FATE OF ^{14}C-D-MANNOHEPTULOSE IN THE RAT

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Received October 5, 1970

Summary: Uniformly labeled ^{14}C-D-mannoheptulose was administered subcutaneously to rats. 82 - 87% of the injected radioactivity was excreted within 6 hours in the urine as mannoheptulose and only about 3% was found as $^{14}CO_2$ in the expired air; 8 - 14% of the mannoheptulose was recovered from the carcass. The total recovery as mannoheptulose in carcass and excreta amounted to 95 - 96%. No radioactive metabolite was detected in the internal organs, blood or urine. It is concluded that mannoheptulose itself, rather than a degradation product, acts as a diabetogen.

Introduction

Our previous investigations have shown that parenteral administration of mannoheptulose to laboratory animals evokes a diabetic syndrome[1]. Excretion studies revealed that 67% of the administered mannoheptulose appeared in the urine, as measured by the Dische orcinol method, within 24 hours[2]. The present study attempts, with the help of labelled mannoheptulose, to answer the question: Is mannoheptulose metabolized in mammals, and if so, is mannoheptulose itself the diabetogen or is a metabolite of mannoheptulose involved in its diabetogenic action?

Materials and Methods

Male Wistar-derived albino rats of the Biodynamics Department strain, 200 g in weight, were used in all metabolic studies. The animals were fasted for 18 - 24 hours and given water ad libitum during the time they were kept in the metabolism cages.

Delmar (Scientific Lab., Maywood, Ill.) all glass metabolic cages were used with the following modifications: a) The scrubbers for trapping $^{14}CO_2$ in

the expired air were replaced by glass columns of 50 mm I.D. and 40 cm effective height, packed with 750 g 4 mm glass beads and 120 ml 5 N NaOH. Appropriate adapters and tubing connected the scrubber with the metabolic chamber and the vacuum pump. A trap containing saturated $Ba(OH)_2$ served to detect any CO_2 in the emerging air. b) A drying column filled with drierite and silica gel, connected to the air inlet at the top of the metabolic chamber, served to minimize the amount of CO_2 entering the system. A flow meter was attached to the inlet of the drying column and the flow rate maintained during experiments at minimal levels consistent with the physical well-being of the animal. Under these conditions no cloudiness appeared in the $Ba(OH)_2$ trap during control experiments for periods up to 24 hours. c) A conical glass diverter was used to effect more efficient separation of urine from feces.

Uniformly labelled ^{14}C-D-mannoheptulose was a gift of Dr. R. C. Bean (University of California, Riverside), who prepared it photosynthetically[3] by subjecting Avocado leaves to $^{14}CO_2$. The radioactive mannoheptulose was purified by the Riverside group by treatment with glucose oxidase and ion exchange to remove gluconic acid as well as other ionic material. We subjected the radioactive material to paper chromatography in four systems; I, n-butanol: ethanol: borate buffer (40:11:19); II, n-butanol: ethanol: water (40:11:19); III, phenol: water (pH 5.5, NH_3); and IV, ethylacetate: pyridine: water (8:2:1). System I is the only one able to separate glucose from mannoheptulose[4]. This separation occurs only during prolonged runs, the time required for separation being shortened by higher borate concentrations. Using saturated $NaB_4O_7 \cdot 10\ H_2O$, separation is achieved in 20-24 hours. Analysis of chromatograms on the Vanguard Autoscanner 880 showed that the material contained traces of radioactive impurities. Purification was achieved by application of preparative scale paper chromatography, applying 30μCi (equivalent to about 2 mg) to Whatman number 1 paper, using system II. Non-radioactive mannoheptulose served as a marker. The eluates in water were combined and assayed for radioactivity in the Packard Scintillation Spectrometer Model 3002 as described by Lindner[5].

On re-chromatography, less than 0.1% radiochemical impurities were found. Quench corrections were made by re-counting the samples after adding a known amount of U-^{14}C- D- glucose.

Results

Efficiency of the recovery of radioactivity of CO_2 was determined in the following manner: to the water reservoir sidearm of the chamber was attached a vessel containing 100 - 150 mg. $Na_2{}^{14}CO_3$ in solution (100μCi), the anticipated amount of CO_2 expired by a 200 g rat during one hour. All connections were of glass. A small buret containing 0.1 N HCl fitted into this vessel and was opened to permit flow of acid at such a rate as to liberate the total CO_2 in approximately one hour. In three experiments recoveries were 94, 97 and 101%.

Preliminary experiments were carried out with small amounts of radioactive mannoheptulose (1μCi per animal). The objective of these trials was to determine whether any $^{14}CO_2$ could be detected in the expired air following administration of ^{14}C- mannoheptulose. Three rats were used. The animals were allowed to become accustomed to the chamber for about 2 hours before injection. Up to 0.5% of the injected radioactivity appeared in the expired air as CO_2 within 2 hours. Analysis of the organs showed that the pancreas contained 0.04% ^{14}C-mannoheptulose; in the nephrectomized animal, its concentration was increased tenfold, but the liver content of mannoheptulose was similarly increased.

In a subsequent experiment, each of two animals received 3 ml of ^{14}C-D-mannoheptulose (15μCi). The animals remained in the cages for 6 hours. Every 2 hours, the scrubbing columns were changed, the volume of the NaOH solutions measured and a sample taken for counting. The columns were washed twice with 100 ml distilled water and the radioactivity in the washings was also determined. After 6 hours, the urine was removed from the collecting vessel and the apparatus rinsed with distilled water. These washings were combined with the urine, bringing the volume to 50 ml, from which samples were taken for counting. The animals were killed and organs removed and

weighed. The organs were homogenized in a known volume of 0.3 N $Ba(OH)_2$ solution in a Ultra-Turrax homogenizer and deproteinized with 5% $ZnSO_4 \cdot 7\ H_2O$. After centrifugation, samples of the supernatant were taken for counting. In these experiments, the pancreas was included with the carcass.

Table 1. Distribution of ^{14}C-D-mannoheptulose in the Rat

Animal No.	1	2	3	4	5
			Nephrectomized		
Radioactivity injected*	1μCi	1μCi	1μCi	15μCi	15μCi
Carrier injected (mannohept.)	-	100 mg	-	-	-
Duration of Experiment	2 hr	2 hr	2 hr	6 hr	6 hr
Recovery of Radioactivity**					
Urine	63.6	80.1	-	87.2	82.0
Expired CO_2	0.5	0.1	0.3	3.3	3.4
Liver	1.8	1.5	22.9	0.5	0.7
Kidney	-†	-†	-	0.6	1.5
Spleen	0.03	0.03	-†	0.02	0.03
Pancreas	0.04	0.04	0.35	-†	-†
Intestine	-†	-†	-†	1.4	0.9
Remaining carcass	24.7	11.9	68.1	5.2	11.5
Total Recovery**	90.7%	93.7%	91.7%	98.2%	100.0%

*Specific Activity = 3.3 mCi/m-mole.

**Percentage of injected dose, measured as mannoheptulose (urine, organs and carcass) or CO_2 (expired air).

†Treated with remaining carcass.

The remainder of the carcass was dropped into a 2 liter beaker containing 800 ml 2N KOH and boiled for 30 minutes. A portion of the solution was removed, cooled, neutralized, centrifuged and aliquots deproteinized with $Ba(OH)_2$ and $ZnSO_4$ in a manner similar to that used on tissues.

Radiochromatography of Urine and Tissues.

Chromatography of urine samples from the animals was performed in solvent systems I and II. These showed only a single component, migrating at the rate of authentic D-mannoheptulose markers. Repeated scanning at highest sensitivity revealed no radioactive component differing in chromatographic mobility from D-mannoheptulose. Chromatography of the liver homogenates, other tissues and the carcass also revealed only a single radioactive component, D-mannoheptulose.

Discussion

Evidence has been presented that uniformly labelled ^{14}C-D-mannoheptulose is metabolized in the rat only to a very small extent. 80 - 90% of this sugar is excreted unchanged in the urine within 6 hours.

In Avocado leaves[6], infiltration studies with labelled mannoheptulose and glucose revealed that "catabolic products (malate, asparagine, glutamate, alanine) were similar but differed quantitatively. However, despite active synthesis of mannoheptulose and glucose, no interconversion of hexose and heptulose structures was detected even during incubation of 36 hours."

In view of the very limited degradation of mannoheptulose in the rat, the elucidation of its catabolic pathway presents great difficulty. Since we were unable to detect any metabolite of mannoheptulose in tissues, blood or urine, we are inclined to conclude that mannoheptulose itself acts as a diabetogen.

Acknowledgment

The authors are grateful to Mr. Albert Almoznino for skilled technical assistance.

This work was supported in part by N.I.H. Research Grant AM 05701-05 MET.

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