Metabolism of maltitol by conventional rats and mice and germ-free mice, and comparative digestibility between maltitol and sorbitol in germ-free mice

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The metabolism of maltitol (4-α-D-glucosylsorbitol) was assessed in fasting conventional (C) rats, C mice and germ-free (GF) mice, using [U-^{14}C]maltitol. The radiorespirometric patterns of $^{14}CO_2$ collected for 48 h after the administration of labelled maltitol were characterized by a constant rate of $^{14}CO_2$ production lasting 4 h for both C rats and mice. The pattern for the GF mice showed a peak at the second hour followed immediately by a slow decrease. The percentage recovery of $^{14}CO_2$ was significantly lower for the GF mice (59%) compared with C animals (72–74%). Urine, faeces and intestinal contents after 48 h totalled 19% of the administered radioactivity in the C rats and mice and 39% in the GF mice. The digestibility of maltitol and the absorption of sorbitol in GF mice was also assessed. The caecum and small intestine of GF mice, 3 h after administration of equimolar quantities of maltitol (140 mg/kg body-weight) or sorbitol (70 mg/kg body-weight), contained 39 and 51% of the ingested dose respectively, present mostly in the caecum as sorbitol. The α-glucosidase (maltase) (*EC* 3.2.1.20) activity of the small intestine was appreciably higher (1·5–1·7 times) in the GF mice than in the C mice. These results suggest that the enzymic activities in the small intestine of mice and rats are sufficient to hydrolyse maltitol extensively. Consequently, the slow absorption of sorbitol seems to be an important factor limiting the overall assimilation of maltitol in the small intestine.

Maltitol metabolism: Sorbitol metabolism: Polyol digestion: Mouse: Rat

Many alternative bulk sweeteners have been proposed over the last 20 years and several of them are found in so-called 'sugar-free' products in various countries. These bulk sweeteners, which are mostly polyols, are characterized by a low cariogenicity and are absorbed and metabolized to a lesser extent than sucrose (Imfeld, 1977; Ziesenitz & Siebert, 1987). The disaccharide alcohol maltitol has a sweetness approaching that of sucrose in intensity and quality (Fabry, 1988). In addition, many studies have shown that maltitol does not cause a decrease in human dental plaque pH to the same extent as dietary carbohydrates (Würsch & Koellreutter, 1982; Imfeld & Lutz, 1984), nor does it promote the synthesis of dental plaques (Saxer, 1984). Maltitol has been shown to be slowly digested by the enzymes of the small intestine (Würsch & Del Vedovo, 1981; Rosiers *et al.* 1985; Ziesenitz & Siebert, 1987) and it produces a blunted blood glucose response when compared with sucrose (Felber *et al.* 1987). Due to its slow digestion, part of the polyol and its breakdown products reach the large intestine and are metabolized by the colonic bacteria (Beaugerie, 1987; Würsch *et al.* 1989). The dose that can be tolerated without undesirable symptoms such as flatus production and abdominal discomfort has been estimated to be between 20 and 30 g maltitol syrup (580 g maltitol/kg) (Abraham *et al.* 1981).

Only a few methods are available to discriminate between host and microbial

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contribution to total carbohydrate utilization (Ziesenitz & Siebert, 1987; Würsch & Anantharaman, 1989). Among them, studies with radiolabelled substrates have provided valuable information on substrate absorption and metabolism in the body by following the recovery of labelled CO_2, and by the analysis of radioactivity in the urine and faeces and of its incorporation in the organs. It has been applied to the study of the metabolic fate of [^{14}C]maltitol in rat and man (Rennhard & Bianchine, 1976; Zanolo & Giachetti, 1987). This method, however, does not always allow the quantification of the unabsorbed fraction, because the $^{14}CO_2$ recovered from expired breath arises from the metabolism of the sugar by the host and by the intestinal bacteria, and from the metabolism by the host of the short-chain fatty acids produced by the (colonic) bacteria. This problem can be circumvented by using germ-free animals (GF), thus limiting the metabolism to that of the absorbed sugars.

The present paper describes the metabolic fate of [^{14}C]maltitol administered to conventional (C) rats and mice and GF mice. In addition, the in vitro digestibility of maltitol by the intestinal enzymes of both types of mice was compared and finally the in vivo hydrolysis and absorption in the small intestine of GF mice was assessed and compared with the absorption of pure sorbitol.

MATERIALS AND METHODS

[U-^{14}C]maltitol with a specific radioactivity of 1000 kBq/mg (Amersham International plc, Amersham, Bucks) exhibited a radiochemical purity higher than 98% when analysed with a radioactivity detector (Berthold) after thin-layer chromatography (Würsch & Roulet, 1982). Crystalline maltitol (Malbit cryst ECS; > 98%) was obtained from Melida (Milan, Italy) while other chemicals were obtained from Merck (Darmstadt, West Germany). The sorbitol kit was purchased from Boehringer (Mannheim, West Germany).

Metabolism of [U-^{14}C]maltitol

C male Sprague–Dawley rats (240 (SD 80)g), female C mice (36 (SD 13) g) and female GF mice OF1 (32 (SD 8) g; IFACREDO, L'Arbresle, France) were fed *ad lib.* before the test on a basal purified AIN 76 diet containing maize starch as the main source of carbohydrate (Anon., 1977). The GF mice were born and were housed in a sterile isolator and their pelleted diet was sterilized by γ-irradiation (30 kGy). Maltitol was supplied as a solution in water (50 mg/ml for rats and 10 mg/ml for mice). For the experiment with GF mice, the solution was sterilized by autoclaving at 115° for 10 min. After an overnight fast, 180 kBq [U-^{14}C]maltitol with 140 mg unlabelled maltitol/kg body-weight were administered to the animals by gastric intubation. A precise quantity of solution containing the amount of substance to be administered was weighed into individual tubes. The total radioactivity administered was calculated from the radioactivity prepared in the tubes and the radioactivity remaining after the administration of the solution. Immediately after administration, C rats and mice were placed in individual glass metabolism cages (2·5 litre volume) from where expired CO_2 was trapped in a methanol solution containing ethanolamine (120 g/l) (Würsch *et al.* 1979). The air flow was set to 0·8 litres/min. In the case of GF mice, sterilized (autoclaved) glass metabolism cages including sterile solutions (sterilized by membrane filtration; Millipore, Molsheim, France), were transferred through a lock chamber into the isolator. During the experiment, the animals had no food but had free access to water.

Urine was collected over the 48 h test period and radioactivity in a representative portion was measured using a liquid-scintillation counter (Betarack: LKB, Uppsala, Sweden). Radioactivity in the faeces and the intestinal contents was measured after combusion of the material (Biological Oxidizer, OX-300; Harvey Instruments Corp., Hillsdale, NJ, USA).

A portion of the faeces of C rats was dispersed in 10 vol. 50% ethanol and allowed to stand overnight at 20°. The suspension was centrifuged and the sediment washed with the same volume of solvent. Radioactivity was measured in the combined supernatant fractions and the residue. The combined supernatant fractions were acidified with 1 ml 0·5 M-sulphuric acid, evaporated to dryness, heated at 100° for 4 h, and finally the radioactivity was measured in the residue.

Intestinal content of polyols in the GF mice

Overnight fasted GF female mice OF1 (27 (SE 3) g) received 140 mg maltitol/kg body-weight or 70 mg sorbitol/kg body-weight in a precise volume approximating 0·5 ml water by stomach tube. After 3 h, they were killed by diethyl ether anaesthesia, and the stomach, small intestine, caecum and colon were removed immediately. The contents of the first two organs were washed with 3 ml distilled water, while the caecum and colon were homogenized with a Polytron homogenizer (Kinematica, Lucerne, Switzerland) in 4 ml distilled water. An internal standard of 1 mg *myo*-inositol and an equal volume of ethanol were added to the washout solutions and the mixture was centrifuged, evaporated to dryness under vacuum and the same volume of water was added. Free sorbitol was determined enzymically (Beutler, 1984). A portion was freeze-dried and the sugars were analysed by gas–liquid chromatography as described elsewhere (Preuss & Thier, 1982), using maltitol and *myo*-inositol as reference standards.

Disaccharidase activity in the small intestine

Six C and six GF mice were killed after an overnight fast and their small intestines were carefully withdrawn. The lumen was washed by passing 5 ml cold saline (9 g sodium chloride/l) through the intestine. The intestine was then opened and the interior carefully scraped with a spatula. The material was suspended in 1 ml 50 mM-sodium phosphate buffer, pH 7·0 containing Triton (10 g/l). The suspension was homogenized with a Polytron homogenizer and was centrifuged 1 h later at 3000 ***g*** for 30 min (4° throughout). Protein was measured as described by Lowry *et al.* (1951).

Disaccharidase activity was measured as follows: 50 μl 100 mM-maltose or maltitol and 50 μl enzyme solution (jejunum solution diluted 1:30 for maltose determination) were incubated for 10 min for the jejunum solution and for 30 min for the caecum and colon solutions at 37°. Released glucose was measured using the glucose oxidase (*EC* 1.1.3.4)–peroxidase (*EC* 1.11.1.7) reagent (Dahlqvist, 1964). The activity was expressed as the amount of substrate hydrolysed per min.

Statistical analysis

Variability is expressed as standard error (SE) of the mean. Values were analysed using the unpaired *t* test.

RESULTS

The radiochemical balance study of maltitol

The rate of $^{14}CO_2$ collection in C rats and mice was constant between the second and the fifth hour after the administration, with a slight delay in the C rats (Fig. 1). The pattern of $^{14}CO_2$ collection in GF mice was similar to that in C mice during the first 3 h. After a maximum reached at 2 h, the rate of $^{14}CO_2$ collection fell significantly thereafter. At 8 h, the cumulative recovery of $^{14}CO_2$ was 33% lower ($P < 0{\cdot}025$) in GF mice when compared with the C mice.

The total expired $^{14}CO_2$ over 8 and 48 h was similar for both groups of C animals (Table 1). The urinary loss of radioactivity, in great part as sorbitol, in C rats and mice was 4·2

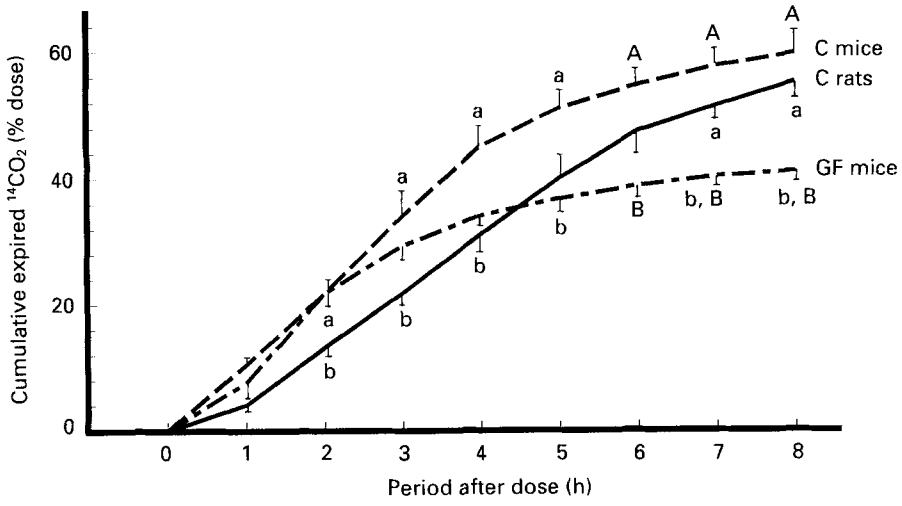

Fig. 1. Mean cumulative excretion of $^{14}CO_2$, after an intragastric dose of [U-^{14}C]maltitol and 140 mg/kg unlabelled maltitol; by conventional (C) rats (—), C mice (- - -) and germ-free (GF) mice (- · - · -). Values represent the means, with their standard errors represented by vertical bars. [a, b] Means with different letters were significantly different ($P < 0.025$). [A, B] Means with different letters were significantly different ($P < 0.005$).

Table 1. *Percentage recovery of radioactivity following intragastric administration of 140 mg [U-^{14}C]maltitol/kg to conventional (C) rats and mice and germ-free mice*

(Mean values with their standard errors for three rats or four mice/group)

	C rats		C mice		Germ-free mice	
	Mean	SE	Mean	SE	Mean	SE
CO_2						
8 h	54·7	2·4	59·3	3·4	40·7	1·2
8–48 h	17·3	1·3	15·3	1·1	18·2	1·6
Total	72·2[A]	2·3	74·6[a]	4·1	59·0[b, B]	1·4
Urine	4·2	0·4	5·9	0·8	10·7	1·1
Intestinal contents	3·4	1·2	10·2	3·0	21·1	0·1
Faeces	11·7†	1·2	3·2	1·1	7·3	0·06
Total balance of radioactivity	92·0	2·0	95·5	4·0	98·0	0·06

[a, b] Means with different superscript letters were significantly different ($P < 0.025$).
[A, B] Means with different superscript letters were significantly different ($P < 0.01$).
† 83 (SE 8)% of these faeces were soluble in 50% ethanol.

and 5·9% respectively over 48 h. The radioactivity recovered in the faeces and in the intestinal content was 15·1 and 13·4% respectively. Neither maltitol nor sorbitol was found, but a large part of the radioactivity (83% in C rats) was found to be soluble in ethanol–water and volatile in the acidic medium, suggesting that the radioactivity was incorporated into short-chain fatty acids and in the bacterial cell mass. Negligible radioactivity was found in the stomach. The total recovery of radioactivity in expired $^{14}CO_2$, urine and faeces was greater than 90%, indicating that a very low incorporation of the label into the carcass occurred.

Table 2. *Recovery (% of administered dose) of sorbitol and maltitol in the intestinal tract of germ-free mice, 3 h after intragastric administration of sorbitol or maltitol (70 mg/kg and 140 mg/kg respectively)*

(Mean values with their standard errors)

	Sorbitol				Maltitol				Total (%)	
	Small intestine		Caecum		Small intestine		Caecum			
	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Expt 1										
Sorbitol (*n* 5)	45	6·5	30	7·0	—		—		75[a]	9·4
Maltitol (*n* 5)	25	1·6	14	2·0	2	1·0	0·4	0·3	41[b]	1·7
Expt 2										
Sorbitol (*n* 5)	6	2·0	44	9·0	—		—		51	9·4
Maltitol (*n* 8)	6	1·8	28	1·9	0·0		5	2·0	39	2·8

[a, b] Means with different superscript letters were significantly different ($P < 0·01$).

Table 3. *Disaccharidase specific activities (units†) of brush border enzymes of the small intestine, caecum and colon of conventional (C) and germ-free (GF) mice*

(Mean values with their standard errors for six mice)

	Maltose		Maltitol	
	Mean	SE	Mean	SE
C mice				
Small intestine	280[a]	20	10[b]	0·7
GF mice				
Small intestine	377[c]	8	15[d]	0·5
Caecum	8	2·6	2	0·7
Colon	22	3·2	2	0·5

[a, b, c, d] Means with different superscript letters were significantly different ($P < 0·001$).
† 1 unit = 1 μmol substrate converted/min per g protein.

Digestion and absorption in GF mice

The intestinal contents were analysed 3 h after administration of cold maltitol or sorbitol to GF mice. Table 2 shows the recovery obtained in two experiments. In the first experiment the small intestine still contained a significant proportion of sugar alcohols, whereas in the second experiment practically all the unabsorbed sugar alcohols were in the caecum and colon. In both experiments most animals had completely digested the maltitol and no free glucose was detectable in the whole intestine.

In the first experiment the animals were quiet, whereas in the second experiment they moved constantly in the cage due perhaps to a less quiet environment. This activity was associated with a stimulation of the transit as well as the absorption of sorbitol. The proportion of sorbitol absorbed was calculated from the values in Table 2. The total unabsorbed sorbitol includes free sorbitol and the sorbitol moiety of the undigested maltitol that remained in the intestinal tract. The mice that received maltitol, absorbed 21 (SE 3·1)% of the total sorbitol in the administered maltitol (i.e. 73·5 mg/kg) in the first experiment and 33 (SE 3·6)% in the second experiment. These values were not significantly

different from those obtained when sorbitol was administered, when the absorption reached 27 (SE 22)% and 49 (SE 8)% respectively in the two experiments. In the second experiment with maltitol, the recovery of carbohydrate in the caecum reached 39 (SE 2·8)%, which was 37% higher ($P < 0·0025$) than the radioactivity recovered in the faeces and intestinal contents of GF mice after 48 h (Table 1).

Disaccharidase activities in the intestine

The disaccharidase activities in the different parts of the intestinal tract of the C and GF mice are presented in Table 3. The specific activity of α-glucosidase in GF mice small intestine (measured with maltose as substrate) was 135% of that found in the C mice small intestine ($P < 0·01$) and the total activity was 153%. The difference was even larger for maltitol (150 and 166% respectively). The α-glucosidase activities in the caecum and the colon were found to be very low when compared with those in the small intestine.

DISCUSSION

The purpose of this study was to determine the extent of hydrolysis of maltitol and the absorption of the liberated sorbitol in the small intestine of rodents, since several earlier studies have suggested that the reduced availability of maltitol was due to reduced hydrolysis of the disaccharide alcohols (Dahlqvist & Telenius, 1965; Oku *et al.* 1971).

With C animals, it is not easy to interpret the time-course of expired $^{14}CO_2$ produced from radioactive substrates administered intragastrically. There is a significant overlap between the $^{14}CO_2$ profiles produced by the catabolism of the absorbed carbohydrates by the host and of the short-chain fatty acids generated by the bacteria in the caecum, as already shown by Rennhard & Bianchine (1976). However, comparison of the $^{14}CO_2$ profiles from maltitol with those of lactitol can help to distinguish between the two sources of CO_2. Lactitol is poorly digested in the small intestine of rats and as a consequence most of the substrate reaches the caecum intact (Dharmaraj *et al.* 1987; Nilsson & Jägerstad, 1987). A metabolic study of [^{14}C]lactitol in fasting rats showed that $^{14}CO_2$ excretion started only 2–4 h after administration, indicating that lactitol was entirely degraded by the intestinal flora (D. C. Leegwater, unpublished results). Expired $^{14}CO_2$ only reached 16·5% of the administered radioactivity after 5 h and 48·5% after 24 h. Therefore it is very likely that in the present study the $^{14}CO_2$ exhaled during the first 3 h after administration of maltitol to the rats was produced essentially by the metabolism of absorbed glucose and sorbitol.

The extent of hydrolysis of maltitol and the absorption of sorbitol can be measured best in GF animals.

Excretion of polyol after oral administration of maltitol to fasting GF rats has been quantified by Lian-Loh *et al.* (1982). At a dose of 2 g/kg body-weight, only 11·5% was recovered in the faeces after 24 h, 95% as sorbitol, indicating that maltitol was almost completely hydrolysed in the intestine of these animals. A comparable investigation carried out with palatinit (an equimolar mixture of D-glucosyl-α(1–6)-D-glucitol and D-glucosyl-α(1–1)-D-mannitol) showed that after 24 h 7·6–11·8% of the ingested dose was excreted in the faeces essentially as mannitol and sorbitol (Ziesenitz & Siebert, 1987), again suggesting that it is the utilization of the monosaccharide alcohols that limits overall utilization. However, the large intestinal contents were not analysed in either study. An alternative hypothesis given by Ziesenitz & Siebert (1987) is that this extensive hydrolysis and these low levels of excretion were due to a higher activity of disaccharidase in the intestines of these animals than in conventional ones, as reported by others (Kawai & Morotomi, 1978; Whitt & Savage, 1980) and also found in our own study where the hydrolysis and subsequent

absorption continued in the caecum and in the large intestine. This latter step is unlikely in conventional animals because of the rapid utilization of the polyols by the intestinal flora. At 3 h after ingestion of maltitol by the GF mice practically all the product was digested, but a significant proportion of the released sorbitol was recovered in the caecum. Our results do not allow us to deduce that the reduced disaccharidase activity found in the C mice would decrease the extent of maltitol hydrolysis, because some GF animals had no more maltitol in the gastrointestinal tract, indicating that sufficient enzyme activity was present. However, the present experiment has shown that the sorbitol produced during digestion is only partially absorbed when compared with the sorbitol actually given to animals. The absorption of sorbitol produced from maltitol was much less than 50%. Therefore, the lower recovery of radiolabelled product in the faeces collected from GF mice after maltitol administration, when compared with the polyols present in the caecum 3 h after administration of maltitol, suggests that some of the sorbitol is also absorbed in the caecum and the colon in the GF animals. From these results we can conclude that 40% or more of the maltitol administered to fasting C mice reached the caecum—mostly as sorbitol—and was fermented by the intestinal flora.

The level of recovery of sorbitol 3 h after its administration is in agreement with that reported by Ertel *et al.* (1983) who recovered 55% in the intestinal tract of C rats. Grossklaus (1987), however, recovered only 22% of the polyol after 1 h and less than 10% 3 h after administration of 150 mg sorbitol/kg to C rats.

The comparison between the recovery from the intestine of sorbitol and maltitol leads to some interesting conclusions. Less than half of the administered sorbitol and about one-third of the sorbitol released from maltitol (which was 93% hydrolysed) were eventually absorbed after 3 h under our experimental conditions. This suggests that, for maltitol, the absorption of sorbitol is a significant rate-limiting step in the overall digestion and absorption of maltitol.

The polyols were administered in liquid form to fasting animals, as is generally the procedure in such studies (Schnell-Dompert & Siebert, 1980; Lian-Loh *et al.* 1982). In these conditions, the transit time is more rapid than when they are taken within a solid meal (Read *et al.* 1982; De Vries *et al.* 1988) and one could expect a lower digestibility of maltitol.

However, even in the conditions used in our study, practically all the maltitol ingested was hydrolysed. On the other hand, sorbitol absorption was found to be lower in mice with a slower intestinal transit than in mice with a more rapid intestinal transit. It is, therefore, not certain that the quantity of sorbitol absorbed is related to mouth-to-caecum transit time.

The quantitative direct measurement of non-absorbed polyol in human beings and large animals has been attempted using various approaches. Rérat *et al.* (1987) quantified the absorption of glucose and sorbitol in the portal blood of pigs after administration of hydrogenated glucose syrup of 55 sorbitol equivalent. All the glucose moiety and only 6·8% of the sorbitol moiety were absorbed, indicating that the maltitol in the syrup was completely hydrolysed and only a small proportion of the sorbitol was absorbed. In humans, Beaugerie (1987) found quite different results: after administration of 19 g maltitol to human volunteers, 90% of the maltitol was hydrolysed and 24% of the ingested dose was recovered at the terminal ileum. As a comparison, only 21% of the sorbitol was recovered after ingestion of 10 g of sorbitol. By contrast, Würsch *et al.* (1990), by measuring the H_2 exhaled after consumption of various polyols, found that maltitol produced 35–40% less H_2 than lactitol, which is reported to be essentially neither digested nor absorbed (Dharmaraj *et al.* 1987). Furthermore, sorbitol produced 16% less H_2 than a double load of maltitol (Würsch *et al.* 1990). These results indicate that a significant part

of sorbitol or maltitol ingested reached the large intestine, which corresponds to our findings in the GF mice.

The results presented here strongly suggest that maltitol, after hydrolysis, and equally sorbitol, are only partially absorbed in the small intestine and that the slow rate of sorbitol absorption is an important factor which limits the availability of maltitol.

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