Energy Utilization of Dietary Sorbose in Growing Rats

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The energy value of L-sorbose in rats was estimated. Sorbose was shown to be stable under incubation with 0.05 M hydrochloric acid (pH about 1.3, 37 °C, 120 min). The in vitro absorption rate of sorbose was lower than that of glucose. When ^{14}C-labeled sorbose was injected intravenously (6.0 mg/100 g of body weight), about 50% was excreted in urine and about 30% was expired as CO_2 in 24 h. ^{14}C-Labeled sorbose or ^{14}C-labeled glucose was orally administered (20 mg/100 g of body weight) to rats fed diets with or without antibiotics. The recovery of $^{14}CO_2$ in glucose-administered rats was significantly higher than that of $^{14}CO_2$ in sorbose-administered rats with or without antibiotics. The recovery of expired $^{14}CO_2$ in rats without antibiotics was significantly higher than that of the corresponding antibiotics-treated group. Large amounts of sorbose were recovered in urine (16–19%) and feces (9–16%). The remainder which was not recovered in radioactivity after labeled sorbose doses would provide its full energy, so that available energy of sorbose for the rat could be calculated as about 1.3–2.2 kcal/g.

INTRODUCTION

L-Sorbose is an isomer of D-fructose and is actually found in apple vinegars, passion fruits, and the berries of the mountain ash, *Sorbus aucuparia*, belonging to the Rosaceae family (Martin and Reuter, 1947; McComb, 1975). It can be produced industrially from sorbitol or glucose by fermentation with acetate forming bacteria (Beshkov et al., 1973). There have been many studies on the physiological properties of sorbose: slow absorption rate (Verzár and Laszt, 1935; Annegers, 1964), lowered plasma insulin level (Siebert et al., 1980; Furuse et al., 1989), and high excretion into urine (Beyreiss et al., 1966; Furuse et al., 1989).

Dietary sorbose lowered blood glucose levels and prevented incidence of diabetes in the nonobese diabetic mouse (Furuse et al., 1991a). In this study, the differences in final body weight gains (6 weeks) for 200 g of sorbose/kg of diet group and control group were significant, although no significant difference was observed in daily food intake between the two diets. In laying hens, body weight gain, food intake, serum triglyceride, cholesterol, LDL, VLDL, and chylomicron levels were significantly reduced to the increment of sorbose in dose-dependent fashion (Furuse et al., 1990). Dietary sorbose, as a sweetener as well as a bulking agent, seemed to be a suitable sugar for the obese and diabetic with special reference to lower body fat deposition, body weight gain, and energy utilization rate without reducing protein utilization in growing rats (Furuse et al., 1989). It has been recognized that maltitol was a poorly digestible sugar, but the recovery of expired $^{14}CO_2$ in rats administered [U-^{14}C]sorbose was 60% of that in [U-^{14}C]maltitol-administered counterparts (20-mg dose/100 g of body weight), although neither body weight gain nor food consumption (6 weeks) was significantly different in sorbose and maltitol groups at the level of 100 g/kg of diet (Tamura et al., 1991).

The present study was conducted to clarify an energy utilization mechanism, in terms of the availability of sorbose in the upper gastrointestinal tract, the metabolism in the macroorganism, and the fermentability by the hindgut microorganisms. Further study was also carried out to elucidate the estimation of energy utilization of sorbose in rats by the oral administration of [U-^{14}C]sorbose compared to [U-^{14}C]glucose as a control.

MATERIALS AND METHODS

Animals. Male Wistar rats (ST strain) used in the present study were purchased from the Shizuoka Experimental Animal and Agricultural Cooperative, Hamamatsu, Japan.

Quantitative Analysis of Sugars. Amounts of sugars were analyzed with a high-performance liquid chromatograph (Shimadzu LC-6A pump, Shimadzu Co. Ltd., Tokyo). As the mobile phase, 0.5 M borate buffer (pH 8.7 adjusted with NaOH) was used and delivered at a flow rate of 0.4 mL/min. Sugars were separated at 65 °C on commercially available prepared columns of TSK-gel Sugar AXI (Tosoh Co. Ltd., Tokyo) packed with a strong cation-exchange resin.

The column effluent and 10 g/L arginine solution as the fluorometric detection reagent delivered by another LC-6A pump at a flow rate of 0.4 mL/min were led to a chemical reaction bath at 150 °C (Mikami et al., 1983). The detection limit for both L-sorbose and D-glucose was approximately 5 ng. Arabinose was used as the internal standard. Samples were injected at least three times so that their standard error (SE) did not exceed 1.0% of the averaged value.

Stability of Sorbose. To estimate the chemical and physical stability of sorbose in gastric acid, a solution of sorbose (20 mg/mL) was mixed with an equal volume of 0.1 M HCl, and thereafter the solution was incubated for 120 min at 37 °C (pH about 1.3) to investigate possible destruction by the gastric acid, as described by Nilsson and Björck (1988). At 0, 30, 60, 90, and 120 min, sorbose concentration in the solution was measured (four replicates at each time).

In Vitro Absorption Rate of Sorbose. According to the method of Baker et al. (1961), the in vitro absorption rate of sorbose was measured by using the everted gut sac. Rats (5 weeks of age; 86–132 g, mean 111 g) were fasted overnight before the small intestine was removed under diethyl ether anesthetization. The small intestine was immediately everted over a glass rod and divided into six segments. The everted guts were immediately placed in a 300-mL conical beaker with air-bubbled solution (146 mM NaCl and 4.0 mM KCl). Each segment was filled with 1.0 mL of the Krebs–Ringer–Henseleit buffer and placed in a 100-mL conical beaker with 20 mL of the buffer with sorbose or glucose at 3, 10, 30 mg/mL under 95% O_2 and 5%

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CO_2. Each beaker was placed in a shaking water bath maintained at 37 °C for 1 h. Four or five rats were used for each incubation. After incubation, the outside of the sac was rinsed with distilled water. The gut sac was cut open, drained, and rinsed with distilled water to measure the entry amount of sugar.

Intravenous Injection of Sorbose. To investigate the metabolism of sorbose in the body, two experiments were done by intravenous injection of sorbose. First, to trace the amount of sorbose per se excreted into urine, 0.1 mL of sorbose (4.1 mg/mL) per 100 g of body weight in saline was injected into five animals (each 140 g); each rat was injected by microsyringe with 0.57 mg of cold sorbose whose levels were sufficient to determine quantitatively by the HPLC system mentioned above. To determine the amounts of sorbose per se, this was much more sensitive than the isotopically labeled TLC method. After injection, each rat was transferred to a metabolic cage in a room kept at 25 °C. The urine was collected at 3, 6, 9, 12, and 24 h after injection.

Second, to clear the fate of sorbose injected intravenously, [U-^{14}C]sorbose diluted with unlabeled sorbose solution (60 mg/mL) was injected to five rats (159–171 g, mean 165 g). Seventy-four kilobecquerels in 0.1 mL (i.e., 6.0 mg of sorbose)/100 g of body weight was injected to each rat. The rats were immediately transferred to glass metabolic cages (Metabolica, Sugiyamagen Co, Tokyo). The expired CO_2 was trapped with monoethanolamine (Tokunaga et al., 1981) and was collected at 0, 4, 8, 12, and 24 h. Urine and feces were collected separately. Urine was sampled at the same time of the expired CO_2, and feces were obtained over 24 h. Fecal radioactivity was counted after exhaustive extraction with distilled water at least four times. The analysis of radioactivity in the expired $^{14}CO_2$, urine, and feces was described previously (Tamura et al., 1991).

Energy Utilization of Sorbose and Contribution of Gut Microflora. The energy utilization of sorbose in rats was investigated in comparison with glucose. Simultaneously, the contribution of the gut microorganisms to the utilization of sorbose was studied. Twelve rats (4 weeks of age) were divided into three groups of four rats each. The first and second groups were fed a control diet ad libitum for 7–10 days. The composition (grams per kilogram diet) of the control diet was as follows: cornstarch 650; casein 200; DL-methionine 3; cellulose 50; corn oil 50; AIN76 (1977) mineral mixture 35; AIN76A (1980) vitamin mixture 10; choline bitartrate 2. The third group was fed a similar diet ad libitum for 7–10 days except for the inclusion of nebacitin, an antibiotic drug composed of bacitracin and neomycin sulfate in a 2:1 w/w ratio. Nebacitin was supplemented at a level of 7 g/kg of diet. [U-^{14}C]Glucose diluted with unlabeled glucose solution (200 mg/mL) was administered with a gastric tube to the first group (141–171 g, mean 155 g). [U-^{14}C]Sorbose diluted with unlabeled sorbose solution (200 mg/mL) was administered similarly to the second (141–172 g, mean 156 g) and third (149–163 g, mean 155 g) groups. These labeled materials, both having 98% purity, were purchased from Amersham Japan, Tokyo. Seventy-four kilobecquerels in 0.1 mL/100 g of body weight was administered to each rat. After the administration, rats were transferred to the glass metabolic cage and treated as described above. Each rat was fed the experimental diet with or without antibiotics ad libitum and allowed free access to water both prior to and after the intubation. The sample of the expired CO_2 was obtained at 0, 2, 4, 6, 8, 10, 12, and 24 h after the injection. Urine and feces were collected separately over 24 h. Radioactivity of sorbose per se in urine and feces was analyzed with the thin-layer chromatography as described previously (Tamura et al., 1991).

Statistical Procedure. Data were subjected to analysis of variance, and significance of difference between means was determined according to Duncan's multiple range test or Student's t-test using a commercially available statistical package (SAS, 1985).

RESULTS

Stability of Sorbose. Sorbose was completely stable under 0.05 M hydrochloric acid (pH about 1.3, 37 °C), since the recovery of sorbose was 100.2 % (SEM 0.2) after 2 h of incubation.

Table I. In Vitro Transport of Sorbose and Glucose in Everted Sacs of Rat Intestine[a]

sugar	concn, mg/mL	no. of rats	amt transported sugars, mg/h	rel value
glucose	3	4	21.9 ± 3.1	100
sorbose	3	5	7.7* ± 0.4	35
glucose	10	5	40.5 ± 3.7	100
sorbose	10	4	21.0* ± 1.8	52
glucose	30	4	138.2 ± 14.8	100
sorbose	30	4	86.1* ± 6.0	62

[a] Each segment contained 1 mL of solution. The amounts of transported sugars are expressed as milligrams per hour glucose or sorbose in serosal side of the intestine ± SEM. Relative value was the in vitro absorption rate of sorbose/that of glucose. * Significantly different from the corresponding glucose value at $p < 0.05$.

Table II. Recovery of Radioactivity in Expired CO_2, Urine, and Feces after Intravenous Injection of [U-^{14}C]Sorbose[a]

	hours after injection	radioactivity, % of injection
urine	0–4	44.6 ± 1.7
	4–8	3.6 ± 0.4
	8–12	2.1 ± 0.4
	12–24	1.2 ± 0.2
	0–24	51.6 ± 1.3
expired CO_2	0–4	19.8 ± 1.4
	4–8	6.7 ± 0.7
	8–12	1.7 ± 0.5
	12–24	0.9 ± 0.4
	0–24	29.0 ± 0.7
feces	0–24	1.4 ± 0.1
total	0–24	82.0 ± 2.0

[a] Values are means ± SEM.

In Vitro Absorption Rate of Sorbose. Table I shows the in vitro absorption rate (mean ± SEM) of sugars in the everted gut sac. At 3.0 mg/mL, the rate of sorbose was 35% of that of glucose. As the sorbose concentration was increased, the absorbed rate was increased linearly. Although the relative value (the in vitro absorption rate of sorbose/that of glucose) became larger to the increment of sugar concentration, the difference in in vitro absorption rate between the two sugars was significant ($p < 0.05$) at any level.

Intravenous Injection of Sorbose. Total recovery of sorbose in urine over 24 h was 46% (SEM 7.2) after intravenous injection of 0.57 mg of unlabeled sorbose. Sorbose was completely excreted into urine within 9 h after injection. Table II shows the recoveries of radioactivity in expired CO_2, urine, and feces of rats injected intravenously with [U-^{14}C]sorbose. Over 24 h, recoveries of radioactivity in expired CO_2 and urine reached 29% and 52% respectively, and most of both (more than 90%) were excreted within 8 h. Only a small amount of radioactivity was detected in feces.

Energy Utilization of Sorbose and Contribution of Gut Microflora. The time course of radioactive recovery of expired CO_2 in rats administered orally with [U-^{14}C]-sorbose or [U-^{14}C]glucose is indicated in Figure 1. The values for expired $^{14}CO_2$ in rats with glucose were significantly higher than those in rats with sorbose. The expired $^{14}CO_2$ over 24 h was significantly lower in rats with dietary antibiotics than in corresponding counterparts. The total recovery of radioactivity in expired CO_2, urine, and feces over 24 h is given in Table III. Both urinary and fecal recoveries of radioactivity were small in glucose-administered rats. Recoveries of radioactivity in urine and in urinary sorbose itself were not affected by dietary antibiotics. Dietary antibiotics significantly reduced the radioactivities of feces and fecal sorbose itself.

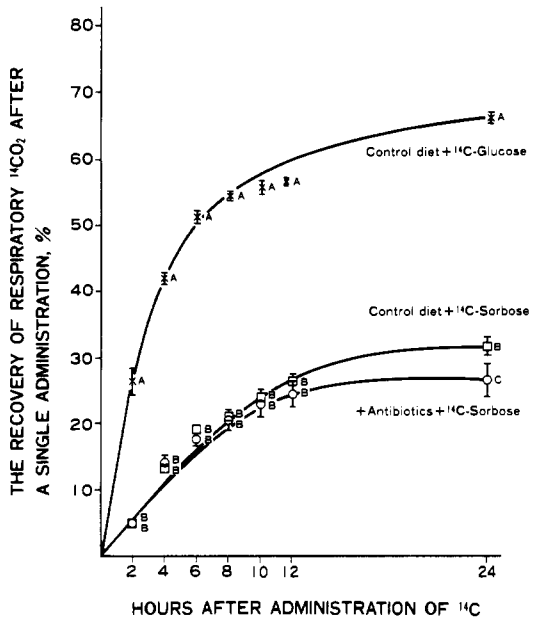

Figure 1. Recovery of expired $^{14}CO_2$ after a single oral administration of uniformly ^{14}C-labeled sugars to rats. (○) [U-^{14}C]-Sorbose administered to rats fed the control diet with Nebacitin; (□) [U-^{14}C]sorbose administered to rats fed the control diet; (×) [U-^{14}C]glucose administered to rats fed the control diet. Values are means of four rats, and vertical bars represent standard errors. Means not sharing a common superscript letter at the same time are significantly different at $p < 0.05$.

Table III. Recovery of Radioactivity in Expired CO_2, Urine, and Feces over 24 h after Oral Administration of [U-^{14}C]Glucose and [U-^{14}C]Sorbose to Rats[a]

	radioactivity, % of administration		
labeled material:	sorbose		glucose
diet:	control	+antiobiotics[b]	control
no. of rats:	4	4	4
expired CO_2	32.8[a] ± 1.1	27.1[b] ± 2.5	66.1[c] ± 0.6
urine	16.6[a] ± 0.9	18.8[a] ± 1.0	2.7[b] ± 0.2
as sorbose	13.8 ± 1.0	14.8 ± 1.6	
feces	32.7[a] ± 2.6	20.2[b] ± 1.6	1.8[c] ± 0.4
as sorbose	15.7[a] ± 1.3	8.9[b] ± 0.7	
total	82.2[a] ± 0.8	66.1[b] ± 4.4	70.6[b] ± 0.6

[a] Values are means (%) ± SEM. Means not sharing a common superscript letter are significantly different at $p < 0.05$. [b] Nebacitin was supplemented to the control diet at 7000 ppm.

DISCUSSION

Physical and chemical characteristics of sorbose might not be altered in the stomach of rats, because sorbose was completely stable under incubation with 0.05 M hydrochloric acid (pH about 1.3, 37 °C, 120 min). On the other hand, in vitro absorption rate of sorbose from the intestine was low compared with that of glucose, irrespective of the same molecular weight. This in vitro absorption rate was consistent with the report by Verzár and Laszt (1935), who observed that in situ in rats the amount of sorbose absorbed was 28% of that of glucose. These slow absorption rate of sorbose were due to the fact that sorbose is absorbed by passive diffusion, because the amounts of sorbose absorbed increased linearly as the increment of sorbose concentration (Table I), which was consistent with the study by Ilundarin et al. (1979). In any event, large amounts of sorbose per se might reach the hindgut. The contribution of the gut microflora to the utilization of dietary sorbose could be expected. This possibility was studied and is discussed in the following.

Several investigators (Oku et al., 1971, 1984; Elia et al., 1987) reported that sugars, which are not metabolized easily in the host, were rapidly excreted in urine after the intravenous injection. In the present study, about half the amount of sorbose was excreted in urine within 9 h and thereafter no sorbose was detected. However, this experiment did not explain the whereabouts of the rest of sorbose. Thus, a tracer technique was used in another experiment. About 30% of sorbose was metabolized and expired as CO_2 over 24 h after injection and about 50% was excreted rapidly in urine; 20% remained in the body. Some sorbose could be metabolized in the internal organs of rats, since Wursch et al. (1979) measured the rest of the label in carcasses after oral administration of radiolabeled sorbose and recovered 10–20% of the radioactivity in internal organs and intestinal content. This deposition might correspond to our results.

It has been recognized that certain antibiotics drastically decrease intestinal microbial activity (Eggum et al., 1979; Nilsson and Björck, 1988). The recovery of radioactivity in expired CO_2 after oral administration of [U-^{14}C]sorbose was significantly lower in nebacitin-treated rats than in untreated control. Although sorbose was reported less fermentable in rumen microbe (Czerkawski and Breckenridge, 1969) and chicken gut microflora (Furuse et al., 1991b), some sorbose was fermented by the hindgut microorganisms as expected above.

The fecal recovery of radioactivity and that as sorbose itself in feces were significantly decreased by the antibiotics treatment. Transit time of food in nebacitin-treated rats might become longer, since the hindgut content in nebacitin-treated rats increased (Chawla et al., 1976; Eggum et al., 1979).

From the results of the present study, the available energy of sorbose in the rat can be calculated by two different ways.

(1) The first was based upon the difference between the recoveries of expired radiolabeled CO_2 in nebacitin-treated and untreated rats. Eggum et al. (1979) reported that ATP per intestinal content was reduced to 20% by nebacitin treatment and intestinal content increased 3 times. The activity of intestinal microorganisms in nebacitin-treated rats thus seemed to be 60% (0.2 × 300) of that in untreated rats. Dietary nebacitin caused 40% reduction in microbial activities, and consequently the recovery of expired $^{14}CO_2$ in nebacitin-treated rats (27.1%) was significantly lower than that in untreated rats (32.8%) as shown in Table III. Consequently, 14.3% [(32.8 − 27.1)/0.4] of the sorbose administered was broken down by the gut microflora. The remaining 18.5% (32.8% − 14.3%) was metabolized by the host itself. The recovery of radioactivity in expired CO_2 was 66.1% in radiolabeled glucose (4 kcal/g) treated groups (Table III). Rats could obtain 1.1 kcal/g of sorbose (4 × 18.5/66.1) by themselves. Furthermore, rats could utilize 0.4 kcal/g of sorbose by fermentation, and this value was obtained from the following postulations. When carbohydrates in the hindgut were completely fermented to short-chain fatty acids, CO_2, and CH_4, the energy value was reduced to about 66% (Wolin, 1960; Oku, 1988). It was also indicated that energy utilization efficiency of acetate was 58–70% in sheep (Black et al., 1987) and 69% in humans (Hosoya et al., 1988), although the energy efficiency for other short-chain fatty acids has not been reported to our knowledge. Accordingly, it was speculated here that energy utilization efficiency of sorbose was reduced to 70% by fermentation, and the value for 0.4 kcal/g was calculated as 4 kcal/g × 0.66 × 0.7 × 14.3/66.1. The energy value of sorbose was thus taken as 1.5 kcal/g (1.1 + 0.4). Furthermore, the remaining 17.8% of sorbose (100 − 82.2, Table III) was postulated to be completely metabolized, and the value of 0.7 kcal/g (4

× 0.178) was calculated. The energy value of sorbose was less than 2.2 kcal/g (1.5–2.2).

(2) The second calculation was based upon the process relating digestion, absorption, metabolism, and microbial fermentation. Sorbose was completely stable in the hydrochloric acid solution. The in vitro absorption efficiency was 35%, and the remaining 65% reached the hindgut. About 29% (recovered as expired $^{14}CO_2$ in Table II) of absorbed sorbose was metabolized in the body, and then 0.41 kcal/g of sorbose was metabolized; this value could be calculated as 4 kcal/g × 35% × 29%. Of the 65% of sorbose reached the hindgut, 15.7% was excreted in the feces (Table III). Postulating the remaining 49.3% being fermented, it would be calculated, by use of coefficients of 0.66 and 0.7 as described in the first calculation, as follows: 4 kcal/g × 49.3% × 0.66 × 0.7 = 0.91. The energy of sorbose could be more than 1.3 kcal/g (0.41 + 0.91). In addition, the remaining 18% located in the body after intravenous injection of [^{14}C]sorbose was postulated to be completely metabolized and the value of 0.25 kcal/g (4 × 0.35 × 0.18) was calculated. The energy value of sorbose was less than 1.6 kcal/g (1.3–1.6).

Thus, the value for available energy of sorbose for the rat was between 1.3 and 2.2 kcal/g.

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