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In-vivo studies on C_2 organic acids in the tissues of rats injected with xylitol and glucose

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With 1 figure and 2 tables

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The metabolism of the metabolically related C_2 acids glycollate, glyoxylate and oxalate has been studied thoroughly with regard to primary hyperoxaluria and oxalosis (1, 2).

Oxalate has been implicated as the cause of the reported toxicity of xylitol administered parenterally in man (3, 4), although these reports have not been substantiated by other work (5). The functional significance of these C_2 acids, particularly glycollate, in carbohydrate metabolism has been indicated by the finding of greatly increased renal excretion of glycollate after xylitol infusion in man (5).

Except for the C_1 and C_2 carbon atoms of ascorbic acid the only known precursor of oxalic acid in mammalian system is glyoxylate.

Glyoxylate is formed from glycine by transamination or by oxidative deamination, by the oxidation of glycollate and from L-hydroxyproline via 2-hydroxy-4-oxo-glutarate.

The major endogenous source of glycollate is glycolaldehyde, formed directly from hydroxypyruvate or from ethanolamine via the glycine-serine pathway (6). Intracellular mechanisms by which glycollate and possibly glyoxylate and oxalate are formed from xylitol are not known, and the present study was undertaken to determine whether in the intact rat xylitol injection leads to an intracellular increase of the C_2 acids. The effect of xylitol injection on amino acids was also examined.

Materials and methods

Experimental procedure

Fasted male rats (200 g) were injected intravenously with [U-^{14}C] glucose or [U-^{14}C-xylitol] (40 µci; 252 µmole), purified before use by ion exchange chromatography. Two hours after injection the animals were killed by decapitation, liver, kidney, heart, and brain were removed as quickly as possible and frozen in liquid nitrogen. The organs were ground thoroughly in a precooled iron mortar, homogenized with 0.2M perchlorid acid, and centrifuged. The supernatant solution was neutralized with 2M potassium hydroxide to remove the excess perchlorate.

An aliquot of the neutralised supernatant was applied to tandem columns consisting of a cation-exchange column (1 × 9 cm of lewatit s 1080 in the hydrogen

form, 100–200 mesh, from E. Merck, Darmstadt) (7) followed by an anion exchange column (1 × 10 cm of Lewatit M 5080 in the acetate form 100–200 mesh, from E. Merck, Darmstadt).

The columns were eluted with 8 × 10 ml portions of water and then separated. The cation exchange column was eluted with 8 × 10 ml portions of 2M aqueous ammonium hydroxide and the anion exchange column five times with 8 × 10 ml portions each of aqueous formic acid in the increasing concentrations 0,2M, 0,5M, 1M, 4M, and 4M to which 2M ammonium formiate was added. Monitoring of the radioactivity of the individual fractions showed that 100 % of the radioactivity applied to the column was recovered. Elution patterns in terms of dpm/ml for each fraction were drawn and the distribution of activity in the various organs and fractions determined. Fractions showing the maximal radioactivity were selected and analysed for glucose, glycogen, xylitol, organic acids, and amino acids as appropriate using the methods outlined below.

Analytical methods

Glucose was measured by a standard method using hexokinase, glycogen by the modified method of Good et al. (8) and xylitol by the modified method of Maurer and Christophus (9). Organic acids were determined in formic acid fractions using gas chromatography and mass spectrometry (10). Detailed examinations of the glycollate region of the chromatogram was made by cyclic scanning for seven ions simultaneously as described previously (5). Oxalic acid was also measured enzymatically in the neutralised supernatant by the modified method of Mayer et al. (11). Thin-layer chromatography was used for identifying labelled amino acids.

Results

After intravenous injection of either ^{14}C-xylitol or ^{14}C-glucose, the majority of the radioactivity was located in the liver and blood (table 1). The radioactivity after chromatography on the tandem ion exchange system was primarily distributed in the water (neutral components), ammonia (amino acids) and 0.2M formic acid (weak organic acids) fractions (table 1, fig. 1).

These fractions were analysed in greater detail as described above, the 0.2M formic acid fractions contain weak acids including lactate and glycollate, pyruvate normally occurs in the 1M formic acid fraction, and di- and tricarboxylic acids and phosphate esters in the 4M formic acid + 2M ammonium formate fractions.

Only glucose and glycogen but no xylitol could be detected in the neutral fractions eluted from the tandem column. Application of xylitol led to an increase in glucose and glycogen content in liver when compared to application of glucose: glucose contents were 10.5 (glucose injected) and 14.6 (xylitol injected) µmol/g liver, and glycogen contents were 130 (glucose injected) and 176.5 (xylitol injected) µmol/g liver.

Table 2 lists the level of organic acids observed in the 0.2M formic acid fractions from the liver and kidneys of both ^{14}C-glucose and ^{14}C-xylitol injected rats. Glycollic acid was observed only in the tissues of the xylitol injected animals. No oxalic acid could be detected enzymatically in the tissues of any of the injected animals. Glyceric acid occurred at detectable levels only in the liver of the xylitol-injected rats.

Thin-layer chromatography and amino acid analysis of the ammonium hydroxide fractions revealed that among the amino acids claimed to be

Table 1. Recovery and distribution of radioactivity in different organs of the rat after intravenous injection of [U-^{14}C]-Xylitol and [U-^{14}C]-Glucose.

Organ		Radio-activity of injected dose %	Distribution of Radioactivity (%) in fractions from ion exchange chromatography eluted with the following compounds:						
			H_2O	2N NH_4OH	0.2N HCOOH	0.5N HCOOH	1N HCOOH	4N HCOOH	4N HCOOH + 2N NH_4OOH
Liver	^{14}C-Xylitol	1.4	36	27	22	2.9	3.6	3.3	4.7
	^{14}C-Glucose	2.1	63	17	11	2.7	2.4	1.1	3
Kidney	^{14}C-Xylitol	0.6	20	16	56	2.6	2.7	1.2	1.7
	^{14}C-Glucose	0.33	40	27	27	3	3	1.4	1.8
Heart	^{14}C-Xylitol	0.16	14	23	40	3.6	6.1	7.7	5.7
	^{14}C-Glucose	0.28	18	25	45	4.6	3.6	0.6	3.4
Brain	^{14}C-Xylitol	0.29	3	75	19	1.8	1	–	0.5
	^{14}C-Glucose	0.62	2	64	17	6.9	5.7	2.2	2
Blood	^{14}C-Xylitol	1.27	56	8	35	–	0.5	–	–
	^{14}C-Glucose	1.7	47	47	5	0.4	3.8	0.7	0.6

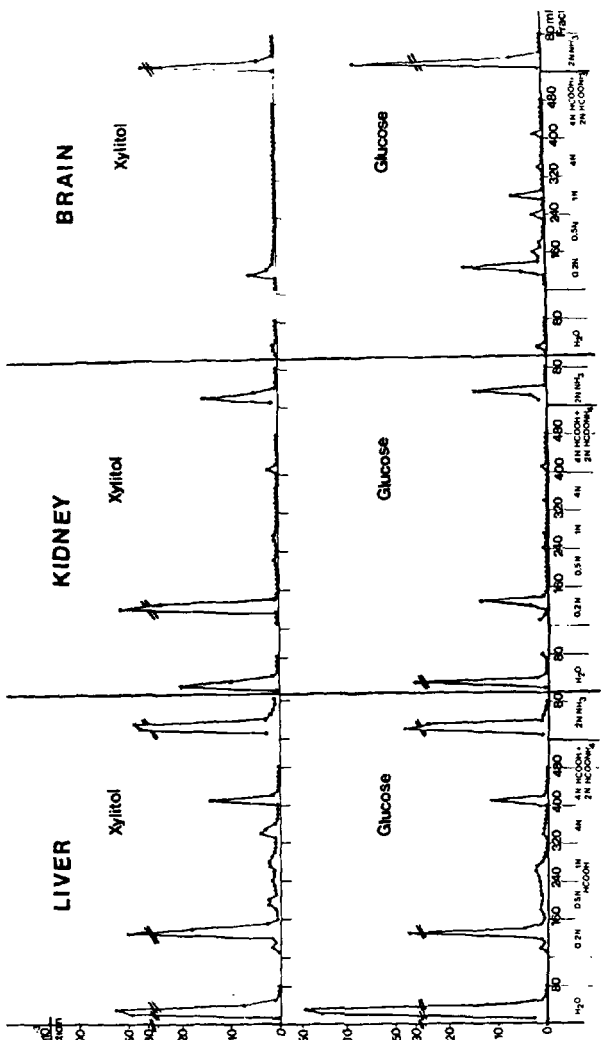

Fig. 1. Chromatographic separation of intermediates in perchloric acid extracts of rat organs after intravenous injection of ^{14}C-labelled glucose or ^{14}C-xylitol.

Table 2. Organic acids in the tissues of rats injected with ^{14}C-xylitol or ^{14}C-glucose.

Acid	µg*) per g wet tissue Liver Xylitol	Glucose	Kidney Xylitol	Glucose
Lactic	1313	1448	1348	955
Glycollic**)	99	Nil+)	101	Nil
3-Hydroxybutyric	98	134	Nil	Nil
Succinic	394	386	428	351
Glyceric	6	Nil	Nil	Nil
Tetronics***)	47	34	175	88
Gluconic (plus some glucuronic)	57	80	Nil	Nil

*) Assuming the GC detector response is unity with respect to that of the n-tetracosane internal standard. Response factors of the derivatives of these acids relative to n-tetracosane range from 0.8 to 1.1.

**) Estimated from the mass fragmentograms of the specific ions for lactate and glycollate.

***) Erythronic plus threonic acids, with erythronic predominating in all cases.

+) Not observed; the detection limits for the acids shown here are about 2 µg per g wet tissue.

precursors of oxalate (12) only glycine and aspartic acid were found to be significantly labelled after injection with either substrate. There were no significant differences in the amount of labelling after glucose or xylitol injection.

Discussion

The incorporation of injected glucose or xylitol into liver, heart, kidney, brain and blood is low (table 1), not exceeding 5 % in total. This suggests that 2 hours after a single dose of the substrates they have been primarily converted into CO_2 or excreted in the urine. A small amount may also be incorporated into skeletal muscle, adipose tissue, or diaphragm. The elution patterns from the tandem ion exchange system were similar for both substrates.

In the neutral fractions from liver and kidney only glucose and glycogen but no xylitol could be detected indicating that the injected amount of ^{14}C-xylitol has been either converted to glucose, degraded or excreted in the urine.

Utilization of xylitol in liver (13, 14) and kidney (15) has also been reported by other authors. The increase in the glucose and glycogen content in livers after xylitol injection suggests that most of the xylitol has been converted to glucose.

The fractions containing amino acids and weak organic acids were of greatest interest in determining the influence of injected xylitol on known oxalate precursors.

A number of amino acids, glycine, serine, tryptophan, hydroxyproline phenylalanine, tyrosine, aspartate, and asparagine have been identified as

oxalate precursors (12), glycine being the predominant amino acid precursor (1, 2, 12). In the present experiments radioactivity was detected in glycine, with no difference between glucose- and xylitol-injected rats. It would appear therefore that xylitol does not produce an altered metabolism of glycine.

The most significant finding was the occurrence of glycollic acid in both liver and kidney after xylitol injection (table 2). No glycollic acid was observed after glucose injection. This finding is in accord with previous in-vivo studies in humans infused with glucose and xylitol (5). Pathways leading from xylitol to glycollate need to be further investigated.

The main endogenous source of glycollate are glycollaldehyde derived from ethanolamine via the glycine-serine pathway or from hydroxypyruvate, and glyoxylate. Production from glyoxylate via glycine appears unlikely in the absence of increased radioactivity in the glycine of the liver and kidney.

The oxidation of glycollaldehyde to free glycollate via the transketolase reaction by natural oxidants has been discussed in plants (16). Whether this mechanism underlies the present work or whether glycollate is formed from intermediates of the glycolytic pathway, i.e. from 3-phosphoglycerate, remains to be clarified.

This work has shown that xylitol injected into rats produces an increase in liver and kidney glycollate without an associated increase in oxalate. This is in accord with the occurrence of glycollic aciduria without hyperoxaluria in xylitol-infused patients and adds support to the suggestion that the renal and extrarenal oxalosis reported in xylitol-infused patients (3, 4) is due to factors other than the metabolism of xylitol itself and unrelated to thiamine or pyridoxine nutritional status (5, 17).

Summary

Oxalic, glyoxylic, and glycollic acid were determined in rat liver and kidney after injection with [U-^{14}C]-xylitol or [U-^{14}C]-glucose. Neither glucose nor xylitol led to the formation of oxalic and glyoxylic acid, yet glycollic acid was found in both tissues after injection with xylitol.

Possible pathways leading from xylitol to glycollic acid are discussed.

Zusammenfassung

Oxal-, Glyoxyl- und Glykollsäure wurden in Rattenleber und -niere nach Injektion von [U-^{14}C]-Xylit oder [U-^{14}C]-Glucose bestimmt. Weder Glucose noch Xylit führten zu einer Oxal- oder Glyoxylsäurebildung. Glycollsäure wurde jedoch in beiden Geweben nach Injektion von Xylit gefunden.

Mögliche Stoffwechselwege vom Xylit zur Glykollsäure werden diskutiert.

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References

1. Watts, R. W. E., J. Roy. Coll. Physcns. Lon., **7**, 161 (1973).
2. Williams, H. E., and L. H. Smith Jr., Amer. J. Med. **45**, 715 (1968).
3. Thomas, D. W., J. B. Edwards, J. E. Gilligan, J. R. Laurence, and R. G. Edwards., Med. J. Australia **3**, 1238 (1972).
4. Schröder, R., W. F. De Lacroix, J. Franzen, P. J. Klein, and W. Müller., Acta Neuropathol., **27**, 181 (1974).
5. Hauschildt, S., R. A. Chalmers, A. M. Lawson, K. Schulthis, and R. W. E. Watts., Amer. J. Clin. Nutr. **29**, 258 (1976).
6. Dean, B. M., R. W. E. Watts, W. J. Westwick, Clin. Sci. **35**, 325 (1968).
7. Katz, J., K. Brand, S. Goldberg, and D. Rubinstein, Cancer Res. **34**, 872 (1974).
8. Good, C. A., H. Kramer, and M. Somogyi, J. Biol. Chem. **100**, 485 (1933).
9. Maurer, C., and P. Christophus, Z. Klin. Chem. Klin. Biochem., **11**, 535 (1973).
10. Chalmers, R. A., and R. W. E. Watts, Analyst. (Lond.), **97**, 958 (1972).
11. Mayer, G., D. Markow, and F. Karp, Clin. Chem., **9**, 334 (1963).
12. Hagler, L., and R. H. Herman, Amer. J. Clin. Nutr. **26**, 758 (1973).
13. Müller, F., E. Strack, E. Kuhfahl, and D. Dettmer, Z. Ges. Exp. Med., **142**, 338 (1967).
14. Bässler, K. H., G. Stein, and W. Belzer, Biochem. Z. **346**, 171 (1966).
15. Quadflieg, K. H., and K. Brand, Z. Ernährungswiss., **15**, 346 (1976).
16. Asami, S., and T. Akazawa, Plant and Cell Physiol., **16**, 805 (1975).
17. Oshinsky, R. J., Y. M. Wang, and J. Van Eys, J. Nutr. **107**, 792 (1977).

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