Utilization and Excretion of a New Sweetener, Fructooligosaccharide (Neosugar), in Rats

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ABSTRACT In order to study the digestibility of the fructooligosaccharide "Neosugar," [U-^{14}C]Neosugar or [U-^{14}C]sucrose was orally administered to germfree, conventional and antibiotic-treated rats and the radioactivities of expired $^{14}CO_2$, urine and feces were determined 24 h later. More than 50% of the Neosugar was expired as CO_2 in conventional rats. This was the same as for sucrose, but the time course was delayed by about 2 h. In germfree rats, no $^{14}CO_2$ was released for the first 8 h, and $^{14}CO_2$ released after 8 h probably reflected bacterial colonization of the gut. The radioactivity of the urine was about 3–4% in all groups, but that of the feces from germfree rats was about eight times higher than the level in conventional rats. When [U-^{14}C]Neosugar was anaerobically incubated with the cecal contents of conventional rats, more than 10% of the added Neosugar was metabolized to CO_2, about 66% to volatile fatty acids and about 7% to microbes. More than 58% of 1-^{14}C-volatile fatty acids such as acetic acid, propionic acid or butyric acid injected directly into the cecum of conventional rats was excreted as CO_2 within 24 h. These results indicate that Neosugar given orally to rats is metabolized mainly to volatile fatty acids and CO_2 by intestinal microorganisms, and the volatile fatty acids produced are absorbed and further converted to CO_2 in the body. Thus, the data indicate that Neosugar is partially utilized as an energy source. *J. Nutr.* 119: 553–559, 1989.

INDEXING KEY WORDS:

- *sweetener* • *fructooligosaccharide*
- *Neosugar* • *conventional rats*
- *germfree rats* • *microorganism*
- *volatile fatty acids*

A newly developed sweetener, "Neosugar," is a mixture of fructooligosaccharides consisting of approximately 28% 1-kestose (GF_2), 60% nystose (GF_3) and 12% 1^F-β-fructofuranosyl nystose (GF_4). Its formula is illustrated in **Figure 1**. The sweetness of Neosugar is about half that of sucrose, and its characteristics are similar to those of sucrose (1–3). GF_2, GF_3 and GF_4 occur naturally in many kinds of plants such as onion, asparagus root, tubers of the Jerusalem artichoke and wheat (3). At present, Neosugar can be manufactured from sucrose by a fungal fructosyltransferase (1, 2).

The fructooligosaccharides are not hydrolyzed in the rat by digestive enzymes such as disaccharidases of intestinal mucosa and α-amylase of pancreatic homogenates (4). Neosugar injected intravenously is rapidly excreted into the urine without degradation, indicating no decomposition by the hydrolyzing enzymes of the internal organs (4). Furthermore, when rats are fed a diet containing Neosugar for a prolonged period, body weight gain and serum triacylglycerol levels are lower than those of rats fed the control diet without Neosugar. Fecal weight increases significantly and the gastrointestinal transit time is shortened. The excretion of fecal volatile fatty acids is also increased (5).

These results suggest that Neosugar is partially utilized as an energy source and that it has attributes similar to dietary fiber. However, there have been no direct observations of whether or not orally administered Neosugar is metabolized in the body. The purpose of the present study was to clarify the metabolic fate of orally administered [U-^{14}C]Neosugar in rats and to observe the conversion of Neosugar to volatile fatty acids and other derivatives by intestinal microorganisms.

MATERIALS AND METHODS

Animals and diets. Conventional male Wistar rats (Nisseizai, Tokyo) weighing 240 g were fed a nonpurified diet (MF, Oriental Yeast, Tokyo) or experimental diets with or without Neosugar as shown in **Table 1** for 2–3 wk (5). To decrease intestinal microorganisms, several conventional male rats weighing about 240 g were given drinking water containing penicillin G (50 units/ml) and chloramphenicol (50 μg/ml) for 1 wk before use. Germfree male rats weighing about 120 g were

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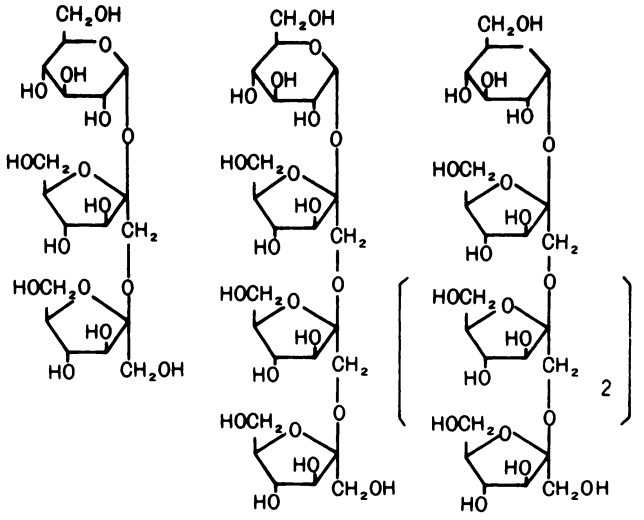

FIGURE 1 Chemical structure of fructooligosaccharides.

purchased from Clea Japan (Tokyo). All animals were fasted overnight before dosing, and during the metabolic experiment, the nonpurified diet without Neosugar and water were given ad libitum.

Preparation of [U-^{14}C]Neosugar. [U-^{14}C]Neosugar was prepared enzymatically from [U-^{14}C]sucrose (specific activity 555 mCi/mmol). One half of a milliliter of 25% (wt/v) sucrose solution (250 μCi) was incubated with 0.36 units of purified fructosyltransferase (*Aspergillus niger* origin) at 40°C for 8 h (1). The reactants were applied onto an activated charcoal column and eluted step-wise with adequate concentrations of ethanol solution. The ^{14}C-materials in each fraction were identified by thin layer chromatography with Kiesel gel 60 (E. Merck AG., Darmstadt, West Germany) (7). The yield of [U-^{14}C]Neosugar was 30.2% GF_2, 50.0% GF_3 and 19.8% GF_4.

Administration of [U-^{14}C]Neosugar and determination of radioactivity. Three μCi of [U-^{14}C]Neosugar (2.1 mg) in 0.6 ml of 0.9% NaCl solution was administered to rats with a gastric tube. Immediately after the dose, each rat was transferred to a glass metabolic cage (Metabolica, Sugiyamagen, Tokyo) kept at 25 ± 1°C (4). The expired $^{14}CO_2$ was trapped with 500 ml of monoethanolamine refreshed at 6 and 12 h, and sampling (1 ml) was carried out at 1, 2, 4, 6, 8, 10, 12 and 24 h. The radioactivity of 0.2 ml of monoethanolamine was determined in a liquid scintillation counter (Model LS-300, Beckman, Palo Alto, CA) after the addition of 10 ml of scintillation cocktail with 0.4% Omnifluor (New England Nuclear, Boston, MA) in a toluene:triton X-100 (2:1) solution. Urine and feces were collected separately in the bottles of the metabolic cage at intervals of 2 or 3 h. The feces were homogenized in 3–5 volumes of distilled water using a polytron (Brinkman, Westburg, NY) and centrifuged at 10,000 × *g* for 15 min to obtain the supernatant. One milliliter of the supernatant or the urine was mixed with 10 ml of the scintillation cocktail to measure the radioactivity. The counting efficiency was 70–80%.

TABLE 1

Diet composition

Ingredients	Control diet	Neosugar diet
	%	
Corn starch[1]	67	57
Neosugar[2]	0	10
Milk casein[1]	21	21
Corn oil[1]	7	7
Mineral mixture[1]	4	4
Vitamin mixture[3]	1	1

[1]Clea Japan (Tokyo). Mineral mixture (mg/100 g diet): $CaCO_3$, 1355; KH_2PO_4, 1730; $CaHPO_4 \cdot 2H_2O$, 6; $CuSO_4 \cdot 5H_2O$, 1.26; $CoCl_2 \cdot 6H_2O$, 0.4; $Ca(IO_3)_2$, 1.54; $MnSO_4 \cdot 4H_2O$, 15.4.
[2]Meiji Seika Kaisha (Tokyo), composed of 28% 1-kestose, 60% nystose and 1F-β-fructofuranosyl nystose 12%.
[3]AIN-76 mixture (6).

Injection of 1-^{14}C-volatile fatty acids into the cecum. Two and one-half μCi of [1-^{14}C]acetic acid, [1-^{14}C] propionic acid or [1-^{14}C]butyric acid (all sodium salts) was injected directly into the cecum of conventional rats (about 200 g body wt) after a laparotomy performed under ether anesthesia. Immediately after the incision was closed with wound clips, the rats were transferred to the glass metabolic cages. The $^{14}CO_2$ expired over 24 h was collected and measured as described above.

Identification of ^{14}C-materials derived from [U-^{14}C] Neosugar in the urine. ^{14}C-Materials excreted in the urine were separated by activated charcoal:celite (1:1) column chromatography (1 × 10.5 cm). A mixture of 1.8 mg fructose, 8.4 mg glucose, 3.0 mg sucrose, 6.9 mg GF_2, 6.9 mg GF_3 and 3.0 mg GF_4 was added as a tracer to 3 ml of urine. ^{14} C-Materials in the urine were eluted with 80 ml of distilled water and 70 ml of 20% ethanol solution. The effluent (5 ml) was collected in a tube and 1 ml of each fraction was used to determine the radioactivity. The sugars in each fraction were identified by thin layer chromatography as described above.

Metabolism of [U-^{14}C]Neosugar by the cecal contents in vitro. Cecal contents from the rats fed the experimental diets for 2 wk were suspended in 4 volumes of phosphate buffered saline (pH 7.5) containing 0.1 M KH_2PO_4, 0.1 M Na_2HPO_4, 0.9% NaCl and 2% ascorbate-cysteine solution (a mixture of 3.4% L-ascorbate and 0.5% L-cysteine chloride, pH 7.4) to maintain anaerobic conditions (8). The suspension (30 ml) was incubated with 0.5 μCi of [U-^{14}C]Neosugar (100 mg) at 37°C for 6 h, bubbling with nitrogen gas (50 ml/min). The $^{14}CO_2$ produced was accumulated in 50 ml of monoethanolamine, which was refreshed at 3 h, and the radioactivity was measured as described above. After

centrifugation of the incubated reactant, the precipitate was washed three times with distilled water and resuspended in an adequate volume of distilled water. The suspension (0.5 ml) was solubilized with 1 ml of Protosol (New England Nuclear, Boston, MA) at 50°C for 6 h to measure the radioactivity incorporated into the intestinal microorganisms. A portion of the supernatant was used to measure volatile fatty acids.

Determination of volatile fatty acids in feces. To measure volatile fatty acids, supernatant incubated with cecal contents (1 ml) was extracted with 2 ml of ethylether after the addition of 1 ml of distilled water, 20 μl of 50% H_2SO_4 solution and 0.75 g of NaCl (9). This extraction was carried out twice. The ether phases were combined and dried after the addition of 50 μl of 10 *N* NaOH to prevent the escape of volatile fatty acids. The sodium salts of the volatile fatty acids were redissolved in 100 μl of distilled water and separated by thin layer chromatography using a Kiesel gel 60 plate. The plates were developed with acetone:*n*-butanol:*t*-butanol:NH_4OH (2:1:1:1) solution. Volatile fatty acid spots were detected with sprayed 0.04% bromcresol purple solution in formaldehyde:ethanol (1:5) and exposure to NH_3 vapor (10). To measure the radioactivity, each spot was scraped. The recovery rates were 48% acetic acid, 85% propionic acid and 88% butyric acid.

Chemicals. [1-^{14}C]Acetic acid sodium salt (56.0 mCi/mmol), [1-^{14}C]propionic acid sodium salt (58.4 mCi/mmol) and [1-^{14}C]butyric acid sodium salt (13.4 mCi/mmol) were purchased from New England Nuclear and [U-^{14}C]sucrose (555 mCi/mmol) was obtained from Amersham International (Buckinghamshire, England). Neosugar and fructosyltransferase from *Aspergillus niger* were kindly provided by Meiji Seika Kaisha Ltd. (Tokyo, Japan). All other reagents employed were analytical grade.

Statistical analysis. Data were analyzed by analysis of variance and Duncan's multiple-range test. An effect was considered to be significant at $P < 0.05$.

RESULTS

Metabolism of [U-^{14}C]Neosugar in conventional rats. About 55% of the radioactivity given as [U-^{14}C]Neosugar to conventional rats was expired as $^{14}CO_2$ within 24 h; 3.2% and 3.0% was execreted in urine and feces, respectively (**Table 2**). About 56% of the radioactivity given as [U-^{14}C]sucrose was expired as $^{14}CO_2$ within 24 h; 4.6% and 4.8% was excreted in urine and feces, respectively (Table 2). The total amounts of radioactivity recovered as $^{14}CO_2$ within 24 h were not significantly different between Neosugar and sucrose groups. The time sequence for $^{14}CO_2$ release in animals given [U-^{14}C]Neosugar was delayed for about 2 h compared to that of [U-^{14}C]sucrose (**Fig. 2**).

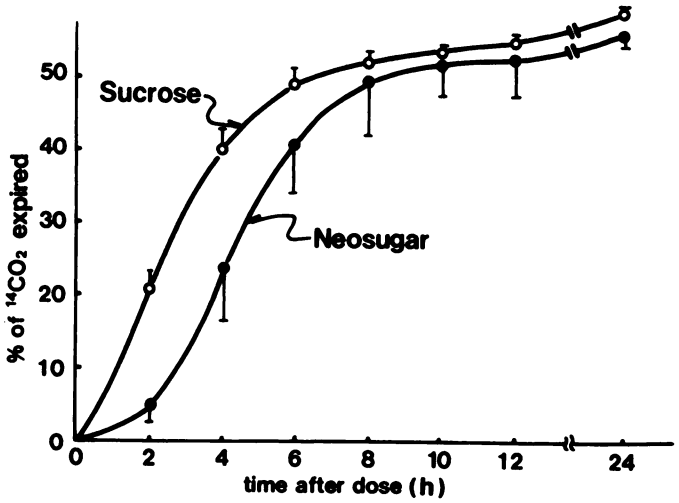

FIGURE 2 Time course of expired $^{14}CO_2$ from conventional rats orally administered [U-^{14}C]Neosugar or [U-^{14}C]sucrose. [U-^{14}C]Neosugar (3 μCi/2.1 mg) in 0.6 ml of 0.9% NaCl solution or [U-^{14}C]sucrose (2 μCi/2.8 mg) in 0.8 ml of 0.9% NaCl solution was orally administered to conventional rats. Immediately after dosing, rats were transferred to metabolic cages and the expired $^{14}CO_2$ was collected for 24 h. Each point represents the mean ± SD of 2–3 rats/group.

Our data indicate that orally administered Neosugar and sucrose are converted to CO_2 to a similar extent. However, the 2-h delay in $^{14}CO_2$ release in animals given Neosugar suggests a difference in the overall processes by which these two compounds are converted into CO_2.

Influence of intestinal microorganisms on Neosugar metabolism. Contrary to our expectations, more than

TABLE 2

Metabolism of [U-^{14}C]Neosugar and [U-^{14}C]sucrose administered orally to conventional, antibiotic-treated or germfree rats

	[U-^{14}C]Neosugar			[U-^{14}C]sucrose[1]		
^{14}C-materials	Conventional	Antibiotic-treated	Germfree	Conventional	Antibiotic-treated	Germfree
			% of total radioactivity administered			
$^{14}CO_2$	54.6 ± 3.4	40.3 ± 1.0	36.3 ± 6.2	56.5 ± 3.5	60.0 ± 1.7	68.2 ± 2.2
^{14}C in urine	3.2 ± 0.4	3.6 ± 0.4	3.6 ± 0.1	4.6 ± 0.1	5.3 ± 0.6	2.4 ± 0.3
^{14}C in feces	3.0 ± 1.0	7.6 ± 2.7	25.5 ± 2.7	4.8 ± 1.0	3.0 ± 0.7	2.7 ± 0.6

[1]Each value represents the mean ± SD. [U-^{14}C]Neosugar (2.4–3.0 μCi) or [U-^{14}C]sucrose (2 μCi) was orally administered to three conventional rats (body wt ~ 240 g), two antibiotic-treated rats (body wt ~ 250 g) and two germfree rats (body wt ~ 120 g) using a gastric tube.

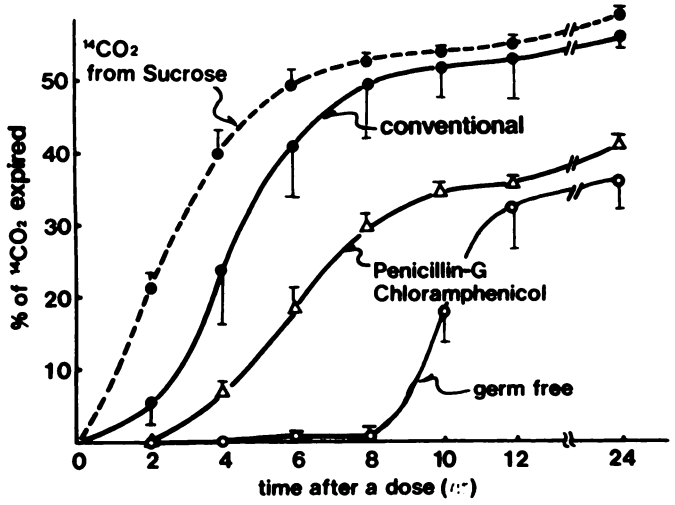

FIGURE 3 Time course of expired $^{14}CO_2$ from conventional, antibiotic-treated and germfree rats orally administered [U-^{14}C]Neosugar. Each point represents the mean ± SD for 2–3 rats/group. See Table 2 for total percent of $^{14}CO_2$ expired. Values for sucrose (broken line; data from Fig. 2) are given in order to compare with those of Neosugar.

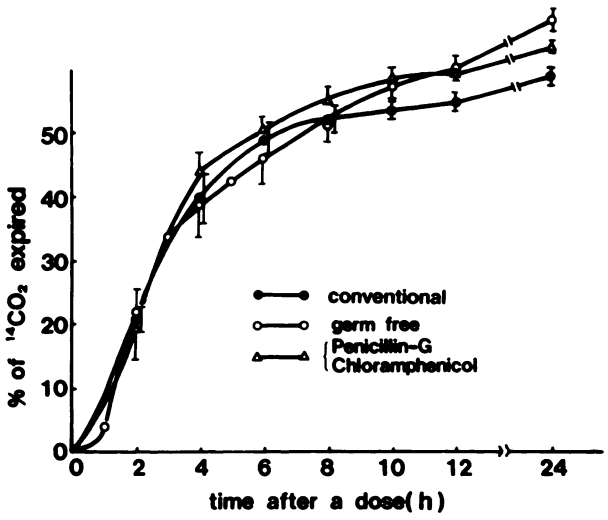

FIGURE 4 Time course of expired $^{14}CO_2$ from conventional, antibiotic-treated and germfree rats orally administered [U-^{14}C]sucrose. Each point represents the mean ± SD of two rats. See Table 2 for total percent of $^{14}CO_2$ excreted.

50% of the Neosugar administered orally was metabolized to CO_2 within 24 h, in spite of it not being hydrolyzed by intestinal mucosal enzymes. One reason appears to be that the intestinal microorganisms metabolize Neosugar in a fashion similar to that for dietary fiber (11–14). Therefore, the metabolism of Neosugar was investigated using rats given antibiotics and germfree rats.

When [U-^{14}C]Neosugar was orally administered to rats given antibiotics, there was a greater delay in $^{14}CO_2$ release than with conventional rats. The amount of $^{14}CO_2$ expired within 24 h in animals treated with antibiotics was significantly lower than that of conventional rats (**Fig. 3**). The amount of radioactivity in the urine was similar to that of conventional rats, but that in feces was about double (Table 2).

Germfree rats given [U-^{14}C]Neosugar expired little $^{14}CO_2$ in the first 8 h after the dosage (Fig. 3). The radioactivity of expired $^{14}CO_2$ within 24 h was about 40% of the total amount given. The radioactivity excreted in the feces was much higher than that of the other two groups, although ^{14}C-materials excreted in the urine was not different (Table 2). On the other hand, the metabolism of [U-^{14}C]sucrose was slightly stimulated by the absence of intestinal microorganisms. ^{14}C-Materials excreted in urine and feces were slightly lower in germfree rats than in conventional rats (Table 2, **Fig. 4**).

These observations suggest that Neosugar is metabolized by intestinal microorganisms in the gastrointestinal tract, although the nature of this process and the metabolic products are unknown.

Identification of ^{14}C-materials from [U-^{14}C]Neosugar in urine. In order to confirm the contribution of intestinal microorganisms to $^{14}CO_2$ production, the urine samples were divided into two time periods; 0–7 h and 12–24 h after [U-^{14}C]Neosugar dosage. The ^{14}C-materials in the urine collected 0–7 h after the dosage were almost entirely disaccharides and/or oligosaccharides and a small amount of nonsaccharic material (**Fig. 5**).

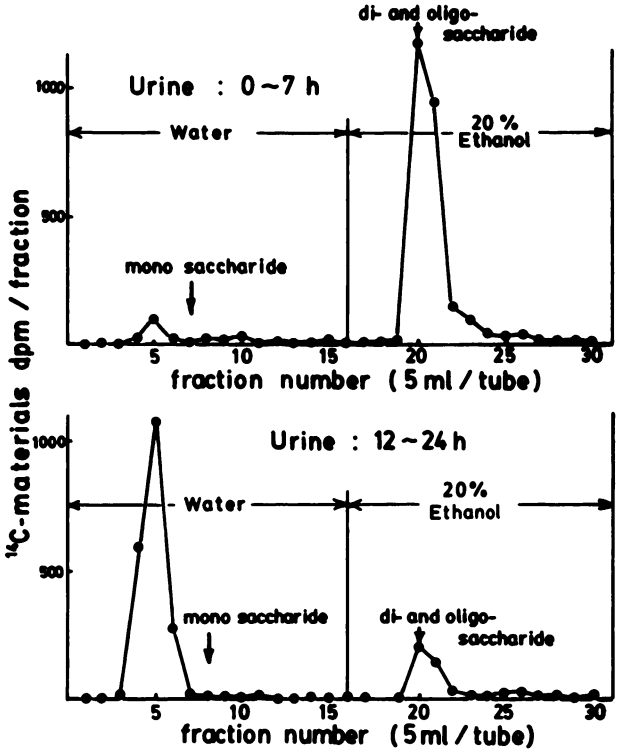

FIGURE 5 Identification of ^{14}C-materials in the urine obtained from germfree rats orally administered [U-^{14}C]Neosugar. Urine (3 ml) was collected for two time periods, 0–7 h and 12–24 h, after [U-^{14}]Neosugar was orally administered to germfree rats. Each point represents the mean of duplicate determinations.

In the urine collected 12–24 h after the dosage, there were large amounts of nonsaccharic ^{14}C-material but only small amounts of disaccharides and/or oligosaccharides. These results also suggest that Neosugar administered orally is metabolized to other materials by intestinal microorganisms.

Metabolism of [U-^{14}C]Neosugar by intestinal microorganisms in vitro. In order to clarify the metabolism of Neosugar by intestinal microorganisms, a suspension of the cecal contents of rats fed a Neosugar-free diet or the diet containing 10% Neosugar was anaerobically incubated with [U-^{14}C]Neosugar, and the products were then identified. More than 10% of the radioactivity added to the reaction medium was converted to $^{14}CO_2$ during the 6-h incubation (**Table 3**). In rats fed a Neosugar-free diet, about 66% of the [U-^{14}C]Neosugar was metabolized to the ether-extractable ^{14}C-materials, most of them volatile fatty acids.

Among volatile fatty acids from [U-^{14}C]Neosugar, the most abundant was butyric acid (29%), followed by acetic acid (17%), propionic acid (16%) and valeric acid (5%). Except for the radioactivity incorporated into the precipitates, no significant difference in the percent of the products from Neosugar was found between rats fed a Neosugar-free diet and rats fed a Neosugar diet (Table 3).

The production of $^{14}CO_2$ from [U-^{14}C]Neosugar in vitro indicates that a portion of the expired $^{14}CO_2$ from rats given [U-^{14}C]Neosugar involves the action of intestinal microorganisms.

Metabolism of [1-^{14}C]acetic acid, [1-^{14}C]propionic acid or [1-^{14}C]butyric acid injected directly into the cecum of conventional rats. It appears that the volatile fatty acids produced from Neosugar are absorbed from the lower gut and utilized as an energy source or that the volatile fatty acids may be further metabolized to more polar substances by intestinal microorganisms.

In order to confirm that volatile fatty acids are absorbed from the cecum and further metabolized in the body to other substances, [1-^{14}C]acetic acid, [1-^{14}C]propionic acid or [1-^{14}C]butyric acid was directly injected into the cecum of conventional rats and expired $^{14}CO_2$ was observed during 24 h. As shown in **Table 4**, all volatile fatty acids tested were readily metabolized, and 60–70% of the injected volatile fatty acids were expired as $^{14}CO_2$ within 24 h. The radioactivity in the urine was 3.1–3.9% and that in the feces was 6–8% (Table 4).

These results suggest that volatile fatty acids are rapidly absorbed from the cecum and metabolized to CO_2, and that the expired $^{14}CO_2$ from conventional rats given orally administered [U-^{14}C]Neosugar involves $^{14}CO_2$ from volatile fatty acids produced from Neosugar by intestinal microorganisms.

TABLE 3

^{14}C-Products from [U-^{14}C]Neosugar after incubation with cecal contents from conventional rats fed a diet with or without Neosugar[1]

^{14}C-products	Cecal contents	
	Neosugar-free diet	Neosugar diet
	% of total radioactivity in the medium	
$^{14}CO_2$	12.5 ± 3.4	11.8 ± 3.8
^{14}C in microbes[2]	6.2 ± 0.2	10.8 ± 1.6[3]
^{14}C-volatile fatty acids	66.1 ± 2.2	71.5 ± 6.6
acetic acid	16.9 ± 3.0	17.9 ± 1.2
propionic acid	15.8 ± 3.8	18.9 ± 10.8
butyric acid	28.7 ± 3.6	29.3 ± 6.2
valeric acid	4.8 ± 1.6	5.4 ± 2.6
Others	11.8 ± 4.6	5.2 ± 5.2
Recovery	95.8 ± 1.8	98.6 ± 4.0

[1]Each value represents the mean ± SD of four rats with duplicate determinations.

[2] ^{14}C in microbes shows that the radioactivity was incorporated into the precipitate of the reactant.

[3]Significantly different from the Neosugar-free diet group at $P < 0.01$.

TABLE 4

Metabolism of [1-^{14}C]acetic acid, [1-^{14}C]propionic acid and [1-^{14}C]butyric acid injected directly into the cecum of conventional rats[1]

^{14}C-materials	Volatile fatty acids injected[2]		
	Acetic acid	Propionic acid	Butyric acid
	% of total radioactivity injected		
CO_2	67.1 ± 6.3	69.0 ± 4.1	58.9 ± 12.9
Urine	3.9 ± 0.7	3.1 ± 0.2	3.5 ± 0.9
Feces (supernatant)	4.5 ± 1.9	2.9 ± 2.4	3.5 ± 2.6
Feces (precipitate)	3.4 ± 0.7	2.7 ± 0.3	3.7 ± 1.2

[1]Each value represents the mean ± SD of three rats.

[2]Each [1-^{14}C]volatile fatty acid (2.5 μCi) was directly injected into the cecum of anesthetized conventional rats. $^{14}CO_2$, urine and feces were collected in glass metabolic cages for 24 h.

DISCUSSION

Neosugar is not hydrolyzed by digestive enzymes (4). Prolonged intake of Neosugar decreases the body weight gain, lowers triacylglycerol and increases the fecal weight and fecal excretion of volatile fatty acids (5).

Gastrointestinal transit time is also shortened as with dietary fiber. Therefore, we thought that Neosugar might not be metabolized in the body when orally administered to conventional rats. Contrary to our expectations, more than 50% of the Neosugar administered was expired as CO_2 within 24 h, just as for sucrose (Fig. 2).

There were two reasons why we suspected that Neosugar administered orally was metabolized and excreted as CO_2. Because a fructooligosaccharide such

as inulin is unstable in acidic pH (15), we speculated that Neosugar may be hydrolyzed to monosaccharides by the gastric juices during passage through the stomach. However, the results obtained with germfree rats administered [^{14}C]Neosugar (Fig. 3) eliminated this possibility. The excretion of $^{14}CO_2$ was scarcely detected until 8 h after ingestion whereas sucrose, a digestible saccharide, was fully metabolized even in germfree rats (Fig. 4). The results indicate that hydrolysis of Neosugar in the stomach does not occur in rats.

Secondly, we suspected that Neosugar may be fermented by intestinal microorganisms and that products such as volatile fatty acids are absorbed from the lower intestine and further metabolized to CO_2. As expected, the data obtained from the experiments using conventional, antibiotic-treated and germfree rats demonstrated that Neosugar is largely metabolized by intestinal microorganisms (Table 2). This is similar to the fermentation of nondigestible polysaccharides such as cellulose in ruminants (16) and suggests that microorganisms play an important role in degrading nondigestible carbohydrates in the nonruminant.

Nevertheless, when [U-^{14}C]Neosugar was orally administered to germfree rats, more than 30% of the total radioactivity was expired as $^{14}CO_2$ between 8 and 24 h after dosage (Fig. 3), perhaps for the following reason. Since germfree rats were housed in metabolic cages using conventional conditions during the experiment, microbial colonization of the germfree rats might start at the beginning of the metabolic experiment. Thus, the microorganisms should convert Neosugar to other metabolizable materials. This consideration is supported by the observation that the ^{14}C-materials in the urine during 0–7 h after the dose of Neosugar were almost all disaccharides and/or oligosaccharides, and those in the urine during 12–24 h were mainly nonsaccharic.

It is thought that disaccharides such as maltose, sucrose and lactose and oligosaccharides are not absorbed directly from the small intestine without digestion. However, maltitol, a disaccharic alcohol which is resistant to digestive enzymes, is detected in the blood of humans after oral administration (17). In the present study, disaccharides and/or oligosaccharides were also detected in the urine during 0–7 h after the oral dose of [U-^{14}C]Neosugar. This suggests the possibility that small amounts of disaccharides and/or oligosaccharides which are resistant to the digestive enzymes of intestinal mucosa are absorbed from the small or large intestine without any degradation.

More than 10% of the Neosugar added to the reaction medium was metabolized to $^{14}CO_2$ when [U-^{14}C]Neosugar was anaerobically incubated with the cecal contents of conventional rats for 6 h (Table 3). Also, the gas produced in the ileum and the cecum contained $^{14}CO_2$ which could be trapped with monoethanolamine when [U-^{14}C]Neosugar was directly injected into the duodenum of conventional rats (data not shown). This CO_2 production by intestinal microorganisms suggests that the metabolically available energy of Neosugar is reduced in a manner corresponding to the quantity of CO_2. This idea is further supported by the observation that long-term feeding of Neosugar decreases body weight gain, while digestible saccharides such as starch (5) do not.

Miller and Wolin (16) have demonstrated fermentation equations in rumen and human feces. In the fermentation equations, the only metabolizable fraction, i.e., source of energy, of fermentative by-products are the volatile fatty acids such as acetate, propionate and butyrate. Total combustion energy of volatile fatty acids produced from 1 g of substrate is calculated to be about 10.9 kJ/g (2.6 kcal/g). Accordingly, this indicates that the apparent metabolizable energy of nondigestible saccharide is reduced to at least 66% of the combustion energy when it is fermented by bacteria. Neosugar given orally is not degraded by digestive enzymes and readily fermented. Therefore, the metabolically available energy of Neosugar is much less than that of digestible saccharide. The net metabolically available energy seems to be even less than the apparent metabolizable energy because the efficiency ratio of volatile fatty acids is not 100%.

Whether volatile fatty acids produced in the gut act only as energy sources or whether they have other physiological actions is not known. They may influence lipid metabolism, perhaps by reducing serum triacylglycerol and cholesterol levels (5, 18, 19), because Neosugar has dietary fiber-like attributes in spite of being a low-molecular-weight oligosaccharide. In preliminary experiments, Neosugar did not inhibit the intestinal absorption of cholesterol in a manner similar to that of a dietary fiber such as glucomannan (data not shown).

Hidaka et al. (3) have demonstrated that Neosugar is selectively utilized by *Bifidobacterium* in vitro. In the fermentative experiment reported herein, the radioactivity incorporated into the cecal precipitate was greater in the Neosugar-adapted animals than in the non-adapted rats (Table 3). This therefore appears to be dependent on the proliferation of *Bifidobacterium* activated by Neosugar feeding. This suggests that a chronic intake of Neosugar may improve the environment of the intragastrointestinal tract by increasing *Bifidobacterium* and decreasing putrefactive microorganisms.

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