Utilization and Metabolism of \([U^{14}C]4'\text{Galactosyllactose} (O-\beta-D-Galactopyranosyl-(1\rightarrow4)-O-\beta-D-Galactopyranosyl-(1\rightarrow4)-D-Glucopyranose)\) in Rats

Koutaro OHTSUKA,1 Keisuke TSUJI,2 Yasue NAKAGAWA,3 Hiroshi UEDA,1 Osamu OZAWA,1 Takatsugu UCHIDA,1 and Tomio ICHIKAWA2

1 Nissin Sugar Mfg. Co. Ltd., Koutou-ku, Tokyo 135, Japan 2 The National Institute of Health and Nutrition, Shinjuku-ku, Tokyo 162, Japan 3 Jissen Women’s University, Hino, Tokyo 191, Japan

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Summary \(O-\beta-D-Galactopyranosyl-(1\rightarrow4)-O-\beta-D-galactopyranosyl-(1\rightarrow4)-D-glucopyranose\) (designated as 4'GL) are produced from lactose with Cryptococcus laurentii OKN-4. Excretion and metabolism of 4'GL in rats were examined using a radioisotope technique. \([U^{14}C]4'GL\) was synthesized from \([U^{14}C]\)lactose by Cryptococcus laurentii OKN-4. The \(^{14}\text{CO}_2\) in expired air was counted after oral administration of \([U^{14}C]4'GL\) or \([U^{14}C]\)lactose in conventional rats, rats treated with antibiotics and germ-free rats. The rate of \(^{14}\text{CO}_2\) excretion from conventional rats given \([U^{14}C]4'GL\) was slower than that from those administered \([U^{14}C]\)lactose. When \([U^{14}C]4'GL\) was orally administered to rats given antibiotics, there was a 2-h delay in \(^{14}\text{CO}_2\) excretion, as compared to conventional rats. In germ-free rats, total excretion of \(^{14}\text{CO}_2\) from \([U^{14}C]4'GL\) decreased to about one-third of that of conventional rats during a 24-h period. Radioactivities in the serum, liver, and carcass of the \([U^{14}C]4'GL\) oral administration group were lower than those of the \([U^{14}C]\)lactose oral administration group. Radioactivities in the feces and urine however, were higher in \([U^{14}C]4'GL\) group than in \([U^{14}C]\)lactose group.

Key Words galactooligosaccharide, Cryptococcus laurentii, lactose substitutes, \([U^{14}C]4'\text{galactosyllactose}, [U^{14}C]\)lactose, \(^{14}\text{CO}_2\) excretion, metabolism of galactooligosaccharide, radioisotope technique, bioavailability, dietary fiber

A strain identified as Cryptococcus laurentii OKN-4 was found to produce \(O-\beta-D-galactopyranosyl-(1\rightarrow4)-O-\beta-D-galactopyranosyl-(1\rightarrow4)-D-glucopyranose\) (hereinafter designated as 4'GL) from lactose (1). The administration of 4'GL to
healthy adult men induced a significant increase in the number of fecal Bifidobacterium and a decrease in Bacteroidaceae and Enterobacteriaceae (2). Moreover, 4’GL feeding to elderly constipated patients improved the constipation and the intestinal microflora without causing side effects (3).

4’GL was not hydrolyzed by salivary and pancreatic α-amylase in vitro, and only a very small amount of 4’GL was digested by the homogenate of the rat small intestinal mucosa. Also 4’GL was not hydrolyzed by the artificial gastric juice (pH 1.0) after 6 h incubation at 37°C (4). These in vitro observations suggest that utilization of 4’GL as an energy source in the body might be difficult. No in vivo studies have been conducted however, to determine if 4’GL is utilized in the body.

In the present study, we attempt to elucidate the possibility of utilization of 4’GL for energy in the rat by a radioisotope technique following ingestion of [U-14C]4’GL.

METHODS

Chemicals. [U-14C]Lactose (specific activity 2.5×10¹⁰ Bq/mmol) were purchased from New England Nuclear. Monoethanolamine, 2-methoxyethanol, POPOP, DPO, Triton X-100, toluene, 1-butanol, pyridine, ethanol, lactose, (NH₄)₂SO₄, KH₂PO₄, MgSO₄·7H₂O, meat extract, yeast extract, and charcoal were obtained from Wako Pure Chemical. Penicillin G and chloramphenicol were obtained from Sigma Chemical. Protosol was obtained from Du Pont. Thin-layer chromatography (TLC) aluminum sheet (Kieselgel 160 20×20 cm) was obtained from Merck. 1,3-Dihydroxynaphthalene was obtained from Tokyo Kasei Kogyo. Celite 545 was obtained from Junsei Chemical.

Preparation of [U-14C]4’GL. [U-14C]4’GL was prepared from [U-14C]lactose as shown in Fig. 1, according to the method reported previously (1). The mixture contained 0.3 ml of [U-14C]lactose (1.1×10⁷ Bq/158 μg), 0.7 ml of culture medium (lactose 5.0 g, (NH₄)₂SO₄ 0.5 g, KH₂PO₄ 0.1 g, MgSO₄·7H₂O 0.05 g, meat extract 0.2 g, yeast extract 0.2 g, water 70 ml) and 0.1 ml of preculture of Cryptococcus

\[
\text{[U-14C]Lactose (1.1×10⁷ Bq) 0.3 ml, culture medium 0.7 ml}\n\]

\[
\downarrow\text{Cryptococcus laurentii OKN-4}\n\]

\[
\downarrow\text{48 h incubated at 30°C}\n\]

Carbon-celite column

\[
\begin{align*}
\text{Water} & \quad 10 \text{ ml} \\
\text{5% Ethanol} & \quad 50 \text{ ml} \\
\text{10% Ethanol} & \quad 100 \text{ ml}
\end{align*}
\]

\[
\downarrow\text{Concentration}\n\]

\[
\text{[U-14C]4’GL (2.3×10⁶ Bq/9.92 mg) in 31 ml}\n\]

Fig. 1. Preparation of [U-14C]4’GL from [U-14C]lactose.

laurentii OKN-4 was incubated for 2 days at 30°C on a shaker.

[U-14C]4'GL was purified from the culture medium on a charcoal column. Charcoal chromatography was performed on a 10 × 200 mm column packed with charcoal and Celite 545 (1:1). The [U-14C]lactose and [U-14C]4'GL were eluted with 5% ethanol and 10% ethanol at a flow rate of 8 ml/h, respectively. Generally, 0.8 ml fractions were collected by drop counting with an automatic fraction collector.

The identity of the 4'GL was confirmed by thin-layer chromatography (TLC) analysis with a developing solvent of 1-butanol:pyridine:water (60:25:15) and detected by spraying a 0.2% 1,3-dihydroxynaphthalene solution. 4'GL fraction was concentrated by a rotary vacuum evaporator and prepared to 74 kBq/ml.

Purity analysis of the [U-14C]4'GL and [U-14C]lactose. The purified [U-14C]-4'GL and [U-14C]lactose were developed by TLC as described above and the radioactivity was analyzed using a Radiochromanalyzer JTC (Aloka).

Animals and diet. Conventional male SD rats (Nippon Clea, Tokyo), weighing about 230 g, were raised on a AIN-76 diet with or without 4'GL as shown in Table 1 for 2 weeks and then used in the studies. To decrease intestinal microorganisms, several conventional male rats weighing about 230 g were given drinking water containing penicillin G (50 units/ml) and chloramphenicol (50 µg/ml) for 1 week before use (5). Germ-free male Wistar rats weighing about 80 g were purchased from Nippon Clea. All animals were starved overnight before administration of [U-14C]4'GL or [U-14C]lactose.

Administration of [U-14C]4'GL and measurement of radioactivity.

1) 14CO2 in expired air: Rats received 0.5 ml of [U-14C]4'GL (37 kBq/0.16 mg 4'GL) in saline solution with a gastric tube. Immediately after the dosing, each rat was transferred to a glass metabolic cage designed to separate urine and feces at 25 ± 1°C. Radioactive CO2 in expired air was trapped for 24 h in 200 ml of monoethanolamine·2-methoxyethanol solution (1:2, v/v) (6), which was replaced at 4 and 12 h after the dosing. The sampling solution (0.2 ml) containing trapped 14CO2 in monoethanolamine·2-methoxyethanol was taken out at 2, 4, 6, 8, 10, 12,

Table 1. Composition of diet.

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>5% 4'GL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Corn starch</td>
<td>65.0</td>
<td>60.0</td>
</tr>
<tr>
<td>4'GL</td>
<td>—</td>
<td>5.0</td>
</tr>
<tr>
<td>Cellulose</td>
<td>3.0</td>
<td>3.0</td>
</tr>
<tr>
<td>Casein</td>
<td>22.0</td>
<td>22.0</td>
</tr>
<tr>
<td>l-Methionine</td>
<td>0.3</td>
<td>0.3</td>
</tr>
<tr>
<td>Corn oil</td>
<td>5.0</td>
<td>5.0</td>
</tr>
<tr>
<td>Salt mixturea</td>
<td>3.5</td>
<td>3.5</td>
</tr>
<tr>
<td>Vitamin mixturea</td>
<td>1.2</td>
<td>1.2</td>
</tr>
</tbody>
</table>

a AIN-76 TM, Oriental Yeast, Tokyo. Vitamin mixture contains choline tartarate, 0.2 g/100 g.
and 24 h after the dosing. An aliquot of the sample (0.2 ml) was used to measure radioactivity in 10 ml of scintillation cocktail. Scintillation cocktail was composed of 0.01% POPOP and 0.5% DPO, dissolved in a mixture of 37% Triton X-100 and 63% toluene (6). The radioactive samples were measured in a Packard 3255 TRI-CARB liquid scintillation spectrometer. The radioassays were performed at least in duplicate and samples were counted twice.

2) 14C-Compounds in serum, urine, feces, and various organs: Blood was collected when the rats were sacrificed by decapitation. The serum was obtained by centrifugation of the heparinized blood at 3,000 rpm for 20 min. 0.1 ml of serum was solubilized with 0.2 ml of Protosol at 50°C for 1 h and then decolorized by the addition of 0.5 ml of H2O2. The serum weight was calculated from body weight as 3.8%. When the rats were killed, the liver, stomach, small intestine, caecum, and colon were removed immediately. The feces, intestine and its contents, liver, and carcass were homogenized in an adequate volume of distilled water using a polytron homogenizer (Brinkman, Westburg, NY). In this experiment, the carcass means the body-exenterated liver, small intestine, caecum, and colon. An aliquot of the homogenate was solubilized with 1 ml of Protosol at 50°C for 1 h and then decolorized by the addition of 0.5 ml of H2O2. Ten milliliters of scintillation cocktail was added to each of the following: 0.2 ml of the solubilized serum, 0.5 ml of urine, 0.2 ml of the solubilized feces, 0.2 ml of the solubilized intestine and its contents, 0.2 ml of the solubilized liver, and 0.2 ml of the solubilized carcass, prior to determination of the radioactivity in a liquid scintillation counter. Scintillation cocktail was composed of 0.01% POPOP and 0.5% DPO, dissolved in a mixture of 37% Triton X-100 and 63% toluene (6). The counting efficiency of the respiratory CO2, urine, feces, intestine and its contents, liver, serum, and carcass were 86, 84, 78, 77, 78, 84, and 80%, respectively.

Analysis of 4’GL in feces. Five conventional rats were raised on 5% 4’GL diets as shown in Table 1 for 2 weeks. Feces from the last day were collected and dried at 80°C. The dried feces were homogenized in adequate volume of distilled water and 4’GL was extracted. 4’GL was determined using HPLC according to the method reported previously (1).

Results were expressed as means±SE.

RESULTS

Preparation of synthesized [U-14C]4’GL

Adding 0.7 ml of culture solution to 0.3 ml (1.1×107 Bq) of [U-14C]lactose solution, Cryptococcus laurentii OKN-4 was cultured by shaking at 30°C for 48 h. After separating and purifying [U-14C]4’GL from the culture solution using a charcoal celite column, 2.3×106 Bq of [U-14C]4’GL was obtained. After developing the purified [U-14C]4’GL on TLC and causing it to develop color with 1,3-dihydroxynaphthalene solution, the radioactivity distribution was analyzed using a Radiochromanyzer JTC. As a result, 4’GL spots agreed with the radioactivity.
distribution. There was no radioactivity in sites other than 4'GL spots, and thus it was possible to obtain 99.9% pure [U-14C]4'GL. The specific activity of [U-14C]-4'GL was $1.17 \times 10^8$ Bq/mmol.

$^{14}$CO$_2$ in expired air from rats orally administered [U-14C]4'GL and [U-14C]lactose 1) $^{14}$CO$_2$ in expired air from conventional rats. Figure 2 shows the time course of $^{14}$CO$_2$ excretion after [U-14C]4'GL or [U-14C]lactose administration over the 24 h collection period. Thirty seven kBq of [U-14C]4'GL or [U-14C]lactose in 0.5 ml of saline was administered to rats. 49% of the radioactivity was recovered as $^{14}$CO$_2$ within 24 h in rats given [U-14C]4'GL. The rate of excretion $^{14}$CO$_2$ from [U-14C]4'GL was very slow during first 2 h. Maximum excretion rates of $^{14}$CO$_2$ were found between 6-8 h after ingestion.

For comparison, $^{13}$CO$_2$ in expired air after administration of [U-14C]lactose rapidly increased during the first 2 h, and 43.2% of radioactivity was recovered within 24 h. The excretion of $^{14}$CO$_2$ from [U-14C]4'GL increased more slowly than that from [U-14C]lactose during the first 2 h, and was delayed for about 5 h. The cumulative excretion of $^{14}$CO$_2$ from [U-14C]4'GL for 24 h was a little higher than that from [U-14C]lactose, but there was no significant difference between [U-14C]-4'GL and [U-14C]lactose.

![Fig. 2. Time course of $^{14}$CO$_2$ excretion from conventional and antibiotics administered rats after an oral dose of [U-14C]4'GL or [U-14C]lactose. [U-14C]-4'GL or [U-14C]lactose were orally administered 37 kBq to each rat, respectively. The values are expressed as a percentage of the radioactivity excreted to the radioactivity administered. Four male SD rats (7 weeks of age) per group were used. Each point represents the mean±SE of 4 rats/group. O, [U-14C]4'GL; ●, [U-14C]lactose.](image-url)
2) $^{14}$CO$_2$ in expired air from rats treated with antibiotics. When [U-$^{14}$C]-4'GL or [U-$^{14}$C]lactose was orally administered to conventional rats, $^{14}$CO$_2$ in expired air from [U-$^{14}$C]4'GL was delayed for about 5 h compared to that of [U-$^{14}$C]lactose. This result suggests that 4'GL was not absorbed from the upper gastrointestinal tract and had reached the lower gut, where the intestinal microorganisms fermented 4'GL to smaller molecules or $^{14}$CO$_2$, which was then absorbed from the large intestine. The metabolism of 4'GL was therefore studied using rats given antibiotics, which decreases the number of intestinal microorganisms.

Figure 2 shows the time course of $^{14}$CO$_2$ appearance in exhaled gas from rats treated with antibiotics after [U-$^{14}$C]4'GL or [U-$^{14}$C]lactose administration over the 24 h collection period. When [U-$^{14}$C]4'GL was orally administered to antibiotics-treated rats, there was a 2 h delay in $^{14}$CO$_2$ excretion, as compared to conventional rats. The amount of $^{14}$CO$_2$ in expired air within 24 h in antibiotics rats was 25% lower than that in conventional rats.

When [U-$^{14}$C]lactose was administered to rats given antibiotics, however, there was no significant difference in $^{14}$CO$_2$ excretion between rats treated with

Fig. 3. Time course of $^{14}$CO$_2$ excretion after an oral dose of [U-$^{14}$C]4'GL or [U-$^{14}$C]lactose to germ-free rats. [U-$^{14}$C]4'GL or [U-$^{14}$C]lactose were orally administered 37 kBq to each rat, respectively. The values are expressed as a percentage of the radioactivity excreted to the radioactivity administered. Four male Wistar rats (4 weeks of age) per group were used. Each point represents the mean±SE of 4 rats/group. ○, [U-$^{14}$C]4'GL; ●, [U-$^{14}$C]lactose.
Table 2. Excretion and distribution of the radioactivity in rats after oral administration \([U-^{14}C]4'\text{GL}\) or \([U-^{14}C]\text{lactose}\).

<table>
<thead>
<tr>
<th></th>
<th>Body weight (g)</th>
<th>Total respiratory CO(_2) excretion (%)</th>
<th>Total urinary excretion (%)</th>
<th>Total fecal excretion (%)</th>
<th>Intestine and its contents (%)</th>
<th>Liver (%)</th>
<th>Serum (%)</th>
<th>Carcass (%)</th>
<th>Total (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Conventional rats</td>
<td></td>
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<tr>
<td>Lactose ((n = 4))</td>
<td>195.4 ± 8.1</td>
<td>43.23 ± 0.54</td>
<td>3.19 ± 0.21</td>
<td>1.47 ± 0.40</td>
<td>6.66 ± 0.25</td>
<td>1.91 ± 0.08</td>
<td>0.99 ± 0.44</td>
<td>23.18 ± 1.02</td>
<td>80.63 ± 0.97</td>
</tr>
<tr>
<td>4'GL ((n = 4))</td>
<td>196.2 ± 8.5</td>
<td>48.78 ± 3.51</td>
<td>4.77 ± 0.67</td>
<td>4.11 ± 1.35</td>
<td>8.70 ± 1.10</td>
<td>1.43 ± 0.08</td>
<td>0.62 ± 0.01</td>
<td>12.83 ± 0.40</td>
<td>81.20 ± 2.75</td>
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<tr>
<td>Germ-free rats</td>
<td></td>
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<tr>
<td>Lactose ((n = 4))</td>
<td>81.4 ± 2.8</td>
<td>48.95 ± 0.59</td>
<td>4.05 ± 0.93</td>
<td>0.71 ± 0.20</td>
<td>15.19 ± 0.60</td>
<td>1.19 ± 0.06</td>
<td>0.73 ± 0.03</td>
<td>14.30 ± 0.38</td>
<td>85.13 ± 0.96</td>
</tr>
<tr>
<td>4'GL ((n = 4))</td>
<td>83.4 ± 0.5</td>
<td>17.72 ± 2.95</td>
<td>5.50 ± 0.71</td>
<td>0.50 ± 0.20</td>
<td>58.31 ± 6.52</td>
<td>0.56 ± 0.09</td>
<td>0.43 ± 0.12</td>
<td>7.71 ± 0.62</td>
<td>90.72 ± 3.96</td>
</tr>
</tbody>
</table>

Rats were not given the diet for 24 h each before and after the dose of \([U-^{14}C]4'\text{GL}\) or \([U-^{14}C]\text{lactose}\). \([U-^{14}C]4'\text{GL}\) or \([U-^{14}C]\text{lactose}\) was orally administered 37 kBC. The values of respiratory CO\(_2\) excretion, urinary excretion and fecal excretion are expressed as % of the radioactivity excreted for 24 h to the radioactivity administered. Distribution of the radioactivity of intestinal and its contents, liver, serum, and carcass are expressed as % of the radioactivity at 24 h after \([U-^{14}C]4'\text{GL}\) or \([U-^{14}C]\text{lactose}\) were administered. The radioassays were performed at least in duplicate and samples were counted twice. Each value represents as the mean ± SE.
antibiotics and conventional rats.

3) $^{14}$CO$_2$ in expired air from germ-free rats. Rats treated with antibiotics excreted little $^{14}$CO$_2$ from [U-$^{14}$C]$4'$GL, as compared with conventional rats. The reduced ability of the antibiotics rats to metabolize $4'$GL indicates that the intestinal microorganisms are essential for the metabolism of $4'$GL by the rat. In the next experiment, therefore the metabolism of $4'$GL was investigated using germ-free rats.

Figure 3 shows the radiorespirometric pattern of the germ-free rats after oral administration of [U-$^{14}$C]$4'$GL or [U-$^{14}$C]lactose under conventional conditions. Germ-free rats given [U-$^{14}$C]$4'$GL expired little $^{14}$CO$_2$ in the first 4h after the administration. About 18% of radioactivity was recovered within 24h. The metabolism of [U-$^{14}$C]lactose, however, followed the same pattern as with conventional rats.

These observations suggest that $4'$GL is metabolized by intestinal microorganisms in the gastrointestinal tract, although the process of $^{14}$CO$_2$ excretion from [U-$^{14}$C]$4'$GL are unknown.

Absorption and excretion of [U-$^{14}$C]$4'$GL

The recovery of radioactivity following oral administration of [U-$^{14}$C]$4'$GL to conventional rats and germ-free rats is shown in Table 2. When [U-$^{14}$C]$4'$GL was administered to conventional rats, the radioactivity in the contents of the gastrointestinal tract, feces, and urine during the 24h period were 8.7, 4.1, and 4.8% of the ingested radioactivity, respectively. These value were higher than that of [U-$^{14}$C]lactose-administered rats. The amount of radioactivity in the liver, serum, and carcass, however, were lower than that of [U-$^{14}$C]lactose.

In germ-free rats, when [U-$^{14}$C]$4'$GL was orally administered to rats, the radioactivity remaining in the gastrointestinal tract contents was 58.3%. This value was much higher than that in [U-$^{14}$C]lactose administered rats. The amount of radioactivity in the liver and carcass, by contrast, were about half the value for [U-$^{14}$C]lactose.

Excretion of $4'$GL in feces

The feces of the five conventional rats raised on a 5% $4'$GL diet for 2 weeks were collected. $4'$GL in the feces were analyzed using HPLC. $4'$GL was not detected from feces of the $4'$GL-fed rats.

DISCUSSION

Regarding the use of digestible saccharides in vivo, such as glucose or fructose, the metabolic mechanism in the human and the rat has been elucidated by using $^{14}$C-labeled compounds (7–9). Meanwhile, regarding indigestible saccharides such as cellulose, those degraded by intestinal microorganisms and the production of short chain fatty acid as metabolites, have been studied using $^{14}$C]cellulose (10,11).
Recently, for the purpose of a comparative study on the energy evaluation of
different saccharides, Tsuji et al. (12) have systematically studied metabolism in
living bodies by administering different $^{14}$C-labeled digestible and undigestible
saccharides such as glucose, fructose, galactose, lactose, maltose, sucrose, cellulose,
and inulin in rats.

In recent years, various oligosaccharides such as lactulose, maltitol, and
fructooligosaccharide, which are hardly digested or absorbed and have additional
effects, have been developed, and their utilization for living bodies has been studied
(5, 13–17). $4'$GL is among these oligosaccharides. It was not hydrolyzed by
digestive enzymes such as the rat small intestinal mucosa or the hog pancreatic
$\alpha$-amylase (4). When $4'$GL was ingested by humans, only Bifidobacterium, which
could be characterized as the beneficial intestinal bacteria, markedly increased (2).
From these results, it may be estimated that $4'$GL is not digested or absorbed in the
small intestine, and its utilization in living bodies is difficult. Its metabolism and
excretion in living bodies, however, remains unclear.

In order to clarify the in vivo behavior of $4'$GL, we orally administered $[U-^{14}$C]4'
GL to conventional rats and measured $^{14}$CO$_2$ excreted into expired air. Com-
pared with $[U-^{14}$C]lactose used as a control, $4'$GL showed a delay of about 5 h in
$^{14}$CO$_2$ excretion. The total $^{14}$CO$_2$ amount excreted for 24 h after administration
however, increased by several percent compared with a $[U-^{14}$C]lactose administered
group. Since lactose is hydrolyzed by rat lactase, $[U-^{14}$C]lactose can theoretically
result in $^{14}$CO$_2$ excretion by living body metabolism. $4'$GL was not hydrolyzed by
small intestinal mucosa in vitro (4), however, and Bond et al. (7) reported that
$^{14}$CO$_2$ excretion into exhaled air was larger by $[^{14}$C]glucose administration to rats
through the cecum than by oral administration. These observations suggest that
intestinal microorganisms take part in $^{14}$CO$_2$ formation from $[U-^{14}$C]4'GL.

A measurable delay in the $^{14}$CO$_2$ excretion from $[U-^{14}$C]4'GL in rats treated
with antibiotics, and a reduction in the total excretion amount in 24 h, are both
results of the present experiment that support this idea. The reduction of $^{14}$CO$_2$
excretion from $[U-^{14}$C]4'GL was only 25%. It seems that all intestinal microor-
ganisms may not be affected by the antibiotics. When germ-free rats were used
under a conventional condition, $^{14}$CO$_2$ excretion from $[U-^{14}$C]4'GL decreased to
36% as compared with $[U-^{14}$C]lactose.

If germ-free rats are used under complete aseptic conditions, it is expected that
$^{14}$CO$_2$ will not be excreted from $[U-^{14}$C]4'GL. In this experiment, however, $^{14}$CO$_2$
was excreted, though the amount was small. This is probably because germ
infection began rapidly after the experiment started under conventional conditions,
and it became impossible to maintain the aseptic condition; bacteria then prolif-
erated to metabolize $[U-^{14}$C]4'GL.

Hosoya et al. (17) reported that when human feces were made to react with
$[U-^{14}$C]fructooligosaccharide anaerobically in vitro, 48% of the fructooligosac-
charide was metabolized to short chain fatty acids such as acetic acid, propionic
acid, and butyric acid by intestinal microorganisms. The energy value of fructo-
oligosaccharide was calculated at 1.5 kcal/g.

4'GL is an indigestible saccharide that is hardly decomposed by digestive enzymes (4). Also in this study, ingested 4'GL was not found from feces in rats. We therefore feel that almost 100% of the ingested 4'GL was fermented in the large intestine by intestinal microorganisms. When 4'GL is utilized by intestinal microorganisms, organic acids are generated (2), so there is a possibility that generated organic acids may be absorbed in the large intestine and utilized as energy.

Wolin and Miller (18) reported the ratio of the amount of sugar by fermentation of human intestinal microorganisms and the amount of the product as in the following formula:

$$34.5 \text{C}_6\text{H}_{12}\text{O}_6 \rightarrow 48 \text{acetate} + 11 \text{propionate} + 5 \text{butyrate} + 23.75 \text{CH}_4 + 34.25 \text{CO}_2 + 10.5 \text{H}_2\text{O}$$

The retention energy of acetic acid, propionic acid, and butyric acid is 209.0, 370.4, and 528.7 kcal/mol, respectively (19). By substituting these values in the formula above, the energy value becomes 2.70 kcal/g. Accordingly, when 1 g of indigestible saccharide such as 4'GL is ingested, if all of it is fermented the total energy amount held by the organic acid produced becomes 2.7 kcal/g. The energy utilization efficiency of organic acids in vivo is unknown at present. According to Black et al. (20), the energy utilization efficiency of acetic acid in sheep is 50–70%. We therefore set the energy utilization efficiency of acetic acid at 70%. While the energy utilization efficiency of propionic acid and butyric acid is thought to differ from that of acetic acid, this has not yet been reported, so we set their efficiency at 70%, the same as that of acetic acid. Using this value, the effective energy of 1 g of 4'GL when fermented was calculated at 1.89 kcal/g (2.70 × 0.70 = 1.89).

In the Standard Tables of Food Composition in Japan (21), the energy utilization efficiency of acetic acid is 69%. Oku (22) calculated the energy value of maltitol and fructooligosaccharide using the Wolin's formula and the value of the Standard Tables of Food Composition. Using this method, the effective energy of 4'GL was calculated at 1.86 kcal/g.

Since these values are based on hypotheses for the organic acid production rate and energy utilization efficiency, more detailed studies are required. There is a possibility, however, that the energy value of 4'GL may be 1.5–2.0 kcal/g.

REFERENCES


METABOLISM OF [U-14C]4'GALACTOSYLLACTOSE


12) Tsuji, K., Ichikawa, T., and Ohtsuka, K. (1988): Metabolism of cellulose and inulin in rat, in Abstracts of Papers, the Annual Meeting of the Japan Society for Bioscience, Biotechnology, and Agrochemistry, Nagoya, April, p. 86.


