrate being collected in a bottle containing conc. HCl(50% w/v); any calculi present were removed from the filter, and the paper was washed with approximately 10 ml. N.HCl, the washings being added to the main bulk of the filtrate.

Analytical methods. The methods used were: (a) for oxalate—isotope dilution analysis—Dean and Griffin (1965), except that Bray's (1960) scintillator solution was used; (b) for urinary uric acid—Liddle, Seegmiller, and Laster (1959); (c) for urinary oxypurines

—Goldfinger, Klinenburg, and Seegmiller (1965), as modified by Watts, Watkins, Matthias, and Gibbs (1966); (d) for urinary creatinine—Peters and Van Slyke (1932).

Results

The urinary oxalate excretion by Patient 1 was unaltered by giving allopurinol, disulfiram, or acetomenaphthone either singly or in the following combinations—allopurinol with disulfiram, and allopurinol with acetomenaphthone (Table I). During the control periods this patient's urinary uric acid was 200-350 mg./24 hr. with a negligible excretion of oxypurines, whereas when allopurinol was given the urinary uric acid level was 75-150 mg./24 hr., the oxypurine excretion being 70-125 mg./24 hr. (calculated as xanthine). These results show that xanthine oxidase was being inhibited by the drug. Allopurinol also failed to influence the urinary oxalate excretion in the study on Patient 2 (Table I), and in an adult patient (Gibbs and Watts, 1967) who was given the drug for 4 months (mean urinary oxalate excretion before and during the treatment being 132 mg./24 hr. (range 102-168; standard deviation 20; 10 observations) and 135 mg./24 hr. (range 81-258; standard deviation 33; 29 observations), respectively (calculated as (COOH)₂ · 2H₂O).

The urinary oxalate excretion by Patient 3 was

TABLE II

Effect of Protein Restriction, Pyridoxine, and Pyridoxine Plus Folic Acid Administration on Urinary Oxalate Excretion in Primary Hyperoxaluria

Period		Drug Régime	Oxalate Excretion					
No.	Duration (dy.)		(mg. (COOH) ₂ . 2H ₂ O/24 hr.)			mg. (COOH) ₂ . 2H ₂ O/mg. Creatinine)		
			Range	Mean	Standard Deviation	Range	Mean	Standard Deviation
1	7	Diet unrestricted except for omission of high oxalate foods, etc. (see text)	172-223	199	21	0·153-0·199	0·175	0·017
2	9	*70 g. protein diet	210-254	234	13	0·195-0·220	0·206	0·009
3	14	†35 g. protein diet	175-245	211	65	0·148-0·213	0·184	0·020
4	17	35 g. protein diet plus pyridoxine HCl 250 mg. 6-hourly	187-234	209	13	0·158-0·200	0·181	0·012
5	10	35 g. protein diet plus pyridoxine HCl 250 mg. 6-hourly and folic acid 5 mg. 6-hourly	228-251	242	8	0·181-0·218	0·204	0·012

* This diet provided a calculated 70 g. protein, 370 g. carbohydrate, 80 g. fat, 35 mg. ascorbic acid, and 2400 cal./24 hr.

† This diet provided a calculated 35 g. protein, 400 g. carbohydrate, 75 g. fat, 30 mg. ascorbic acid, and 2300 cal./24 hr. The high oxalate foods and citrus fruits were both excluded from these diets (see text).

unaltered by large doses of pyridoxine, or by a combination of pyridoxine and folic acid (Table II). It was also shown that doubling this patient's protein intake did not affect her urinary oxalate excretion (Table II).

Discussion

The urinary oxalate excretion increases to adult levels by the age of approximately 12 years (unpublished data from this laboratory), and the present patients all had the grossly increased urinary oxalate excretion characteristic of primary hyperoxaluria. It is unlikely that a therapeutically useful diminution in the urinary oxalate excretion would have been obscured by rapid solution of calcium oxalate urinary calculi during these studies. Thus, in the analogous situation which arises when cystinuric patients are treated with penicillamine, the decrease in the urinary excretion is easily demonstrable when cystine stones are present (Crawhall, Scowen, and Watts, 1963, 1964). It is also of interest that the calcium oxalate is less soluble in urine (about 150 mg./litre at pH 6·0, calculated from the data of Miller, Vermeulen, and Moore (1958)), than cystine (about 300 mg./litre at the same pH value, Dent and Senior (1955)).

The failure of the xanthine oxidase inhibitor allopurinol to reduce the urinary oxalate excretion in primary hyperoxaluria, even though an effective degree of enzyme inhibition was achieved as judged by the results of the uric acid and oxypurine determinations, agrees with our findings in non-hyperoxaluric subjects (Gibbs and Watts, 1966), and indicates that the role of this enzyme in oxalate production is not increased in the disease. Recent studies with hyperoxaluric patients' liver tissue (Gibbs and Watts, 1967) also showed that neither

the over-all catalytic activity of the tissue, nor the extent to which this was mediated by xanthine oxidase, was increased in the disease. Similarly, the present negative results with disulfiram and acetomenaphthone suggest that aldehyde dehydrogenase and aldehyde oxidase, respectively, are either not responsible for the oxidation of glyoxylate to oxalate *in vivo* in primary hyperoxaluria, or that their inhibition can be compensated by increased activity of other enzyme systems.

Pyridoxine either alone or in combination with folic acid did not reduce the urinary excretion of oxalate in the present work. Hockaday *et al.* (1964) also mention that pyridoxine has been ineffective in the treatment of primary hyperoxaluria, though they give no details of the data on which this statement is based. The discrepancy between these results and those of Gershoff *et al.* (1959b) would be explicable if the metabolic pathway, the activity of which was altered by pyridoxine administration, contributes the same absolute amount of oxalate to the total urinary oxalate in primary hyperoxaluria as in normal subjects, this being small by comparison with the total urinary oxalate excretion in primary hyperoxaluria, but forming a large part of a normal subject's urinary oxalate excretion. In these circumstances fluctuations in response to pyridoxine administration, though apparent in normal subjects, might be no greater than the spontaneous fluctuations in the urinary oxalate excretion which occur in primary hyperoxaluria.

McLaurin, Beisel, McCormick, Scalettar, and Herman (1961) report that two patients with primary hyperoxaluria had no symptoms of urinary stones after being given pyridoxine though they both continued to have grossly raised urinary oxalate

levels. The rate at which urinary stones form in patients with primary hyperoxaluria is variable, and there seems no reason to regard the outcome in these cases as having been altered by pyridoxine administration. Ludwig's (1963) patient had a lower oxalate excretion than is usually encountered in the disease except when there is severe renal failure, and it is possible that her hyperoxaluria was due to a different metabolic lesion from that which was present in the patients studied here; it is also possible that some of the changes which he observed could have been due to spontaneous fluctuations in the urinary oxalate excretion.

It is concluded that the recent observations suggesting a relation between pyridoxine intake and the level of urinary oxalate excretion have no therapeutic application in the treatment of primary hyperoxaluria.

Summary

The possibility of reducing the urinary oxalate excretion in primary hyperoxaluria by giving drugs that inhibit some of the enzymes which are known to oxidize aldehydes to carboxylic acids, has been investigated. Allopurinol (a xanthine oxidase inhibitor), disulfiram (an aldehyde dehydrogenase inhibitor), and acetomenaphthone (an aldehyde oxidase inhibitor) did not alter the urinary oxalate excretion. Allopurinol and disulfiram, given together, and allopurinol, with acetomenaphthone, were also ineffective. Thus, these enzymes do not appear to be specifically concerned with oxalate production in primary hyperoxaluria.

Pyridoxine and pyridoxine plus folic acid, which have been previously reported to reduce the urinary oxalate excretion did not lower the urinary oxalate excretion in the present studies.

The urinary oxalate excretion was unaltered by restricting the protein intake.

It is concluded that none of these measures has any place in the treatment of patients with primary hyperoxaluria.

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