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# STUDIES ON THE EFFECT OF XYLITOL ON OXALATE FORMATION

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**Abstract**—The excretion of [$^{14}$C]oxalate after parenteral administration of [U-$^{14}$C]xylitol, [U-$^{14}$C]fructose, [U-$^{14}$C]glucose [U-$^{14}$C]sorbitol and [U-$^{14}$C]glycine has been studied in normally fed rats. All of these compounds were about equally effective as precursors of the urinary oxalate. The effect of xylitol on the $NAD^+$-dependent catalytic oxidation of glyoxylate to oxalate has also been investigated in rat liver cytosol, this reaction being the last step on the oxalate biosynthetic pathway. Xylitol slightly decreased oxalate production from glyoxylate in this system. These results are discussed in relation to the observation that the clinical use of xylitol for parenteral nutrition is sometimes complicated by renal failure with deposits of calcium oxalate in the renal tubules.

Large intravenous doses of xylitol have been used therapeutically as a source of parenteral nutrition in severely ill patients. This practice has been complicated by lactic acidosis, oliguric renal failure after a diuresis during the xylitol infusion, and extensive intratubular deposits of calcium oxalate crystals [1,2]. Similar intratubular calcium oxalate deposits occur in ethylene glycol poisoning [3], and in the terminal stages of primary hyperoxaluria [4], oxalate production and excretion being increased in both of these conditions.

Theoretically, xylitol could increase oxalate production either by providing extra carbon atoms for oxalate biosynthesis, or by changing the balance of the $NAD^+$-dependent coupled oxidation and reduction of glyoxylate to oxalate and glycollate respectively. This communication reports a study of both of these possible mechanisms.

The first step in the metabolism of xylitol, is catalytic oxidation to D-xylulose by D-xylulose reductase [Xylitol:$NAD^+$ oxidoreductase (D-xylulose forming) EC 1.1.1.9]. Carbon atoms numbers 1 and 2 of xylitol could then be converted to oxalate via the active glycolaldehyde fragment, which is transferred from D-xylulose phosphate to D-ribose phosphate by transketolase (sedoheptulose-7-phosphate:D-glyceraldehyde-3-phosphate glycolaldehyde transferase, EC 2.2.1.1).

The D-xylulose reductase reaction generates NADH which can be reoxidised to $NAD^+$ concomitantly with the reduction of pyruvate to lactate which is catalysed by lactate dehydrogenase (L-lactate:$NAD^+$ oxidoreductase EC 1.1.1.27), and although the $NAD^+$ could be recycled by D-xylulose reductase, its presence around the active site of lactate dehydrogenase could promote the oxidation of glyoxylate to oxalate (Fig. 1). This has been investigated by studying the effect of xylitol and pyruvate on the $NAD^+$-dependent catalytic oxidation of glyoxylate to oxalate in rat liver cytosol, which is due to lactate dehydrogenase [5].

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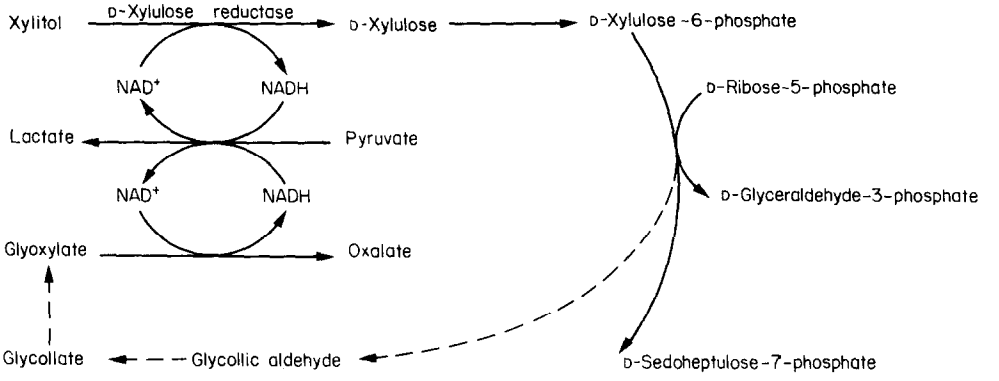


Fig. 1. Schematic representation of the possible coupling of xylitol oxidation to glyoxylate oxidation through pyruvate reduction, and of the possible direct metabolic pathway from carbon atoms numbers 1 and 2 of xylitol via glycollic aldehyde. Glyoxylate is also derived from glycine by transamination and via the glycine–serine–ethanolamine metabolic pathway [19].

Table 1. Conversion of [U-$^{14}$C]xylitol, [U-$^{14}$C]fructose, [U-$^{14}$C]glucose, [U-$^{14}$C]sorbitol and [U-$^{14}$C]glycine to urinary [$^{14}$C]oxalate in the rat

| Injected material | | | | Conversion to oxalate | | | |
|---|---|---|---|---|---|---|---|
| | Dose | | | % Injected dose | | | |
| | $\mu$Ci | $\mu$moles | Route | Day 1 | Day 2 | Day 3 | Day 4 |
| Xylitol | 1·0 | 0·303 | intravenous | 0·037 | 0·081 | 0·080 | 0·013 |
| | 1·0 | 0·303 | intraperitoneal | 0·014 | 0·052 | 0·014 | 0·024 |
| | 2·0 | 213·6 | intravenous | 0·005 | 0·021 | 0·033 | 0·007 |
| | 4·0 | 658·2 | intravenous | 0·105 | 0·007 | 0·004 | 0·003 |
| Fructose | 4·0 | 490·0 | intravenous | 0·064 | 0·004 | 0·007 | 0·003 |
| Glucose | 4·0 | 529·0 | intravenous | 0·029 | 0·004 | | 0·001 |
| Sorbitol | 4·0 | 549·5 | intravenous | 0·109 | 0·007 | 0·006 | 0·002 |
| Glycine | 4·0 | 3990 | intravenous | 0·179 | 0·011 | 0·006 | 0·007 |

## MATERIALS AND METHODS

Analytical grade reagents and glass distilled water were used throughout. [U-$^{14}$C]xylitol (3·3 mCi/m-mole), [U-$^{14}$C]fructose (165 mCi/m-mole), [U-$^{14}$C]glucose (281 mCi/m-mole), [U-$^{14}$C]sorbitol (8·7 mCi/m-mole), [U-$^{14}$C]glycine (114 mCi/m-mole) and sodium [1-$^{14}$C]glyoxylate (7·63 mCi/m-mole) were purchased from the Radio Chemical Centre, Amersham, Bucks. Catalase was purchased from the Boehringer corporation. Sprague–Dawley rats, weighing approx 100 g, were used and housed in separate metabolism cages during the *in vivo* studies. The urine from pairs of similarly treated animals was pooled.

## EXPERIMENTAL AND RESULTS

*The possible direct conversion of injected xylitol to urinary oxalate in the intact rat.* Eight pairs of normal rats were injected with the amounts of $^{14}$C-labelled and non radioactive xylitol, fructose, glucose, sorbitol and glycine shown in Table 1. The animals were allowed free access to food and water before and during the studies. Four successive 24-hr urine collections were made, residual urine was washed from the cages with 5 ml HCl (approx 2·3 M) containing sodium oxalate (0·15 m-mole). $H_2SO_4$ (approx 9·5 M, 0·06 ml/ml urine) was added and calcium oxalate isolated, purified, and its $^{14}$C content measured [6].

Only traces of [$^{14}$C]oxalate were formed when [U-$^{14}$C]xylitol was given either intraperitoneally or intravenously, and although this was somewhat greater when the amount of non radioactive carrier xylitol was increased, it was still only equivalent to about 0·1 per cent of the total dose. Xylitol was not materially superior to fructose, glucose or sorbitol, and somewhat inferior to glycine, as a urinary oxalate precursor in these studies (Table 1).

*The effect of xylitol and pyruvate on the NAD$^+$-dependent catalytic oxidation of glyoxylate to oxalate in rat liver cytosol.* The cytosol fraction (100,000 *g* supernatant) of rat liver was prepared as described previously [5]. A portion (0·5 ml) of this was incubated (45 min, 37°) with: sodium [1-$^{14}$C]glyoxylate (0·64 $\mu$Ci, 0·2 $\mu$mole), non radioactive sodium glyoxylate (10·87 $\mu$mole), catalase (390 international units), and sodium pyrophosphate buffer (0·1 M, pH 7·4), together with NADH (11 $\mu$mole), sodium pyruvate (11 $\mu$mole), NAD$^+$ (11 $\mu$mole) and non radioactive xylitol (11 $\mu$mole) in the combinations shown in Fig. 2. The final total vol was 2·2 ml in each case.

Xylitol alone did not change the amount of oxalate which was formed from glyoxylate in the cytosol. Adding pyruvate and NADH, in order to increase pyruvate reduction with concomitant NAD$^+$ generation, increased the oxidation of glyoxylate to oxalate, but this was slightly reduced in the presence of xylitol. The increased oxalate formation produced by adding NAD$^+$ only or NAD$^+$ together with pyruvate to the cytosol was also slightly reduced by xylitol (Fig. 2).

## DISCUSSION

The present observation that less than about 0·1 per cent of the $^{14}$C injected as [U-$^{14}$C]xylitol was recovered in the urinary oxalate over the course of 4 days even when relatively large amounts of non radioactive xylitol were also injected into normally fed rats shows that, under these conditions, xylitol is not a significant or specific precursor of the urinary oxalate. This conclusion is strengthened by the observation that similar amounts of $^{14}$C were recovered in the urinary oxalate after injecting [U-$^{14}$C]glucose, [U-$^{14}$C]fructose, [U-$^{14}$C]sorbitol and [U-$^{14}$C]glycine. The rate of xylitol infusion which is used therapeutically corresponds to about 1·644 m-mole/kg per hr, which is about one third of the largest dose used as a single injection in the present study. The present findings do not support the suggestion of Thomas *et al.* [1], that xylitol is converted to oxalate via glycolaldehyde to any significant extent. These investigators have recently observed that xylitol increased the urinary oxalate excretion in severely pyrodoxine deficient rats.* Other workers have shown that severe pyridoxine deficiency alone increases the urinary oxalate excretion [7–9]. Hauschildt† found that human subjects, in the clinical situations where xylitol is used, sometimes show minor biochemical evidence of pyridoxine deficiency (activation of erythrocyte glutamate-oxaloacetate aminotransferase by pyridoxal-5′-phosphate). However, these patients did not tend to excrete increased amounts of urinary oxalate. Although very large doses of pyridoxine sometimes reduce the urinary oxalate excretion in primary hyperoxaluria, this has been shown to be unrelated to correction of pyridoxine deficiency [10]. Also,

* D. W. Thomas *et al.* Personal communication (1974).
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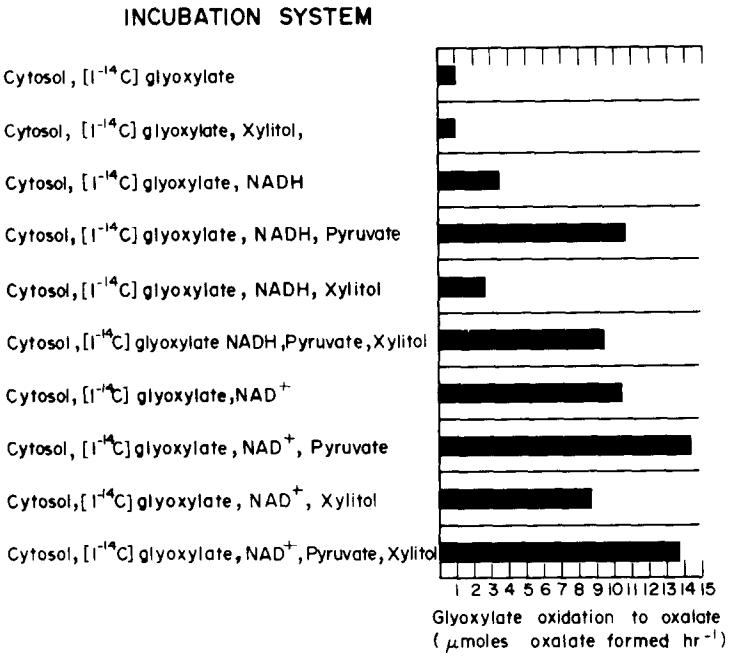


Fig. 2. The effect of xylitol and pyruvate on the $NAD^+$-dependent catalytic oxidation of glyoxylate to oxalate in rat liver cytosol (see text for further details).

there have been no reports of increased oxalate production in the known pyridoxine deficiency syndromes: cystathioninuria, sideroblastic anaemia, xanthurenic aciduria, and infantile convulsions associated with suboptimal pyridoxine intake. Thus, studies of xylitol metabolism in severely pyridoxine deficient animals may not be directly relevant to the problem of the nephrotoxicity of xylitol in clinical practice.

Lactate dehydrogenase catalyses the reduction of glyoxylate to glycollate simultaneously with the oxidation of another molecule of the substrate to oxalate, $NAD^+$ generated by the reductive reaction participating in the oxidative reaction [11]. Pyruvate [11] and hydroxypyruvate [12], which are alternative substrates for the reductive reaction increase oxalate formation. The possibility that a perturbation of the $[NAD^+]/[NADH]$ ratio concomitant with the oxidation of xylitol to D-xylulose might increase oxalate formation indirectly by affecting the lactate dehydrogenase catalysed oxidation of glyoxylate to oxalate, which is the final step in oxalate biosynthesis [5, 13] was investigated because of the negative results obtained in the studies with $^{14}C$-labelled xylitol and other compounds (Table 1). The effect of $NAD^+$, pyruvate, and NADH, were those which were predicted from a consideration of the scheme shown in Fig. 1, and from previous work [5, 11, 12], but xylitol decreased rather than increased oxalate formation presumably by competing for $NAD^+$. This finding agrees with the recent report [14] of reduced cytoplasmic $[NAD^+]/[NADH]$ ratios when the rat's liver is perfused with a medium contining xylitol.

The present results suggest that xylitol induced calcium oxalate nephropathy is related to either the individual patient's clinical state or to his genetic constitution. In the latter case, an enzyme, which catalyses one of the metabolic reactions of glyoxylate other than oxidation to oxalate might be abnormally sensitive to inhibition by xylitol or a xylitol metabolite.

The adverse reactions to methoxyflurane in which there is renal tubule damage and calcium oxalate nephrophathy [15, 16] present a similar problem and are also unexplained in biochemical terms [13].

## REFERENCES

1. D. W. Thomas, J. B. Edwards, J. E. Gilligan, J. R. Lawrence and R. G. Edwards, *Med. J. Aust.* **i**, 1238 (1972).
2. D. W. Thomas, J. E. Gilligan, J. B. Edwards and R. G. Edwards, *Med. J. Aust.* **i**, 1246 (1972).
3. C. A. Pons and R. P. Custer, *Am. J. Med. Sci.* **211**, 544 (1946).
4. E. F. Scowen, A. G. Stansfeld and R. W. E. Watts, *J. Path. Bact.* **77**, 195 (1959).
5. D. A. Gibbs and R. W. E. Watts, *Clin. Sci.* **44**, 227 (1973).
6. D. A. Gibbs and R. W. E. Watts, *J. Lab. clin. Med.* **73**, 901 (1969).
7. S. B. Andrus, S. N. Gershoff and F. F. Faragella, *Am. J. Path.* **35**, 671 (1959).
8. S. N. Gershoff, F. F. Faragella, D. A. Nelson and S. B. Andrus, *Am. J. Med.* **27**, 72 (1959).
9. S. R. Faber, W. W. Feitler, R. E. Bleiler, M. A. Ohlson and R. E. Hodges, *Am. J. clin. Nutr.* **12**, 406 (1963).
10. D. A. Gibbs and R. W. E. Watts, *Clin. Sci.* **38**, 277 (1970).
11. M. Romano and M. Cerra, *Biochim. biophys. Acta* **177**, 421 (1969).
12. H. E. Williams and H. L. Smith, *Science, N.Y.* **171**, 390 (1971).
13. R. W. E. Watts, *Jl. Roy. Coll. Phycns, Lond.* **7**, 161 (1973).
14. H. F. Woods and H. A. Krebs, *Biochem. J.* **134**, 437 (1973).
15. J. A. Frascino, P. Vanamee and P. P. Rosen, *New Engl. J. Med.* **283**, 676 (1970).
16. A. C. Aufderheide, *Archs Path.* **92**, 162 (1971).
17. B. M. Dean, R. W. E. Watts and W. J. Westwick, *Clin. Sci.* **35**, 325 (1968).