Utilization of Orally Administered D-[14C]Mannitol via Fermentation by Intestinal Microbes in Rats

Ryoko Hongo1,2, Sadako Nakamura1 and Tsuneyuki Oku1,8

1Division of Nutritional Science, Graduate School of Human Health Sciences, University of Nagasaki, Siebold, Nagayo, Nagasaki 851–2195, Japan
2Department of Health and Nutrition, Faculty of Health Management, Nagasaki International University, Huis Ten Bosch, Sasebo, Nagasaki 859–3298, Japan

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Summary To investigate the available energy of orally administered [14C]mannitol via intestinal microbes, [14C]mannitol (222 kBq, 105 mg) or [14C]glucose (222 kBq, 105 mg) was administered to conventional rats and antibiotics-treated rats whose intestinal microbes were depleted by drinking water containing antibiotics, respectively. The exhausted CO2, feces and urine were then separately collected at 2, 4, 6, 8, 10, 12 and 24 h after administration of the test solution. In the conventional rats, 45% of administered radioactivity was recovered as 14CO2 in the administration of [14C]mannitol, while 57% of administered radioactivity was recovered as 14CO2 following the administration of [14C]glucose for 24 h. The time sequence for the 14CO2 excretion from [14C]mannitol was delayed as compared to [14C]glucose by about 4–6 h (p<0.05). However, when [14C]mannitol was orally administered to antibiotics-treated rats, only 3% of administered radioactivity was excreted as 14CO2 for 24 h. The total radioactivity of the gastrointestinal contents and feces for 24 h after administration was over 70%, much higher than those of the conventional rats (p<0.05). When a half dose (222 kBq, 52.5 mg) of [14C]mannitol was administered to conventional rats, the recovery as 14CO2 for 24 h (%) was significantly higher than that of a regular dose of [14C]mannitol (105 mg). When cold mannitol (105 mg) was orally administered to the antibiotics-treated rats, about 9% of intact mannitol was excreted in feces within 48 h after administration. However, no intact mannitol was detected in the conventional rats. These results demonstrate that more than 95% of mannitol administered orally is utilized via fermentation by intestinal microbes.

Key Words mannitol, antibiotics, intestinal microbes, fermentation, rats

D-Mannitol (mannitol) is a hexitol (C6H12O6; molecular weight, 182.17) that distributes widely in a variety of plants, seaweeds and fungi (1). Mannitol has been used as a sugar substitute in processed foods, as a diuretic medicine by parenteral administration (2–4) and in an intestinal permeability test to assess intestinal mucosal barrier function (5–8).

Previous studies have demonstrated that about half of administered mannitol is converted to CO2 (9, 10); in spite of being orally administered, hardly any mannitol is absorbed (11, 12). In addition, the metabolic pathway of the production of 14CO2 had not been clarified until the present study. Dwivedi has speculated that mannitol which is not absorbed from the small intestine might be fermented by intestinal microbes, and that the short-chain fatty acids (SCFAs) produced might be utilized by the host (1). Recently, we clarified that the organic acids in cecal contents were increased by feeding of a mannitol-containing diet to conventional rats (13). Ishiyama et al. also reported similar results (14). However, it has not been clarified how intestinal microbes convert orally administered mannitol into CO2.

In previous reports, it was demonstrated that 77–97% of the administered radioactivity was excreted into the urine within 24 h, and only 2–3% of the radioactivity was oxidized to 14CO2 in the intraperitoneal injection of [14C]mannitol in rats (11). These results suggest that mannitol absorbed from the small intestine is not metabolized to produce energy. On the other hand, when 240 mg of [14C]mannitol was orally given to non-fasted conventional rats, 56% of the administered radioactivity was recovered in 14CO2 within 24 h (11). In addition, 40% of the radioactivity was emitted as 14CO2 within 24 h in the oral administration of 500 mg of [14C]mannitol per kg of body weight in fasted conventional rats (9). Although these findings demonstrate that ingested mannitol was metabolized to 14CO2, they did not demonstrate the mechanism or the metabolic pathway of 14CO2 production. Furthermore, the oral administration of mannitol to fasted rhesus monkeys (15) and the feeding of mannitol-containing diets to rats (16) and dogs (17) led to a small increase of liver glycogen (15–17). These results indicate that mannitol is not utilized as an energy source by the absorption in

*To whom correspondence should be addressed.
E-mail: okutsunee@sun.ac.jp
the small intestine. However, the fermentation and utilization of mannitol via intestinal microbes remains ambiguous.

Sugar alcohols such as maltitol or sorbitol, which are not digested or absorbed in the small intestine, are fermented by intestinal microbes and converted to SCFAs, CO₂, H₂ and CH₄. Most of the SCFAs produced are absorbed from the lower intestine and utilized as energy by the host (18–21). We previously clarified that the cecal contents of rats fed a mannitol-containing diet showed a remarkable increase of organic acids (13). This suggests that orally ingested mannitol may be metabolized via fermentation by intestinal microbes. In addition, the decrease of pH value in cecal content and increments of cecal and colon weight were observed in mannitol-fed rats (14, 22). However, there was no evidence in terms of the degree of fermentation and utilization of mannitol via intestinal microbes in vivo experiments.

Oku and Tokunaga et al. clarified that fructooligosaccharide, which is a nondigestible sugar substitute, was metabolized into CO₂ via fermentation by intestinal microbes in experiments in which the [¹⁴C]fructooligosaccharide was orally administered to conventional, antibiotics-treated and germ-free rats and the [¹⁴CO₂] excretion was time-dependently observed (23, 24). Rats given antibiotics-containing drinking water were used as the intestinal microbes-depleted rats. Although the conversion of [¹⁴C]fructooligosaccharide to [¹⁴CO₂] was negligible in antibiotics-treated rats and germ-free rats, about 54.6% of the total radioactivity administered was exhaled as [¹⁴CO₂] within 24 h in conventional rats after the oral administration of [¹⁴C]fructooligosaccharide. These results demonstrate that fructooligosaccharide, which is not digested in the small intestine, is metabolized via fermentation by intestinal microbes (23, 24). In the present study, we investigated the utilization of mannitol via fermentation by intestinal microbes and the degree of fermentation using the same methods as were used with fructooligosaccharide.

The purpose of this study is to directly confirm that mannitol is metabolized to CO₂ via fermentation by intestinal microbes. The degree of fermentation was estimated by the administration of [¹⁴C]mannitol to conventional and antibiotics-treated rats. Although mannitol is clinically used via oral administration as a mucosal permeability marker in the small intestine, the fermentability and available energy of mannitol via intestinal microbes remains unclear. It is essential to estimate the degree of fermentation by orally administered mannitol and to evaluate energy efficacy for safe uses. Furthermore it could contribute to develop the utilization of mannitol. Clarifying the metabolism of orally administered mannitol can contribute to new applications for human health and the treatment of diseases.

**MATERIALS AND METHODS**

1. **Materials.** D-Mannitol (Marinocrystal®, purity >99%), which was kindly provided by Mitsubishi Shoji

| Treatment of rat | Conventional | Antibiotics |
|------------------|--------------|-------------|
| Test solutions   | [¹⁴C]Glucose | [¹⁴C]Mannitol | [¹⁴C]Mannitol |
| Glucose (mg)     | 105.0        | —           | —            |
| Mannitol (mg)    | —            | 105.0       | 52.5         |
|                  |              |             | 105.0        |

Each test material was dissolved in 1.5 mL of sterile water with 222 kBq of respective radioactive compounds. Dose level of 105 mg of mannitol was the maximum permissive dose which did not cause osmotic diarrhea in rats.

Foodtech Co., Ltd. (Tokyo, Japan), mannitol, d-[¹⁴C] (specific activity 1.85 GBq/mmol, American Radiolabeled Chemicals Inc., USA), and glucose, d-[¹⁴C(U)] (specific activity 74 MBq/mmol, American Radiolabeled Chemicals Inc.) were used.

2. **Animals and diets.** Twenty male Wistar rats (8 wk old, 150 g, CLEA Japan, Inc., Tokyo, Japan) were housed in air-conditioned cages (room temperature, 23 ± 1°C; humidity, 50 ± 10%) and maintained on a 12 h light-dark cycle (light, 08:00–20:00; dark, 20:00–08:00). They were raised on a standard solid diet (MF®, Oriental Yeast Co., Ltd., Tokyo, Japan) ad libitum for 3 d to adapt before being used in the administration experiment and then fed a AIN 93-M cellulose-free diet (cellulose was replaced with β-cornstarch). For depletion of intestinal microbes, 5 rats were given the sterilized water containing 50 units/mL of benzylpenicillin potassium (23, 24) (Wako Pure Chemical Industries, Ltd., Osaka, Japan), 2.0 mg/mL of neomycin sulfate (25, 26) (Wako) and 0.5 mg/mL of cefoperazone sodium salt (27, 28) (Wako) ad libitum for 10 d before the oral administration of [¹⁴C]mannitol experiment. The remaining 15 rats were given distilled water ad libitum.

Twelve male Wistar rats (8 wk old, 150 g, CLEA Japan) were fed a standard solid diet ad libitum for 3 d and randomly assigned to 2 groups. Six rats were given sterilized water containing the same dose of antibiotics as mentioned above, and an AIN 93-M cellulose-free diet fed ad libitum for 10 d before the oral administration of [¹⁴C]mannitol experiment. Six rats were given the distilled water without antibiotics and the same diet ad libitum.

3. **Preparation of test solutions.** Table 1 shows the amount of [¹⁴C]mannitol and its radioactivity in the test solutions of oral administration. Based on our preliminary experiments, 105 mg of mannitol was used as the maximum permissive dose which did not cause transitory osmotic diarrhea in rats, and was the appropriate dose level to measure the radioactivity of feces, urine, tissues and organs using a scintillation counter, as well as to analyze the intact mannitol in feces and urine by HPLC.

To clarify the utilizable capacity of [¹⁴C]mannitol via intestinal microbes, 222 kBq of [¹⁴C]mannitol was dissolved in 1.5 mL of sterilized water containing 52.5 mg.
or 105 mg of mannitol. As the control, 222 kBq of $[^{14}\text{C}]$glucose was dissolved in 1.5 mL of sterilized water containing 105 mg of glucose.

4. Oral administration of $[^{14}\text{C}]$mannitol to measure the $[^{14}\text{C}]$distribution into carbon dioxide, gastrointestinal contents, feces, urine, tissues, and organs in conventional and antibiotics-treated rats. To measure the radioactivity from orally administered $[^{14}\text{C}]$mannitol into $^{14}\text{CO}_2$, the contents of the gastrointestinal tracts, feces, urine, tissues, and organs, the rats were fasted for 15 h before the administration of the test solutions and supplied drinking water only. Five conventional rats were each administered the test solution containing 222 kBq (105 mg) of $[^{14}\text{C}]$mannitol or $[^{14}\text{C}]$glucose using a stomach sonde.

In order to estimate the metabolism of mannitol via fermentation by intestinal microbes, 5 antibiotics-treated rats were administered 222 kBq of $[^{14}\text{C}]$mannitol and 105 mg of mannitol/1.5 mL. Immediately after the administration by a stomach sonde, each rat was transferred to the Metabolica apparatus and observed for 24 h. The expired $^{14}\text{CO}_2$ was trapped with 500 mL of monoethanolamine, and sampling (2 mL) was carried out at 2, 4, 6, 8, 10, 12 and 24 h after the administration. Feces and urine were collected at the same time. KOH and soda lime removed CO$_2$ in air, and H$_2$SO$_4$ and silica gel removed H$_2$O.

5. Collection and preparation of expired CO$_2$, feces, urine, contents of gastrointestinal tract, organs, and tissues, and determination of the radioactivity. Immediately after $[^{14}\text{C}]$mannitol or $[^{14}\text{C}]$glucose was administered to each rat, the rat was transferred to a glass metabolic apparatus (Fig. 1) (Metabolica CO-2, Sugiyamagen Co. Ltd., Tokyo, Japan) with an interior temperature maintained at $23 \pm 1 \degree\text{C}$ and $^{14}\text{CO}_2$; urine and feces were collected for 24 h. The rats transferred to the glass metabolic apparatus were fasted for a further 6 h after the administration of the test solution and given only drinking water. The expired $^{14}\text{CO}_2$ was trapped with 500 mL of monoethanolamine (Wako) and 2 mL of monoethanolamine was removed to measure the radioactivity at 2, 4, 6, 8, 10, 12 and 24 h after the administration. After the exhausted $^{14}\text{CO}_2$ was trapped (absorbed) into monoethanolamine for 12 h after administration, it was changed to a new glass container with 500 mL of monoethanolamine, and $^{14}\text{CO}_2$ collection was continued for a further 12 h.

Feces and urine were separately collected in bottles fixed to the metabolic apparatus, and these bottles were changed for new bottles at 2, 4, 6, 8, 10, 12 and 24 h after administration. The rats were killed at 24 h after administration of radioactive material, and the blood, contents of stomach and small intestine, cecum and colon, liver, kidneys, gastrocnemius muscle of the right leg, perirenal and epididymal adipose tissues and small intestinal mucosa were collected to measure the radioactivity incorporated. Serum was obtained by centrifugation at 2,500 $\times g$ for 15 min at 20 $\degree\text{C}$ (High Speed Refrigerated Centrifuge 6900, Kubota Co., Tokyo, Japan).

Five hundred microliters of monoethanolamine containing $^{14}\text{CO}_2$ was taken to a counting vial, and the radioactivity was determined with a liquid scintillation counter (LSC-6000, Aloca Co., Ltd., Tokyo, Japan) after the addition of 5 mL of scintillation cocktail (Hionic-Fluor, Perkin Elmer Japan, Kanagawa, Japan) and 1 mL.
of Cellosolve (Wako). The tissues, organs, contents of stomach, small intestine, cecum and colon, and feces were homogenized using a polytron homogenizer (Kinematica Co., Ltd., Swiss) in 9 volumes of 0.9% NaCl solution. Three hundred microliters of homogenates or serum were taken to a counting vial and solubilized with 0.5 mL of solubilizer (Soluene 350, Perkin Elmer Japan) at 50˚C for 12 h. The feces and contents of the cecum and colon were decolorized by the addition of 0.3 mL of 30% of hydrogen peroxide (Wako). Five milliliters of scintillation cocktail (Pico-fluor 40, Perkin Elmer Japan) was added to the solubilized homogenates and urine in the counting vial, and the radioactivity was determined with a scintillation counter. The counting efficiency was approximately 90%.

6. Measurement of intact mannitol in feces and urine of conventional and antibiotics-treated rats using HPLC.

After 15 h fasting, 6 conventional and 6 antibiotics-treated rats (B.W. 250 g) were orally administered 105 mg of mannitol dissolved in 1.5 mL of sterilized water using a stomach sonde. Immediately after administration, each rat was transferred to a stainless steel metabolic apparatus, and the feces and urine were separately collected at 6, 12, 24, 36 and 48 h after administration.

The feces collected were weighed and homogenized in 9 volumes of 0.9% NaCl solution using a polytron homogenizer (Kinematica Co., Ltd.), and the homogenate was heated at 100˚C for 7 min to inactivate the intestinal microbes and enzymes. After the homogenate was centrifuged by a high-speed refrigerated micro centrifuge (MX-160, Tomy Seiko Co., Ltd., Tokyo, Japan) at 8,500 ×g for 15 min at 20˚C, the supernatant was filtered with a membrane filter (0.22 μm, Millex-GV, Millipore Co., USA) and used for HPLC analysis to measure the mannitol concentration. The total volume of the urine collected was measured and the urine was then heated for 5 min at 100˚C and used for HPLC analysis after filtration. The samples prepared were stored at −20˚C until HPLC analysis.

7. Conditions of HPLC for mannitol analysis.

The amount of intact mannitol in the feces and urine were analyzed by an HPLC system (SCL-10A, Shimadzu Corp., Kyoto, Japan) with a refractive index detector (RID-10A, Shimadzu Corp.), using an Asahipak NH2P50-4E column (4.6 mm I.D. × 250 mm L., Showa Denko Co., Ltd., Tokyo, Japan). The analysis was performed at a constant temperature of 40˚C. The sample was eluted with CH3CN : H2O 75 : 25 at a flow rate of 0.6 mL/min and the injection volume of the sample was 10 μL.

8. Calculation and statistical analysis.

The percentage of radioactivity recovered in the 14CO2, tissues, organs and contents of the gastrointestinal tracts versus oral administered radioactivity were calculated.

Data were calculated as means and standard deviations (SD). After the normality test, the data were analyzed by Student’s t-test or one-way ANOVA and Dunnett’s post hoc test was used to evaluate significant differences among groups. A p-value of less than 0.05 was considered significant. The statistical analysis was performed using SPSS version 16.0 for Windows, Japan (SPSS Inc., Tokyo, Japan) (29).

9. Ethics.

The study protocol was approved by the respective committee on Animal Experiments of the University of Nagasaki, Siebold. The rat experiment was performed according to the guidelines on the care and use of laboratory animals of the University of Nagasaki, Siebold and the standards relating to the care and management of experimental animals (Notification No. 88, April 28, 2006 from the Prime Minister’s office).

RESULTS

1. Conversion of [14C]mannitol into 14CO2 and radioactivity of [14C]mannitol remaining in gastrointestinal contents in conventional rats

In Fig. 2, when [14C]mannitol solution (222 kBq, 105 mg) was administered to a conventional rat, 44.8% of the administered radioactivity was excreted as 14CO2 for 24 h. In contrast, 56.5% of the administered radioactivity was excreted as 14CO2 for 24 h after administration of [14C]glucose (222 kBq, 105 mg). The radioactivity excreted as 14CO2 within 24 h was significantly lower in [14C]mannitol-administered rats than in [14C]glucose administration (p<0.05). In addition, the elimination of 14CO2 at each time point for 24 h after administration was significantly less in the [14C]mannitol-administered rats than in [14C]glucose-administered rats (p<0.05).

Figure 3 shows that 14CO2 excretion in the [14C]mannitol-administered rats started at around 2 h after administration. The peak time of the 14CO2 excretion
from $[14C]$mannitol was delayed as compared to $[14C]$glucose by about 4–6 h.

Figure 4 shows the percentage of radioactivity versus administered amounts in the gastrointestinal contents and feces. When $[14C]$mannitol solution (222 kBq, 105 mg) was administered to a conventional rat, the percentage of radioactivity excreted into feces within 24 h was 6.1%, and excretion by the administration of $[14C]$glucose was 5.4% of radioactivity. The percentages were not significantly different. However, the percentage of radioactivity of the contents of the gastrointestinal tracts in antibiotics-treated rats was significantly higher in conventional rats than in antibiotics-treated rats when $[14C]$mannitol was administered ($p<0.05$). The percentage of radioactivity excreted to the urine within 24 h was 6.3% for $[14C]$glucose and 6.1% for $[14C]$mannitol (Table 2).

Figure 3. Comparison of the time course of expired $^{14}$CO$_2$ between administration of $[14C]$glucose and $[14C]$mannitol in conventional rats. Values were mean±SD ($n=5$). ——, $[14C]$glucose (conventional); ——, $[14C]$mannitol (conventional). $[14C]$Mannitol was orally administered to conventional or antibiotics-treated rats. a–e: Significant differences were evaluated by Student’s $t$-test and values with the same superscripts were significantly different at $p<0.05$.

2. Comparison of conversion from $[14C]$mannitol into $^{14}$CO$_2$ and remaining radioactivity in gastrointestinal contents between conventional rats and antibiotics-treated rats

In the antibiotics-treated rats, the radioactivity expired as $^{14}$CO$_2$ was only 3.3% in 24 h (Fig. 2) and the conversion of $[14C]$mannitol into $^{14}$CO$_2$ was remarkably disturbed by the depletion of intestinal microbes with antibiotics treatment. In contrast, 44.8% of the radioactivity of $[14C]$mannitol administered orally was expired as $^{14}$CO$_2$ in conventional rats in 24 h (Fig. 4). Furthermore, the elimination of $^{14}$CO$_2$ from $[14C]$mannitol at each time point was significantly lower in the antibiotics-treated rats than in the conventional rats ($p<0.05$). The radioactivity recovered in the contents of gastrointestinal tracts is shown in Fig. 4. They were calculated on three sections as follows: stomach, and small intestine; cecum, colon and rectum; feces. They were more than 7-times higher in the antibiotics-treated rats than in the conventional rats administered $[14C]$mannitol ($p<0.05$) (Fig. 4).

3. Incorporation of radioactivity into tissues and organs after oral administration of $[14C]$mannitol to conventional and antibiotics-treated rats

The radioactivities incorporated into the liver, kidneys, and perirenal and epididymal adipose tissues were significantly higher in conventional rats administered $[14C]$mannitol than in conventional rats administered $[14C]$glucose (Table 2) ($p<0.05$). However, the radioactivities incorporated into the organs and tissues of antibiotics-treated rats were significantly less than those in the conventional rats administered $[14C]$mannitol (Table 2) ($p<0.05$).

4. Comparison of the time course of expired $^{14}$CO$_2$ in conventional rats for 24 h after oral administration of 52.5 and 105 mg $[14C]$mannitol

To investigate the utilizable capacity of $[14C]$mannitol via intestinal microbes, $[14C]$mannitol solutions (222 kBq, 105 mg) and (222 kBq, 52.5 mg) were prepared and administered to conventional rats. Figure 5 shows that the conversion of $[14C]$mannitol to $^{14}$CO$_2$ and incorporation of radioactivity into organs and tissues were compared between 52.5 mg and 105 mg of the administration. The cumulative conversion percentages of $[14C]$mannitol to $^{14}$CO$_2$ by the administration of 105 mg and 52.5 mg of $[14C]$mannitol were not significantly different until 2 and 4 h after administration. However the percentage was significantly higher in the 52.5 mg of $[14C]$mannitol than in the 105 mg of $[14C]$mannitol administration at each time point of 6, 8, 10, 12 and 24 h after administration ($p<0.05$). The excretion of $^{14}$CO$_2$ for 24 h after the administration of $[14C]$mannitol was 54.8% of 52.5 mg of $[14C]$mannitol and 44.8% of...
The radioactivity incorporated into the liver, kidneys, serum, gastrocnemius muscle, perirenal and epidydimal adipose tissues, and small intestinal mucosa were significantly higher in the 105 mg of [14C]mannitol administration than in the 52.5 mg of [14C]mannitol administration (p<0.05, data not shown).

These results indicate that when the capacity of fermentation in intestinal microbes is enough to metabolize mannitol, mannitol is quickly fermented, the organic acids are produced, absorbed from the large intestine, and incorporated into the tissues.

5. Excretion of intact mannitol into feces and urine after oral administration of cold mannitol to conventional rats and antibiotics-treated rats

In order to confirm the fecal and urinary excretion of intact mannitol, mannitol was orally administered to both conventional and antibiotics-treated rats, and the feces and urine were collected for 48 h after administration. The difference in the radioactivity recovered in feces between conventional and antibiotics-treated rats is shown in Fig. 6. Nine percent of the intact mannitol was excreted to feces within 48 h in the antibiotics-treated rats, while no intact mannitol was detected in the feces of conventional rats after the administration of 105 mg of mannitol. The difference between the conventional rats and antibiotics-treated rats was significantly different at p<0.05.
rats was significant (p<0.05). These results strongly suggest that orally administered mannitol is decomposed spontaneously by intestinal microbes.

In conventional rats, 1.2% of the administered mannitol was excreted to urine in 48 h, and 1.4% of mannitol was detected in the urine of antibiotics-treated rats. The excretion of mannitol to urine was not significantly different between the conventional and antibiotics-treated rats.

**DISCUSSION**

When [14C]mannitol (105 mg) was orally administered to conventional rats, about 45% of the radioactivity administered was expired as 14CO2, and the time sequence for 14CO2 excretion was delayed as compared to [14C]glucose by about 4–6 h. Oku and Tokunaga et al. demonstrated that a time lag of about 4 h in 14CO2 excretion occurred between the two groups when [14C]sucrose, which is readily digested, and [14C]fructooligosaccharide, which is not digested by intestinal enzymes, were administered separately to conventional rats (23, 24). Therefore, the time lag of 4–6 h of 14CO2 excretion in [14C]mannitol administration to conventional rats is considered to be the period when [14C]mannitol administered orally arrives at the cecum where intestinal microbes live. [14C]Mannitol which arrived at the cecum might be rapidly metabolized by intestinal microbes and metabolized to SCFAs. Finally, SCFAs were metabolized by the host as available energy, because [14C]SCFAs which were directly injected to the cecum of conventional rats were quickly metabolized to 14CO2 (23, 24). When [14C]mannitol was administered to antibiotics-treated rats, only 3.3% of the radioactivity administered was expired as 14CO2 in 24 h; in contrast, 45% of the radioactivity administered was expired as 14CO2 in conventional rats. Additionally, the radioactivity remaining in the gastrointestinal tracts of antibiotics-treated rats was 7 times higher than that in conventional rats. Orally administered [14C]mannitol was incorporated dose-dependently in the liver, gastrocnemius muscle and perirenal and epididymal adipose tissues in conventional rats, while the period of the radioactivity in antibiotics-treated rats was markedly lower than that in conventional rats (p<0.05). Furthermore, when 105 mg of cold mannitol was administered to conventional rats, intact mannitol was not detected in the feces. These results strongly demonstrate that orally administered mannitol is transferred to the large intestine and is readily fermented by intestinal microbes. It is possible that orally administered mannitol was finally utilized by the host, because organic acids markedly increased in cecal content after mannitol feeding in rats (13). It is considered that orally administered mannitol that reaches to the lower intestine might not be metabolized to SCFAs in antibiotics-treated rats whose intestinal microbes were depleted by antibiotics.

In the present study, 105 mg of [14C]mannitol was administered to fasted conventional rats and about 45% of the radioactivity administered was expired as 14CO2 within 24 h (Fig. 2); in contrast, about 55% of the radioactivity administered was expired to 14CO2 in 24 h in the administration of 52.5 mg of [14C]mannitol to conventional rats (Fig. 5). The total of 14CO2 excretion for 24 h depends on the dose level in [14C]mannitol administered rats.

However, although the expiration of 14CO2 continued for 24 h in the administration of the larger amount (105 mg) of [14C]mannitol, the expiration of 14CO2 reached a plateau at 10 h after the administration of the smaller amount (52.5 mg) of [14C]mannitol. The [14C]radioactivities incorporated into the organs and tissues were higher in the administration of 105 mg (500 mg/kg B.W.) of [14C]mannitol than in that of 52.5 mg (250 mg/kg B.W.) of [14C]mannitol (data not shown). We have investigated the dose which did not cause osmotic diarrhea in rats and the maximum permissible dose was 500 mg/kg B.W. in rats (data not shown). We considered this to be the maximum amount of orally administered mannitol which intestinal microbes can completely metabolize to SCFAs, carbon dioxide, hydrogen, methane and others via fermentation in rats (30–33). When 52.5 mg of mannitol is administered, it is rapidly fermented by intestinal microbes and quickly utilized as energy by the host.

We previously clarified that rats fed a mannitol-containing diet showed a remarkable increase in the SCFAs of the cecal contents (13). In addition, Nishiyama et al. reported a similar increase of organic acids of the cecal contents in mannitol-fed rats (14). Furthermore, hardly any 14CO2 was excreted following the administration of [14C]mannitol to antibiotics-treated rats (Fig. 2). Similar results were obtained in the experiments using [14C]fructooligosaccharide (23, 24). When [14C]fructooligosaccharide (74 kBq, 4 mg) was orally administered to conventional rats, about 55% of the radioactivity was expired as 14CO2. However, when [14C]fructooligosaccharide was administered to antibiotics-treated rats or germfree rats, the excretion of 14CO2 was negligible (23, 24). The results obtained in the present study strongly demonstrate that orally ingested mannitol, which is hardly absorbed from the small intestine, is finally metabolized to CO2 via intestinal microbes and utilized as an energy source in the host (9, 10).

When [14C]glucose (222 kBq, 105 mg) was administered to conventional rats, 56.5% of the radioactivity administered was expired as 14CO2 and 5.4% of the radioactivity was expired into feces within 24 h. Furthermore, 1.8% of radioactivity remained in the contents of the gastrointestinal tracts, and 6.3% of radioactivity was expired to the urine in 24 h. Glucose administered orally is quickly absorbed from the small intestine and readily metabolized by the body. Therefore, the radioactivity excreted to urine appears to be derived from the metabolites of glucose absorbed from the small intestine, and the radioactivity of the contents of the gastrointestinal tracts is derived from the metabolites of glucose which are absorbed and excreted to the small intestine with the digestive fluid. On the other hand, when [14C]mannitol was orally administered to
conventional rats, 6.1% of radioactivity was excreted to the urine, 6.1% was excreted into the feces within 24 h, and 6.4% of radioactivity remained in the contents of the gastrointestinal tracts. The radioactivity excreted to the urine (6.1%) might be derived from the metabolites of SCFAs produced from [14C]mannitol by fermentation, because the excretion of [14C] radioactivity was decreased by the antibiotics treatment (1.1%). The radioactivities of feces and contents of the gastrointestinal tract may be the result of SCFAs produced from mannitol being absorbed from the large intestine and further metabolized by some organs, and these metabolites being secreted with digestive fluid. In fact, intact mannitol was not detected in the feces of conventional rats. Almost all of the orally administered mannitol is transferred to the lower intestine, and is completely fermented by intestinal microbes. A small amount might be excreted as the components of the microbes. It was thus estimated that more than 95% of mannitol administered orally was fermented by intestinal microbes.

The fermentation of nondigestible and/or nonabsorbable sugar substitutes by intestinal microbes produces SCFAs, CO2, CH4 and H2 (19–21). The SCFAs are absorbed from the large intestine and further metabolized by the host to produce energy (19–21). This energy value is considered to be less than 50% of the energy coefficient of sucrose (4 kcal/g). Then the available energy is 2 kcal/g when nondigestible/nonabsorbable sugar substitutes are completely fermented by intestinal microbes. Therefore, the available energy of mannitol is considered to be 2 kcal/g, because it is mostly fermented by intestinal microbes.

In conclusion, it was directly demonstrated in the administration of [14C]mannitol to the conventional rats and antibiotics-treated rats that mannitol administered orally was mainly metabolized and utilized via fermentation by intestinal microbes, and the utilisable percentage was estimated to be more than 95% in conventional rats. As in rats, mannitol in humans appears to be utilized via intestinal microbes. In addition, the energy coefficient of mannitol was estimated to be 2 kcal/g in the present study. Mannitol is used in many applications, such as medical and pharmaceutical products and the food industry. The evidence provided by the present study on the utilization of mannitol could contribute to the development of additional health-related uses of mannitol.

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REFERENCES
1) Dwivedi BK. 1991. Sorbitol and mannitol. In: Alternative Sweeteners (Nabors LB, Gelardi RC, eds), p 333–348. Marcel Dekker Inc. New York.
2) Nissenson AR, Weston RE, Kleeman CR. 1979. Mannitol. West J Med 131: 277–284.
3) Livesey G. 2003. Health potential of polyols as sugar replacers, with emphasis on low glycaemic properties. Nutr Res Rev 16: 163–191.
4) Song SH, Viille C. 2009. Recent advances in the biological production of mannitol. Appl Microbial Biotechnol 84: 55–62.
5) Uit JJ, Van Elburg RM, Van Overbeek FM, Mulder CJJ. 1997. Clinical implications of the sugar absorption test: Intestinal permeability test to assess mucusosal barrier function. Scand J Gastroenterol 32: S70–S78.
6) Zhou Q, Zhang B, Verne GN. 2009. Intestinal membrane permeability and hypersensitivity in the irritable bowel syndrome. Pain 146: 41–46.
7) Trehan I, Shulman RJ, Ou CN, Maleta K, Manary MJ. 2009. A randomized, double-blind, placebo-controlled trial of rifaximin, a nonabsorbable antibiotic, in the treatment of tropical enteropathy. Am J Gastroenterol 104: 2326–2333.
8) Haas V, Buning C, Buhner S, Heymann C, Valentini L, Lochas H. 2009. Clinical relevance of measuring colonic permeability. Eur J Clin Invest 39: 139–144.
9) Wick AN, Morita TN, Joseph L. 1954. The oxidation of mannitol. Proc Soc Exp Biol Med 85: 188–190.
10) Gongwer LE. 1979. Unpublished report. Mannitol. In: Dietary Sugars in Health and Disease (quoted by Allison RG), p 16. Report from Life Sciences Research Office, Federation of American Societies for Experimental Biology, Bethesda, MD.
11) Nasrallah SM, Iber FL. 1969. Mannitol absorption and metabolism in man. Am J Med Sci 258: 80–88.
12) Saunders DR, Wiggins HS. 1981. Conservation of mannitol, lactose, and raffinose by the human colon. Am J Physiol 241: 397–402.
13) Hongo R, Nakamura S, Oku T. 2010. Bioavailability of mannitol through intestinal microbes in rats. Jpn Assoc Dietary Fiber Res 14: 13–22 (in Japanese).
14) Nishiyama A, Nishiohka S, Islam SM, Sakaguchi E. 2009. Mannitol lowers fat digestibility and body fat accumulation in both normal and cecrectomized rats. J Nutr Sci Vitaminol 55: 242–251.
15) Ellis FW, Krantz JC. 1941. Sugar alcohols. J Biol Chem 141: 147–134.
16) Carr CJ, Krantz JC. 1938. Sugar alcohols. J Biol Chem 124: 221–227.
17) Todd WR, Myers J, West ES. 1938. On the metabolism of sorbitol and mannitol. J Biol Chem 127: 275–284.
18) Ellwood KC. 1995. Methods available to estimate the energy values of sugar alcohols. Am J Clin Nutr 62: S1169–S1174.
19) Oku T, Nakamura S. 2002. Digestion, absorption, fermentation, and metabolism of functional sugar substitutes and their available energy. Pure Appl Chem 74: 1253–1261.
20) Livesey G. 1992. The energy value of dietary fiber and sugar alcohols for man. Nutr Res Rev 5: 61–84.
21) Cummings JH, Macfarlane GT. 1991. The control and consequences of bacterial fermentation in the human colon. J Appl Bacteriol 70: 443–459.
22) Morishita Y. 1994. The effect of dietary mannitol on the caecal microflora and short-chain fatty acids in rats. Lett Appl Microbiol 18: 27–29.
23) Oku T. 1996. Oligosaccharides with beneficial health effects: A Japanese perspective. Nutr Rev 54: S59–S66.
24) Tokunaga T, Oku T, Hosoya N. 1989. Utilization and excretion of a new sweetener, fructooligosaccharide (neosugar), in rat. J Nutr 119: 553–559.
25) Chonan O, Takahashi R, Watanuki M. 2001. Role of activity of gastrointestinal microflora in absorption of calcium and magnesium in rats fed β1-4 linked galactooligosaccharides. *Biosci Biotechnol Biochem* **65**: 1872–1875.

26) Wang B, Egashira Y, Ohta T, Sanada H. 1998. Effect of indigestible oligosaccharides on the hepatotoxic action of β-galactosamine in rats. *Biosci Biotechnol Biochem* **62**: 1504–1509.

27) Noverr MC, Noggle RM, Toews GB, Huffnagle GB. 2004. Role of antibiotics and fungal microbiota in driving pulmonary allergic responses. *Infect Immun* **72**: 4996–5003.

28) Noverr MC, Falkowski NR, McDonald RA, McKenzie AN, Huffnagle GB. 2005. Development of allergic airway disease in mice following antibiotic therapy and fungal microbiota increase: Role of host genetics, antigen and interleukin-13. *Infect Immun* **73**: 30–38.

29) SPSS Inc. 2007. SPSS Base 16.0 applications guide for Japanese. SPSS Inc Japan, Tokyo (in Japanese).

30) Oku T, Nakamura S. 2007. Threshold for transitory diarrhea induced by ingestion of xylitol and lactitol in young male and female adults. *J Nutr Sci Vitaminol* **53**: 13–20.

31) Oku T, Nakamura S. 2005. Maximum permissive dosage of lactose and lactitol for transitory diarrhea, and utilizable capacity for lactose in Japanese female adults. *J Nutr Sci Vitaminol* **51**: 51–57.

32) Oku T, Nakamura S. 2000. Estimation of intestinal trehalase activity from a laxative threshold of trehalose and lactulose on healthy female subjects. *Eur J Clin Nutr* **54**: 783–788.

33) Oku T, Nakamura S. 2003. Comparison of digestibility and breath hydrogen gas excretion of fructooligosaccharide, galactosyl-sucrose, isomalt-oligosaccharide in healthy human subjects. *Eur J Clin Nutr* **57**: 1150–1156.