Acute Toxicity and Mutagenicity Study on Branched Corn Syrup and Evaluation of Its Laxative Effect in Humans

Yuka KISHIMOTO, Shigeru WAKABAYASHI, Isso MATSUDA, Hiroaki FUDABA and Kazuhiro OHKUMA

Research Institute, Matsutani Chemical Industry Company Ltd., 5–3 Kita-itami, Itami, Hyogo 664–8508, Japan

(Received September 1, 2000)

Summary We developed a branched corn syrup (BCS, average molecular weight: 500, content of indigestible portion: 45%) by heat treatment of indigestible dextrin with hydrochloric acid. To confirm the safety of BCS, we conducted both an acute toxicity test and a mutagenicity test. Moreover, we observed gastroenteric effects of BCS in fifty healthy humans. The results are summarized as follows. 1) There was no death observed after oral administration of BCS in Sprague-Dawley-strain rats. Lethal dose (LD)50 value was estimated to be more than 10 g/kg body weight. 2) No mutagenicity was observed in Salmonella typhimurium TA98, TA100, TA1535, TA1537, or Escherichia coli WP2uvrA. 3) Fifty adults were divided into five groups of ten (five of each sex) and orally administered BCS at 0.2, 0.3, 0.4, 0.5 and 0.6 g/kg body weight as indigestible portion. Although no diarrhea was observed in females, BCS at 0.6 g/kg as indigestible portion caused diarrhea in two out of five males. The maximum non-effective dose of indigestible portion of BCS was estimated to be 0.5 g/kg in males and more than 0.6 g/kg in females.

Key Words branched corn syrup, acute toxicity test, mutagenicity test, laxative effect

The recent flourishing of “Foods for Specific Health Use (FOSHU)” (1), one category of foods for special dietary uses stipulated by article 12 of the nutrition improvement law, has stimulated the development of indigestible saccharide having nutritional functions. The development has been especially active for indigestible oligosaccharides having such functions as bifidobacterial activation (2) or anticaries (3). In addition, dietary fibers for improvement of bowel movement have become available, which include various materials of different sources or different manufacturing methods such as guar gum degradation product, cellulose, polydextrose, wheat bran, psyllium, etc. For manufacturing these indigestible oligosaccharides and dietary fibers, however, enzymological and chemical methods using raw materials such as glucose, sugar, lactose, fruits or grain are generally employed, regrettably resulting in quite a small yield or an insecure provision. The authors previously succeeded in efficiently manufacturing soluble, edible fibers (indigestible dextrin) by heat treatment of cornstarch and reported its clinical usefulness for improvements of bowel movement (4), postprandial blood glucose elevation (5), and serum lipid levels (6). Indigestible dextrin has an average molecular weight of approximately 2,000, which is low as compared with other edible fibers, but still not low enough for some types of processed foods. Recently the authors, using indigestible millet honey of lower molecule than that of indigestible dextrin, have developed “branched corn syrup (BCS)” having the same nutritional function as that of indigestible dextrin, with the intention of expanding its usability for processed foods (7). The present report describes the results of acute toxicity and mutagenicity tests performed to confirm the safety of BCS and also the results of a clinical trial in healthy adults performed to evaluate stool condition and gastroenteric symptoms after oral ingestion of BCS.

METHODS

1. Test substance. BCS was prepared as described in the previous report (7); cornstarch was heated with hydrochloric acid to form dextrin that was subsequently hydrolyzed with hydrochloric acid, and the low molecular substance thus obtained was purified to yield BCS. It has an average molecular weight of 500, dextrose equivalent (DE) of 36, water content of 4%, indigestible portion of 45%, and sweet taste of 33.

2. Acute toxicity test. SD rats (Crl:CD, Charles River Japan, Inc.) at 5 wk of age were individually housed in metal cages in a room maintained at 22±1°C of temperature, 55±10% of relative humidity, 20 air changes/h, and lighting between 6:00 and 18:00. Animals were acclimated for 8 d and 15 healthy animals of each sex were divided into 3 groups randomly (0, 5 and 10 g/kg body weight, 5 animals of each sex per group). The test substance was dissolved in water at 2 concentrations; high concentration solution was for a dose of 10 g/kg, which was the maximum administrable volume, and low concentration solution was for a dose of 5 g/kg, which was obtained by calculation with a factor of 2. After fasting for approximately 16 h, animals were administered the dose orally using a stomach catheter at a volume of 20 mL/kg body weight. Control animals received water, which is listed in Japanese
Pharmacopoeia for injection (JPW: Fuso Pharmaceutical Co., Ltd., Japan) in the same way. Animals were observed for clinical signs and mortality frequently from 0 to 6 h after administration, and twice daily (once in the morning and once in the afternoon) thereafter until 14 d after administration. Body weights were measured on the day of administration (prior to dosing), 1, 2, 3, 5, 7, 11 and 14 d after administration. At the end of the study (on day 14), all surviving animals were euthanized by bleeding from the axillary arteries or abdominal aorta under ether anesthesia and examined grossly.

3. Mutagenicity test (8). Mutagenicity of BCS was examined in an assay system using bacteria. Salmonella typhimurium TA98, TA100, TA1535 and TA1537 used in this study were originally obtained from the Toxicology Department, Residual Agricultural Chemicals Institute, and Escherichia coli WP2uvrA from Japan Bioassay Research Center. The test was performed by the preincubation method with or without a metabolic activation system (direct method). Five dose levels were employed for test in each strain of the bacterial species; high dose was 5,000 µg/plate, which was the upper limit of the dose recommended in the guideline, and 4 lower dose levels of 2,500, 1,250, 625 and 312.5 µg/plate.

Water for injection JPW was used as the negative control, and 2-(2-furanyl)-3-(5-nitro-2-furanyl) acrylamide (AF-2), sodium azide, 9-aminocaridine (9AA) and 2-aminoanthracene (2AA) were used as the positive controls. Both the negative and positive control substances were purchased from Wako Pure Chemical Industries, Ltd., Japan.

1) Direct method (8). One tenth mL of negative control (JPW) was mixed with positive control or test substance, 0.5 mL of 0.1 M phosphate buffer saline, and 0.1 mL of the bacterial suspension culture in this order, and the inoculated medium was shake-cultured at 37°C after being mixed well. After 20 minutes, the test tube was taken out, and soft agar solution (Bacto-Agar, Difco Co., USA) was added and mixed. The cultured medium was then placed in the plate uniformly. Two plates each were used for each concentration of the test, negative control and positive control substances. After having the soft agar solution added, the cultured medium was further cultivated in an incubator at 37°C for 48 to 50 h under protection from light, and then the number of revertants was counted.

2) Metabolic activation method (9). The same procedure as in the direct method was followed except that 0.5 mL of S9 mix was used instead of 0.5 mL of 0.1 M phosphate buffer solution. S9 mix was prepared by mixing the commercially available rat S9 (Oriental Yeast Co., Ltd., Japan) and cofactor I (Oriental Yeast Co., Ltd.) at the time of use.

4. Oral single dose study in healthy adults. Subjects were 50 healthy persons (25 males and 25 females) with ages of 29.1±7.3 y (32.0±8.5 y for males and 26.2±6.4 y for females) and body weights of 58.8±9.9 kg (65.6±7.4 kg for males and 52.0±6.9 kg for females). Subjects were distributed among 5 groups (5 males and 5 females per group) so that the mean body weights were almost equal among groups, and administered BCS at 0.2, 0.3, 0.4, 0.5 and 0.6 g/kg body weight as indigestible portion. The dosing substance was the sterile-packaged BCS solution, which was prepared by weighing out the necessary quantity of BCS for each subject based on the individual body weight and by filling up with warm water to a final volume of 200 mL. Administration was made on a holiday; each subject received 200 mL of BCS solution at 10 a.m. in one dose and was requested to stay in the facility and avoid exercise on that day. No special indication was made for meals taken before and after administration, except that food or beverage containing edible fibers or sugar alcohol possibly affecting defecation was inhibited. An investigation sheet was prepared for each subject, who was requested to record defecation frequency, defecation time, stool condition, gastroenteric symptoms (gurgling sound, distention sensation, abdominal pain, straining, nausea), and menu of diet. Stool condition was divided into 5 types (dry solid, banana-shaped, pasty, muddy and watery); a diagram was given to each subject as a reference when recording. Muddy stool and watery stool were regarded as diarrhea. According to the spirit of Helsinki Declaration, the study contents were precisely explained to the participants and consent was obtained from each subject before the start of the study.

5. Statistical analysis. Results were expressed as mean±standard deviation, and the statistical significance of an intergroup difference was assessed using Student’s t-test with p≤0.05 as a level of significance.

RESULTS

1. Acute toxicity test

No deaths were found during the observation period for either male or female SD rats. On the day of administration, mucous stool was found in one male of the 5 g/kg group at 4 h after administration, and soft stool in one male of the 10 g/kg group at 6 h after administration. On the next day and thereafter, soft stool was found sporadically in males of both groups, but no other noteworthy changes were found. For females, there were no changes found throughout the observation period. Body weight data are shown in Table 1. No noteworthy changes in body weight were found throughout the observation period for either males or females. At necropsy on 14 d after administration, no treatment-related changes were found in any animal of either sex.

Since no deaths were found in the observation period, LD50 values of BCS in rats are considered to be more than 10 g/kg body weight for either sex.

2. Mutagenicity test

Results are shown in Table 2. The number of revertant colonies did not exceed 2-fold the revertant colony count of the negative control in any of the 5 strains of bacteria at any dose level by the direct method or metabolic activation method. For the positive controls, the
number of revertant colonies was 5.7- to 255-fold that of the negative control in their respective strains of bacteria. In addition, the sterility test revealed no contamination in the test system. With these data in consideration all together, the present test system was considered to be normally working, and BCS was considered to have no mutagenicity in the assay system using bacteria.

3. Oral single dose study in healthy adults

Table 3 shows the summarized results on stool condition at the first defecation after ingestion of BCS at 0.2, 0.3, 0.4, 0.5 and 0.6 g/kg body weight as indigestible.

| Intake weight of | 0.2 | 0.3 | 0.4 | 0.5 | 0.6 |
|------------------|-----|-----|-----|-----|-----|
| Dry solid        | male| 0   | 0   | 0   | 0   |
|                  | female| 1   | 1   | 0   | 2   |
|                  | total | 1   | 1   | 0   | 2   |
| Banana-shaped    | male| 4   | 2   | 5   | 0   | 1   |
|                  | female| 4   | 2   | 5   | 2   | 4   |
|                  | total | 8   | 4   | 10  | 2   | 5   |
| Pasty            | male| 1   | 3   | 0   | 5   | 2   |
|                  | female| 0   | 2   | 0   | 1   | 1   |
|                  | total | 1   | 5   | 0   | 6   | 3   |
| Muddy            | male| 0   | 0   | 0   | 0   | 2   |
|                  | female| 0   | 0   | 0   | 0   | 0   |
|                  | total | 0   | 0   | 0   | 0   | 2   |
| Watery           | male| 0   | 0   | 0   | 0   | 0   |
|                  | female| 0   | 0   | 0   | 0   | 0   |
|                  | total | 0   | 0   | 0   | 0   | 0   |

Table 2. Mutagenicity of branched corn syrup (BCS) in the preincubation method.

| Concentration (µg/plate) | S9mix | TA98 (-) (+) | TA100 (-) (+) | TA1535 (-) (+) | TA1537 (-) (+) | WP2uvrA (-) (+) |
|--------------------------|-------|--------------|---------------|----------------|----------------|-----------------|
| Saline                   | 0.0   | 22           | 37            | 85             | 78             | 8               | 8               | 6              | 10             | 22             | 27             |
| BCS                      | 19.5  | 20           | 33            | 96             | 78             | 11              | 6               | 8              | 8              | 8              | 28             | 30             |
| 78.1                     | 25    | 34           | 91            | 87             | 7              | 7               | 7               | 7              | 7              | 7              | 25             | 25             |
| 312.5                    | 22    | 32           | 92            | 102            | 6              | 11              | 6               | 8              | 9              | 8              | 27             | 33             |
| 1,250                    | 27    | 37           | 94            | 79             | 6              | 6               | 6               | 8              | 8              | 8              | 24             | 35             |
| 5,000                    | 23    | 33           | 101           | 88             | 11             | 7               | 7               | 7              | 15             | 29             | 34             | 34             |
| Positive control         |       |              |               |                |                |                 |                 |                |                |                |                 |                 |
| AF2                      | 0.01  | 638          | 478           | 187            |                |                 |                 |                |                |                |                 |                 |
|                          | 0.1   |              |               |                |                |                 |                 |                |                |                |                 |                 |
| SAZ                      | 0.5   |              |               |                | 486            |                 |                 |                |                |                | 1,530           |                 |
| 9AA                      | 80    |              |               |                |                | 1,530           |                 |                |                |                |                 |                 |
| 2AA                      | 0.5   |              |               |                |                | 364             | 159             | 1,636          |                 |                 |                 |                 |
|                          | 1     |              |               |                |                |                 |                 |                |                |                |                 |                 |
|                          | 2     |              |               |                |                |                 |                 |                |                |                |                 |                 |
|                          | 10    |              |               |                |                |                 |                 |                |                |                |                 |                 |

Test substances and positive controls were pre-incubated with (+) or without (−) S9mix at 37°C for 20 min. Then these were cultured at 37°C for 48–50 h. And number of reversion-colonies were calculated. Values are means of two determinations.

AF2: 2-(2-furyl)-3-(5-nitro-2-furyl)acrylamide, SAZ: sodium azide, 9AA: 9-aminoacridine, 2AA: 2-aminoanthracence.

Table 1. Changes in body weight of SD rats by administration of branched corn syrup (BCS) in acute toxicity test.

| Dose (g/kg) | 0 (Control) | 5 | 10 |
|-------------|-------------|---|----|
| Number of rats | 5 | 5 | 5 |

| Male day | 183.0±3.7 | 180.6±3.0 | 178.8±4.7 |
|----------|-----------|-----------|-----------|
| 1        | 209.4±6.3 | 208.0±3.9 | 207.4±4.4 |
| 2        | 220.2±7.9 | 216.8±5.8 | 215.4±6.5 |
| 3        | 230.4±8.0 | 228.2±5.9 | 224.8±3.8 |
| 5        | 248.0±9.8 | 248.8±7.8 | 243.6±4.3 |
| 7        | 264.4±12.3| 266.8±9.3 | 261.0±5.1 |
| 11       | 302.4±18.0| 299.8±13.8| 289.8±18.1|
| 14       | 326.0±18.5| 328.2±14.6| 305.2±33.8|

| Female day | 137.4±5.0 | 137.8±3.1 | 135.0±3.2 |
|------------|-----------|-----------|-----------|
| 1          | 156.2±5.3 | 158.4±4.0 | 156.8±6.3 |
| 2          | 162.6±5.5 | 164.0±2.7 | 162.8±6.4 |
| 3          | 166.0±7.0 | 166.8±3.1 | 167.6±5.4 |
| 5          | 175.0±6.4 | 176.2±5.2 | 175.6±5.5 |
| 7          | 178.0±5.8 | 179.6±6.0 | 179.4±5.0 |
| 11         | 191.0±5.1 | 192.4±6.3 | 195.0±5.2 |
| 14         | 199.8±8.6 | 199.4±9.8 | 203.2±7.0 |

Mean±SD. No significant difference was observed.

Table 3. Effects of branched corn syrup (BCS) on stool conditions in humans.
Fig. 1. Correlation between intake volume of branched corn syrup (BCS) and occurrence of diarrhea.

Subjects ingested 0.2-0.6 g/kg body weight indigestible portion of BCS. ED50 value for diarrhea was estimated from a graph by taking horizontal axis as intake volume and the vertical axis as diarrhea occurrence. C: male, Δ: total. Diarrhea (muddy stool and watery stool) was not observed at 0.2, 0.3, 0.4 or 0.5 g/kg for either males or females. At 0.6 g/kg, muddy stool was observed in 2 out of 5 males, but not in females. Accordingly, the maximum non-effective dose was 0.5 g/kg for males and more than 0.6 g/kg for females. Relationship between incidences of diarrhea and doses is shown in Fig. 1. ED50 values (dose to cause diarrhea in 50% of the subjects) were estimated to be 0.62 g/kg body weight for males and more than 0.6 g/kg for females (0.75 g/kg as a whole). As to gastroenteric symptoms, there were straining at stool (1 subject), gurgling sound (9 subjects), and distention sensation (5 subjects) in males and/or females. These signs were slight and temporary and recovered to normal without any treatment.

DISCUSSION

Dextrin, a product in the hydrolysis of starch, has been utilized as a food for a long time. With this experience, dextrin is recognized as one of the highly safe food materials and ranked as generally recognized as safe (GRAS) in the US Federal Register (CFR) and as A1 in FAO/WHO. As to “indigestible dextrin”, which is the dextrin with a developed branched-structure made by heating dextrin and an intermediate product in the process of BCS manufacturing, previous studies revealed neither deaths even at the maximum single dose of 40 g/kg given to mice nor mutagenicity in an assay in bacteria (10). Probable occurrence of diarrhea due to excessive ingestion of indigestible dextrin was also studied in an oral single dose test in healthy adults, and ED50 value was reported to be 1.48 g/kg body weight (4). The present test material, BCS, is the low molecular product in the hydrolysis of heated dextrin with hydrochloric acid, and has a physicochemical property and a nutritional function intermediate between those of oligosaccharide and edible fibers. In the present study, evaluation of BCS was made of its acute toxicity and mutagenicity and also of the safety in humans by observing the effects of excessive ingestion in healthy adults.

The oral single dose toxicity test in rats revealed no deaths in either male or female rats, and the assay in bacteria with or without metabolic activation revealed no mutagenicity. With these results in addition to the fact that BCS is composed of D-glucose of a clear metabolic pathway, there would be no toxicological problems with respect to BCS.

Excessive ingestion of indigestible hydrocarbon such as indigestible oligosaccharide or sugar alcohol is well known to induce temporary diarrhea (11). This type of diarrhea is considered to occur with an increased transport of the body fluid into the intestine because of an increased intestinal osmotic pressure (12). An osmotic pressure is determined by the molecular weight of a substance, and therefore the osmotic pressure will be higher with a substance of a lower molecular weight when their concentrations are the same. Since BCS is a substance of a lower molecular weight (approximately 500) than that of indigestible dextrin (mean molecular weight of 2,000), a clinical study was performed in 50 healthy adults to learn what would be the effect of this difference on occurrence of diarrhea. The study showed muddy stool in 2 out of 5 males of the 0.6 g/kg group, but no diarrhea in females at any dose level examined. Accordingly, the maximum non-effective dose was 0.5 g/kg body weight for males and more than 0.6 g/kg body weight for females (0.5 g/kg as a whole). And ED50 values are estimated to be 0.62 g/kg body weight for males and more than 0.6 g/kg for females (0.75 g/kg body weight as a whole). These ED50 values were lower
than those for indigestible dextrin. However, we considered further study necessary to confirm the reproducibility of the ED$_{50}$ value. Especially, we should take the dosage level into account when examining the ED$_{50}$ value because we had only conducted the study with dosage levels less than 0.7 g/kg body weight (from 0.2 to 0.6 g/kg body weight).

The osmotic pressures of the test substance solutions were measured using solutions prepared on the basis of actual intakes and group mean body weights. The results are shown in Fig. 2. The osmotic pressure of the test substance solution for the 0.6 g/kg group (incidences of diarrhea were present in this group) was approximately 1,800 mOsm/kg, which is approximately 6-fold that of the body fluid. After oral ingestion, hypotonic solution passes through the stomach without receiving any change into the intestine, but hypertonic solution is diluted to become isotonic in the stomach and then transported into the intestine (13). Since BCS is diluted considerably in the intestine partly because of digestion and absorption of a part of BCS in the small intestine, we cannot tell clearly what would be the correlation between diarrhea and the osmotic pressure of the dosing solution. As to an enteral nutrient, the osmotic pressure should be taken into consideration to prevent diarrhea and is recommended to be below 500 mOsm/kg (14). From these facts, a principal factor to have induced diarrhea in the present study was considered to be inhibition of water absorption due to an increased intestinal osmotic pressure, as was true for sugar alcohol or others.

On the other hand, we found BCS caused diarrhea more easily in males compared to females. In our study two male subjects out of five experienced diarrhea at 0.6 g/kg BCS ingestion but no females. The same finding has been reported (7, 15). The reason is considered to be due to differences between male and female in occurrence of constipation, sensitivity of hormonal and nervous reaction against various kind of stress, eating habit and stuff. Moreover, we calculated the absolute ingestion quantity of BCS from the dosage level and body weight individually. The results were 39.3 g in male and 31.1 g in female. And the osmotic pressure was estimated to be 1,800 mOsm/kg in males and 1,200 mOsm/kg in females, respectively. Despite the dosage levels being the same, the absolute ingestion quantity and osmotic pressure of the BCS solution were different among the individuals. Therefore, it is one of the reasons for difference between male and female in the occurrence of diarrhea.

As to a relationship between molecular weight and the maximum non-effective dose for diarrhea, there are references reporting the maximum non-effective dose to be 0.15–0.3 g/kg for monosaccharides (sorbitol, xylitol) and 0.28–0.3 g/kg for disaccharides (maltitol, pinit) and oligosaccharides (fructo-oligosaccharide, 4′-galacto-oligosaccharide, 6′-galacto-oligo-saccharide) (15, 16). According to Koizumi et al. (17), the maximum non-effective dose of a reducing starch sugar (molecular weight is 450–500, close to that of BCS) was 0.5 g/kg body weight for males. These data suggest that a difference in molecular weights highly affects the maximum non-effective dose for diarrhea. This relationship, however, is not applicable to substances such as erythritole, whose absorption route is different from that of BCS or other substances mentioned in the above. In the present study, ED$_{50}$ value for diarrhea is lower in BCS than that of indigestible dextrin because of the relatively lower molecular weight of BCS. However, the maximum non-effective dose of 0.5 g/kg body weight for males in the present study corresponds to 66 g of BCS for a male weighing 60 kg. Since nutritional effects can be expected with approximately 10 g (7), BCS is considered a relatively safe substance with respect to diarrhea in consideration of its intake in normal dietary life. Corn syrup solid is used in many food products. And BCS has almost the same physical properties as the existing corn syrup solid. BCS is expected to be used in many food applications. In fact, we applied BCS to sweet bean jelly, butter-cake, pudding, butter-cream and so on and we found no prominent differences between existing corn syrup solid and BCS. From now on we will try to apply BCS in place of indigestible dextrin to various processed foods because the molecular weight of BCS is lower than that of indigestible dextrin. Indigestible dextrin is limited to use in processed food due to its molecular weight.

As shown above, BCS revealed no toxicity in either an acute toxicity test or a mutagenicity test. As to its laxative effect, BCS is considered a food material to cause practically no diarrhea when it is ingested within its intended usage (10 g or below).

REFERENCES

1) Japan Health Food & Nutrition Food Association. 1997.
A guide to foods for specified health uses Q & A. p 1–19.

2) Mitsuoka T. 1994. Dietary Control of intestinal flora. In: Intestinal Flora and Diet (Mitsuoka T. ed), p 15–17. Japan Scientific Societies Press, Tokyo.

3) Oshshima T. 1996. Development and application of sweetening carbohydrate for prevention of dental caries —Oligosaccharide—. In: Food Science for Dental Caries Prevention—From Sweetening Sugars to Enzyme Inhibitors—(Oshshima T., Hamada S. eds), p 110–138. Ishiyaku Publishers, Tokyo.

4) Satouchi M, Wakabayashi S, Ohkuma K, Fujiwara K, Matsuoka H. 1993. Effects of indigestible dextrin on bowel movements. Eiyogakuzasshi (Jpn J Nutr) 51: 31–37 (in Japanese).

5) Tokunaga M, Matsuoka H. 1999. Effects of a FOSHU (food for specified health use) containing indigestible dextrin as a functional component on glucose and fat metabolisms. J Jpn Diabet Soc 42: 61–65 (in Japanese).

6) Nomura M, Nakajima Y, Abe Y. 1992. Effects of long-term administration of indigestible dextrin as soluble dietary fiber on lipid and glucose metabolism. Nippon Eijo Syokuryo Gakkaishi (J Jpn Soc Nutr Food Sci) 45: 21–25 (in Japanese).

7) Satouchi M, Wakabayashi S, Ohkuma K, Tsuji K. 1996. Effect of depolymerized pyrodextrin on human intestinal flora. J Bioscience Microflora 2: 93–101.

8) Maron DM, Ames BN. 1983. Revised methods for the salmonella mutagenicity test. Mutation Res 113: 173–215.

9) Matsushima T, Sawamura M, Hara K, Sugimura T. 1976. A safe substitute for polychlorinated biphenyls as an inducer of metabolic activation system. In: In Vitro Metabolic of Metabolic Activation System. (Serres FJ, Fouts JR, Bend JR, Philpot RM, eds), p 85–88, Elsevier Science, Amsterdam.

10) Wakabayashi S, Satouchi M, Ueda Y, Ohkuma K. 1992. Acute toxicity and mutagenicity studies of indigestible dextrin, and its effect on bowel movement of the rat. J Food Hyg Soc 6: 557–562 (in Japanese).

11) Dills WL Jr. 1996. Sugar alcohol as bulk sweetener. Ann Rev Nutr 9: 161–186.

12) Niwa H, Hikichi N, Sakurai E, Ueda K, Fukusei H. 1981. Alteration of biogenic amines, serotonin, histamin and polyamines, in cases of diarrhea induced by sugar alcohols. J Pharm Soc Jpn 101: 567–574 (in Japanese).

13) Terao T, Yamashita Y, Chou N, Sugiuira K, Moriyama Y, Ohsaki H, Nakano S. 1995. Effect of ingestion of two different carbohydrates immediately after exhaustive exercise on muscle glycogen restoration in rats. Jpn J Phys Fit Sports Med 44: 375–384 (in Japanese).

14) Koyama M. 1984. Fact to consider on constituents of semi-digestive nutritious agent —Osmotic pressure—. In: Enteral Nutrition (Ogoshi S, Sato H, eds), p 47. Asakura Shoten, Tokyo.

15) Koizumi N, Fujii M, Ninomiya R, Inoue Y, Kagawa T, Tsukamoto T. 1983. Studies on transitory laxative effects of sorbitol and maltitol. —Estimation of 50% effective does and maximum non-effective does—. Chemosphere 12: 45–53 (in Japanese).

16) Umeki Y, Ozawa Y, Higashikawa H. 1998. Physiological properties and food applications of erythritol. Up-to-date Food Processing 11: 14–17 (in Japanese).

17) Koizumi N, Ninomiya R, Fujita D, Tsukamoto T. 1998. Study on temporary diarrhea due to low calorie sweetener (Reducing starch sugar). Nippon Koushu Eiseigakaizasshi (Jpn Soc Public Health) 35: 629–635 (in Japanese).