

See discussions, stats, and author profiles for this publication at: <https://www.researchgate.net/publication/19085090>

# Gut microflora interactions with xylitol in mouse, rat and man

ARTICLE *in* FOOD AND CHEMICAL TOXICOLOGY · DECEMBER 1985

Impact Factor: 2.9 · DOI: 10.1016/0278-6915(85)90248-0 · Source: PubMed

---

CITATIONS

23

---

READS

67

5 AUTHORS, INCLUDING:



[Seppo J Salminen](#)

University of Turku

315 PUBLICATIONS 16,444 CITATIONS

[SEE PROFILE](#)



[Vincent Marks](#)

University of Surrey

629 PUBLICATIONS 11,145 CITATIONS

[SEE PROFILE](#)

## GUT MICROFLORA INTERACTIONS WITH XYLITOL IN THE MOUSE, RAT AND MAN

S. SALMINEN

Department of Food Chemistry and Technology, University of Helsinki

E. SALMINEN

Department of Radiotherapy and Oncology, University Central Hospital,  
00290 Helsinki

P. KOIVISTOINEN

Department of Food Chemistry and Technology, University of Helsinki,  
00710 Helsinki, Finland

and

J. BRIDGES and V. MARKS

Robens Institute of Industrial and Environmental Health and Safety and Department of Biochemistry,  
University of Surrey, Guildford, Surrey GU2 5XH, England

(Received 31 December 1984; revision received 22 February 1985)

**Abstract**—The influence of dietary xylitol on the quantity and quality of faecal microflora was studied in Wistar albino rats, CD-1 mice and healthy human volunteers. In animals, the effects of xylitol adaptation and of 4-wk xylitol feeding were examined. No major changes in the numbers of total aerobic or anaerobic bacteria, aerobic streptococci, anaerobic streptococci or yeasts were observed, although there was evidence of a dose-dependent decrease in the numbers of aerobic streptococci in the faeces. However, xylitol feeding caused a clear shift in the rodent faecal microbial population from Gram-negative to Gram-positive bacteria. In human volunteers a similar shift was observed even after a single 30-g oral dose of xylitol. All animals were capable of adapting to 20% dietary xylitol and an accompanying enhancement of the ability of caecal and faecal flora to utilize xylitol was observed.

### INTRODUCTION

The development of xylitol as a sugar substitute has been followed by extensive investigations of its safety. Since xylitol is absorbed more slowly from the gastro-intestinal tract than are most other common carbohydrates, it may, under circumstances of high dietary intake, achieve considerable concentrations in the lower bowel (Mäkinen, 1978). However, the microbiological effects of xylitol in the gastro-intestinal tract have received relatively little detailed attention, and therefore we decided to determine what effects, if any, xylitol exerts on the gastro-intestinal microflora of man and of the two species used primarily to investigate xylitol's toxicological properties, namely the mouse and rat (Joint FAO/WHO Expert Committee on Food Additives, 1983; Mäkinen, 1978). Earlier studies in humans (Dubach, Lejune, Forgo & Bueckert, 1974) and in laboratory animals (Krishnan, James, Bais *et al.* 1980a; Krishnan, Wilkinson, Joyce *et al.* 1980b; Wekell, Hartman & Dong, 1980) indicated possible species differences in response to xylitol. Whereas in humans no effects were detected, in rats changes in pathogenic microorganisms as well as changes in the ability of the flora to utilize xylitol were indicated. However, because the experimental designs were not directly comparable, it is not possible to ascertain from the literature whether this indicates a true species difference.

The present study was designed to compare the possible effects of xylitol on the quantity and quality of the intestinal microflora in rats, mice and man, in order to assess the ability of these microflora to degrade xylitol.

### EXPERIMENTAL

#### *Animals and diet*

The animals used were inbred SPF-derived strains of male Wistar albino rats (body weight 170–230 g) and of CD-1 mice weighing 25–30 g. They were bred in the University of Surrey Animal Unit and housed in the University's animal facilities. They were fed *ad lib.* on Spratt's Powdered Laboratory Diet for Rodents (Spratt's Laboratory Services, Barking, Essex, England) supplemented for the test groups with up to 20% xylitol. Supplementation was completed by gradual adaptation, i.e. by gradual replacement of the basal diet with xylitol. Gradual adaptation to sucrose was effected in one control group, to detect any microbial changes due to the carbohydrate addition alone. Another group of animals received a 20% xylitol diet without prior adaptation. Food and water consumption were measured weekly. Details of the feeding patterns used are described in Table 1.

Table 1. Dietary formulations and duration of feeding of each over the 6-wk study period

Duration of feeding (wk)	Dietary regimen (% added carbohydrate*)		
	For adaptation to xylitol (% xylitol)	For non-adapted animals (% xylitol)	For adaptation to sucrose (% sucrose)
2	0	0	0
1	5	20	5
1	10	20	10
1	15	20	15
1	20	20	20

\*Pharmaceutical-grade xylitol (from Xyrofin AG, Baar, Switzerland) or food-grade sucrose (from Finnish Sugar Co., Helsinki), expressed as a percentage of the final diet. The rest was a basal diet (Spratt's Powdered Laboratory Diet for Rodents, produced by Spratt's Laboratory Services), which was unsupplemented as the control diet.

Each group of animals, whether rats or mice, consisted of at least five animals. Housing conditions and animal maintenance were as described by Salminen, Salminen & Marks (1983) and Salminen, Salminen, Bridges & Marks (1983).

#### Human studies

After an overnight fast, six healthy volunteers (three males and three females, 22–35 years of age, average weight 60 kg) were served in random order with a 200-ml drink containing either 30 g xylitol or 30 g glucose. The conduct of the human study was essentially similar to that of the study described by Salminen *et al.* (1982). None of the volunteers had been exposed to a dietary xylitol supplement before.

#### Collection of faecal and caecal specimens

Faecal samples were collected from all the animals at the end of each week of treatment by holding each rat or mouse over a beaker until a faecal specimen was produced. The specimen was then transferred with forceps to a tube containing de-aerated Ringer's diluent. The fresh-weight concentrations were determined by weight difference and the tubes were transferred into an anaerobic glove-box (Forma Scientific, Marietta, OH, USA). Caecal samples from animals killed by cervical dislocation were removed by aseptic surgical procedures and processed in the manner described for faecal samples.

In the human volunteer study, faeces were collected twice before the consumption of the test drink. Each subject was then asked to provide a faecal specimen in a pre-weighed screw-cap aluminium container filled with charcoal-water anaerobic transport medium immediately after the consumption of the test drink. A second sample was taken 6–10 hr after treatment. Samples were brought to the laboratory as soon as possible and were processed immediately in the anaerobic glove-box. Volunteers were asked to keep the period between stool collection and submission for processing to within 2 hr.

#### Quantitative measurement of faecal microflora

Examinations of anaerobic bacteria were conducted in an anaerobic glove-box. All specimens and suspensions were mixed for 30 sec in a vortex mixer and serial tenfold dilutions to  $10^{-7}$  were prepared

from each sample. Microbiological media were prepared, poured into petri dishes and stored in the anaerobic glove-box for at least 3 days prior to use.

Aerobic media were prepared on the open bench. Plating for anaerobic counts was carried out in the anaerobic glove-box, whereas plating for aerobic counts and yeasts was carried out on the open bench. A spring-pipette (Gilson, Villiers-le-Bel, France), graduated in 0.1-ml increments, was used with sterile plastic tips to transfer inocula from dilution tubes to plates. From each suspension, duplicate plates were made at three different dilutions. Plates with counts between 15 and 150, or closest to that range, were used to calculate the numbers of organisms or colony-forming units/ml of original suspension. From the weight of the sample used, the results were calculated as no. of bacteria/g faeces. The culturing methods used were identical to those described by Aranki & Freter (1972) and Brown, Brown, Hyde & Bakner (1978). The following microbiological media were used for the selective culture of faecal flora:

- (i) for total aerobes, a blood agar base plus 70 ml defibrinated horse blood/1000 ml Difco medium;
- (ii) for total anaerobes, neomycin blood agar with added reducing agent: Columbia-agar-based plates completed with the addition of the following to 400 ml Columbia agar—25 ml horse blood, 2 ml neomycin solution (10.00 g/ml), 1 ml haemin (200 mg/100 ml) stock solution, 4 ml 3% sodium formaldehyde sulphoxylate and 4 ml 10% L-cysteine HCl solution;
- (iii) for aerobic streptococci, neomycin blood agar with aerobic incubation;
- (iv) for anaerobic streptococci, neomycin blood agar (the difference between total anaerobes and similar plates after aerobic incubation);
- (v) for yeasts, Sabouraud dextrose chloramphenicol agar.

#### Tests for microbial utilization of xylitol

To test the ability of the caecal and faecal micro-organisms to utilize xylitol, neutral saline solutions containing 5% xylitol as the sole carbon source were inoculated with standard loopsful ( $10 \mu\text{l}$ ) of caecal and faecal suspension and incubated under both aerobic and anaerobic conditions. The ability of the micro-organisms to metabolize xylitol was assessed by measuring the change in pH of the incubation medium indicating production of acid from xylitol. Glucose solutions of the same concentration were used as controls. Additional information on the rate of utilization of xylitol by caecal or faecal suspensions was obtained by analysing the xylitol content of the incubation medium before inoculation and at frequent intervals after the start of the incubation.

#### Determination of xylitol

Caecal or faecal suspensions containing xylitol were concentrated by freeze-drying, purified with an ion-exchange resin and analysed using a high-pressure liquid chromatograph coupled with a refractive index detector (Varo, Westermarck-Rosendahl, Hyvönen & Koivistoinen, 1979).

Table 2. Faecal culture data for control rats and for rats gradually adapted to 20% xylitol or 20% sucrose in the diet

Diet	Treatment wk...	Log no. of colony-forming units/g faeces from rats fed added carbohydrate at dietary levels (%)										
		0	0	0	5	10	15	20	20	20	0	0
		-1	0	1	2	3	4	5	6	7		
<b>Total anaerobes</b>												
Control		10.04 ± 0.64	9.35 ± 0.43	10.20 ± 0.13	9.81 ± 0.79	10.70 ± 0.73	9.40 ± 0.28	9.70 ± 0.23	9.34 ± 0.20	9.70 ± 0.33		
Xylitol (A)		9.92 ± 0.59	9.99 ± 0.81	10.37 ± 0.10	10.24 ± 0.36	9.75 ± 0.70	10.05 ± 0.20	9.54 ± 0.11	9.15 ± 0.11	9.63 ± 0.41		
Sucrose		9.93 ± 0.82	9.77 ± 0.59	10.13 ± 0.93	9.11 ± 0.12	8.59 ± 0.42*	8.02 ± 0.18*	8.57 ± 0.11	9.04 ± 0.20	9.14 ± 0.35		
<b>Anaerobic streptococci</b>												
Control		8.57 ± 0.35	8.67 ± 0.14	8.88 ± 0.68	8.18 ± 0.14	3.94 ± 0.93	7.83 ± 0.95	8.11 ± 0.87	8.00 ± 0.71	8.18 ± 0.53		
Xylitol (A)		8.87 ± 0.14	8.70 ± 0.22	9.32 ± 0.12	8.77 ± 0.38	3.08 ± 0.12	8.15 ± 0.30	7.27 ± 0.23	8.47 ± 0.26	8.20 ± 0.44		
Sucrose		8.84 ± 0.14	8.83 ± 0.79	8.72 ± 0.41	8.57 ± 0.23	3.63 ± 0.68	7.04 ± 0.13	7.91 ± 0.52	7.92 ± 0.20	8.01 ± 0.37		
<b>Total aerobes</b>												
Control		9.32 ± 0.21	10.54 ± 0.39	9.89 ± 0.52	9.10 ± 0.98	10.00 ± 0.20	9.17 ± 0.38	8.15 ± 0.20	8.78 ± 0.51	8.57 ± 0.62		
Xylitol (A)		10.26 ± 0.35	10.32 ± 0.22	9.90 ± 0.14	8.79 ± 0.53	8.99 ± 0.12	8.12 ± 0.18	8.10 ± 0.16	8.10 ± 0.18	9.08 ± 0.15		
Sucrose		10.10 ± 0.11	10.32 ± 0.10	10.18 ± 0.75	9.27 ± 0.19	9.34 ± 0.36	7.60 ± 0.82	9.57 ± 0.75	8.18 ± 0.31	7.58 ± 0.10		
<b>Aerobic streptococci</b>												
Control		10.79 ± 0.10	9.55 ± 0.67	9.24 ± 0.17	9.53 ± 0.39	3.93 ± 0.93	8.43 ± 0.46	8.09 ± 0.18	8.40 ± 0.22	8.32 ± 0.47		
Xylitol (A)		9.89 ± 0.54	9.38 ± 0.10	9.18 ± 0.05	7.05 ± 0.09*	5.21 ± 0.09*	6.38 ± 0.44*	5.43 ± 0.15*	6.55 ± 0.92	7.11 ± 0.21		
Sucrose		9.79 ± 0.10	9.23 ± 0.38	9.48 ± 0.26	8.96 ± 0.78	8.94 ± 0.98	7.45 ± 0.34	5.13 ± 0.12*	6.12 ± 0.40*	7.38 ± 0.30		
<b>Yeasts</b>												
Control		10.10 ± 0.10	9.69 ± 0.27	9.45 ± 0.10	8.98 ± 0.97	8.89 ± 0.67	9.34 ± 0.45	8.93 ± 0.56	9.00 ± 0.34	8.72 ± 0.20		
Xylitol (A)		9.78 ± 0.18	9.75 ± 0.65	9.39 ± 0.29	9.56 ± 0.38	9.67 ± 0.74	8.82 ± 0.68	9.26 ± 0.24	9.80 ± 0.21	8.83 ± 0.11		
Sucrose		10.10 ± 0.18	9.94 ± 0.38	9.59 ± 0.62	8.98 ± 0.14	9.57 ± 0.56	8.55 ± 0.56	9.40 ± 0.33	9.08 ± 0.33	7.96 ± 0.48		

Values are means ± SEM for groups of at least five rats and those marked with an asterisk differ significantly (\* $P < 0.01$ ) from the corresponding control.

A = gradual adaptation to 20% dietary xylitol

Table 3. Faecal culture data for control mice, for mice gradually adapted to a diet containing 20% xylitol or 20% sucrose and for mice given a 20% xylitol diet without prior adaptation

Diet	Treatment wk...	Log no. of colony-forming units/g faeces from mice fed added carbohydrate at dietary levels (%) of		
		0	10	20
		0	2	4
<b>Yeasts</b>				
Control		8.11 ± 0.14	8.27 ± 0.15	9.07 ± 0.21
Xylitol (A)		9.30 ± 0.22	9.73 ± 0.12	8.50 ± 0.33
Sucrose		8.66 ± 0.21	9.96 ± 0.56	9.89 ± 0.23
Xylitol (NA)		9.00 ± 0.17	—	10.22 ± 0.13
<b>Aerobic streptococci</b>				
Control		9.08 ± 0.13	9.40 ± 0.14	9.32 ± 0.20
Xylitol (A)		8.72 ± 0.11	8.19 ± 0.21	7.12 ± 0.89
Sucrose		9.32 ± 0.19	8.74 ± 0.19	8.30 ± 0.21
Xylitol (NA)		8.46 ± 0.21	—	10.13 ± 0.41
<b>Total aerobes</b>				
Control		9.08 ± 0.13	9.76 ± 0.10	9.83 ± 0.21
Xylitol (A)		8.89 ± 0.11	9.59 ± 0.10	10.68 ± 0.39
Sucrose		9.04 ± 0.18	9.45 ± 0.20	10.27 ± 0.10
Xylitol (NA)		9.83 ± 0.31	—	10.66 ± 0.58
<b>Total anaerobes</b>				
Control		10.70 ± 0.31	10.18 ± 0.62	10.27 ± 0.31
Xylitol (A)		10.78 ± 0.22	10.62 ± 0.22	10.68 ± 0.38
Sucrose		10.11 ± 0.20	10.61 ± 0.10	11.17 ± 0.22
Xylitol (NA)		10.98 ± 0.15	—	10.93 ± 0.16

A = Adaptation\* NA = No adaptation†

\*Gradual adaptation of the mice to 20% dietary xylitol.

†Feeding of a 20% xylitol diet without prior adaptation.

Values are means ± SEM for groups of five mice.

### Gram-staining procedure

Standard loopful of caecal suspensions or freshly collected faecal specimens were suspended in water, lightly centrifuged, heat fixed and stained using the Bacto Gram-stain set (Difco Ltd, Poole, Dorset, England). The relative amounts of Gram-positive and Gram-negative bacteria were estimated by a direct microscopic counting method under a high-power light microscope. The organisms were counted in standard fields.

## RESULTS

### Effects of xylitol on the gastro-intestinal flora of rats and mice

During the adaptation and treatment periods freshly voided faeces were collected from each animal

Table 4. pH changes in media inoculated with caecal suspensions from control rats (CO) or from rats adapted to 20% dietary xylitol (XA)

Carbon source	Source of caecal suspension	Final pH of medium*	
		Aerobic incubation	Anaerobic incubation
Glucose	CO	3.75 ± 0.1	3.70 ± 0.1
	XA	4.00 ± 0.2	3.76 ± 0.1
Xylitol	CO	6.20 ± 0.2	5.20 ± 0.1
	XA	5.55 ± 0.1	4.76 ± 0.1
Sorbitol	CO	4.50 ± 0.2	4.70 ± 0.1
	XA	4.45 ± 0.1	4.77 ± 0.1
Sucrose	CO	4.02 ± 0.2	3.85 ± 0.1
	XA	4.20 ± 0.1	4.00 ± 0.2
Fructose	CO	3.85 ± 0.2	3.80 ± 0.2
	XA	4.53 ± 0.2	4.40 ± 0.1
None	CO	6.40 ± 0.1	6.60 ± 0.0
	XA	6.72 ± 0.1	6.70 ± 0.1

\*Ringer's medium with 1% of the named carbohydrate as the sole carbon source and with an initial pH of 7.00 in each case.

Values are means ± SD for three determinations after aerobic or anaerobic incubation for 24 hr.

at regular intervals. For rats, faeces were cultured weekly and for mice every other week. While differences between xylitol-treated and control animals were seen for some components of the faecal flora at various times (Tables 2 & 3), no clear trends were apparent except in the case of aerobic streptococci. Xylitol treatment of rats caused a gradual but apparently reversible decrease in the numbers of aerobic streptococci in the faeces (Table 2), and a similar trend was observed in mice (Table 3). No differences in food consumption were observed between the groups, but water intakes tended to be slightly higher in xylitol-treated rats and mice during the adaptation period. At the end of the adaptation

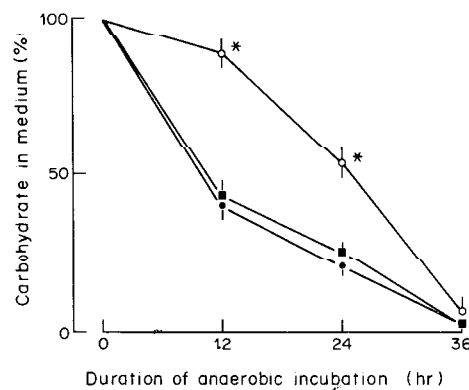


Fig. 1. The metabolism of xylitol (●, ○) and glucose (■) by caecal bacteria from xylitol-adapted rats (●) and from control rats (○, ■), expressed as the percentage recovery of carbohydrate in the medium as measured by HPLC after *in vitro* anaerobic incubation. Each point is the mean ± SD for three determinations, and an asterisk indicates a value significantly different ( $P < 0.001$ ) from the glucose control.

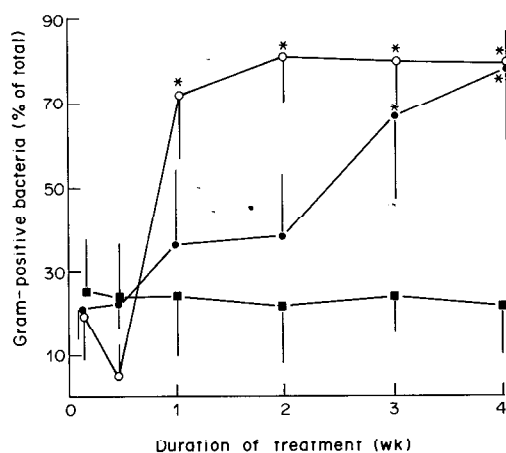


Fig. 2. The percentage of Gram-positive bacteria in the faeces of rats receiving a control diet (■), a 20% xylitol diet without adaptation (○) or a diet designed to adapt the rats gradually to 20% dietary xylitol with 5% stepwise weekly increments (●). Each point is the mean  $\pm$  SD for ten rats and an asterisk indicates a value significantly different ( $P < 0.01$ ) from the control.

period, the food intake of Wistar rats varied from 21 to 28 g/day, resulting in xylitol intakes between 4.2 and 5.6 g/day.

#### Ability of caecal flora to utilize xylitol

Adaptation of rats to 20% dietary xylitol increased the capacity of caecal flora preparations to utilize xylitol as the sole carbon source, as reflected in both acid production (Table 4) and disappearance of xylitol from the fermentation medium (Fig. 1). Considerably more acid was produced from xylitol under anaerobic than under aerobic conditions (Table 4).

#### Effect of xylitol in vivo on composition of intestinal flora in rats

Gradual adaptation to xylitol was associated with a gradual increase in the relative proportion of Gram-positive bacteria in the faeces. Originally, 15–35% of the faecal bacteria were Gram-positive in rats receiving a control diet whereas after the adaptation to 20% dietary xylitol about 70–75% of the bacteria present in the faeces were Gram-positive. A similar shift was seen in animals fed a 20% xylitol diet without adaptation: however, these animals initially developed watery diarrhoea and during the diarrhoea Gram-positive bacteria disappeared from the faeces

almost completely. When diarrhoea began to disappear gradually 7–10 days after treatment with the 20% xylitol diet, the proportion of Gram-positive bacteria increased, reaching the levels observed in rats gradually adapted to xylitol (Fig. 2). In CD-1 mice, adaptation occurred in a similar manner. Both in xylitol-adapted rats and in the rats receiving xylitol without prior adaptation the major changes in the types of bacteria involved an increase in the number of Gram-positive cocci and a decrease in the number of Gram-positive bacilli. However, no clear changes in bacterial type were observed among Gram-negative bacteria.

#### Human studies

Two of the six volunteers reported sudden transient diarrhoea 2–3 hr after xylitol consumption and all six had softer stools and increased stool frequency after xylitol intake.

No significant changes were observed in the total numbers of bacteria estimated either before or 6–10 hr after xylitol treatment (Table 5). With the exception of decreased numbers of yeasts after xylitol intake, all faecal counts were relatively stable. The decrease in the number of yeasts varied greatly, with the lowest counts found in subjects suffering from diarrhoea and flatulence.

The number of Gram-positive bacteria increased from 20–30% of the total to 50–55% of the total after xylitol consumption (Table 5). Although no more xylitol was introduced into the diet, a further reduction in the percentage of Gram-negative bacteria was observed 6–10 hr after xylitol consumption.

#### DISCUSSION

Relatively little attention has been paid to the role of the intestinal bacteria in the adaptation to and absorption of xylitol. This may be due, at least in part, to evidence that in man most oral micro-organisms cannot utilize xylitol and do not adapt to it (Mäkinen, 1978). In contrast, our results indicate that in the caeca of the rat and mouse there are bacteria that are readily able to adapt to utilizing xylitol. Adaptation may occur either by selection of micro-organisms capable of utilizing xylitol or possibly by induction of xylitol dehydrogenases in the bacteria already present. During the period of xylitol exposure the population of micro-organisms changed (Fig. 2). In particular during adaptation the proportion of Gram-positive organisms increased in the

Table 5. Data on cultures of faeces collected from human volunteers before and after ingestion of a drink containing 30 g xylitol in water

Bacteria*	Collection no.† ...	Log no. of colony-forming units/g faeces			
		I	II	III	IV
Total aerobes		10.8 $\pm$ 0.58	10.4 $\pm$ 0.68	10.3 $\pm$ 0.56	10.4 $\pm$ 1.1
Aerobic streptococci		8.2 $\pm$ 0.76	8.1 $\pm$ 0.71	7.9 $\pm$ 0.62	8.2 $\pm$ 0.71
Yeast		9.2 $\pm$ 0.87	9.4 $\pm$ 0.63	7.5 $\pm$ 2.1	7.2 $\pm$ 1.8
Total anaerobes		10.5 $\pm$ 0.66	10.5 $\pm$ 0.59	10.4 $\pm$ 0.49	10.1 $\pm$ 0.56
Anaerobic streptococci		8.4 $\pm$ 0.17	8.3 $\pm$ 0.67	8.3 $\pm$ 0.83	8.5 $\pm$ 1.00

\*Gram-positive bacteria constituted 26.8  $\pm$  8.1, 24.3  $\pm$  9.1, 48.6  $\pm$  10.2 and 39.0  $\pm$  7.9% total bacteria at collections I, II, III and IV, respectively.

†Two collections (I and II) were made before xylitol intake and constituted the controls, while the second two (collections III and IV) were made after xylitol ingestion.

Values are means  $\pm$  SEM for six volunteers.

faeces of rats. In rats given a 20% xylitol diet without prior adaptation, an initial decrease was observed in the number of faecal Gram-positive bacteria. In animals with marked diarrhoea, Gram-positive bacteria disappeared almost completely but started to re-appear as the diarrhoea lessened. After the diarrhoea disappeared, the number of Gram-positive bacteria started increasing until about two-thirds of faecal bacterial were Gram-positive. Towards the end of treatment the faecal microflora population was similar in both gradually adapted rats and rats receiving 20% xylitol diet without prior adaptation. This indicates that the 'washing out' of intestinal Gram-positive bacteria and the xylitol-induced diarrhoea may be connected with each other. Similar changes in Gram-positive bacteria have also been reported by Krishnan *et al.* (1980b) for xylitol, but no data on non-adapted animals receiving 20% xylitol diet and recovering from xylitol-induced diarrhoea have been described previously.

There were few other major changes in the microbial populations. Total numbers of aerobic and anaerobic bacteria remained fairly stable throughout the experiment and corresponded to those generally considered normal (Brown *et al.* 1978). The only significant trend observed was the dose-dependent decrease in the numbers of aerobic streptococci in the faeces of xylitol-treated animals (Table 2). Since data from human studies have shown that oral aerobic streptococci, especially *Streptococcus mutans*, are not capable of growing in xylitol environments (Mäkinen, 1978) it is possible that intestinal Streptococci also lack this ability. From the *in vitro* studies on the metabolism of xylitol, it seems likely that the bacteria concerned are Gram-positive and anaerobic.

Even small changes in the intestinal flora may influence gastro-intestinal conditions and cause increased motility of the intestinal system, thereby producing diarrhoea. Diarrhoea may also be caused by the fermentation of xylitol to low-molecular-weight osmotically active materials altering the environment of the large intestine. Such changes related to diarrhoea have been described by Leegwater, de Groot & van Kalmthout-Kuyper (1974). It should be noted that diarrhoea has also been observed with other polyols (Förster, 1978) but the cause has not been identified.

The results of the human volunteer study are similar to those observed in rats and mice. There was a marked increase in the proportion of Gram-positive bacteria. Since this change was observed in all of the three species used in the study it may indicate a selection of the faecal and intestinal microbes capable of utilizing and metabolizing xylitol. Thus, in respect of the microfloral changes invoked by polyols such as xylitol, rats and mice are probably a relevant model for man. The only other change appeared to occur in the number of yeasts, and it was of interest to observe that the number of faecal yeasts was greatest in the subjects showing signs of diarrhoea and in subjects having a lot of flatulence. Therefore, the growth of

yeasts may play a role in modulating the observed diarrhoea. In general, our results appear to be similar to those described by Dubach *et al.* (1974).

## Conclusion

Oral administration of high doses of xylitol to rats, mice or man caused changes in the faecal bacteria to a population that was capable of surviving in environments containing high xylitol concentrations and able to utilize xylitol as a carbon source. These changes appear to be associated with a marked shift in the faecal population from Gram-negative to Gram-positive organisms. Such effects may be important factors in the tolerance of animals to diets containing high concentrations of xylitol.

## REFERENCES

- Aranki A. & Freter R. (1972). Use of anaerobic glove boxes for the cultivation of strictly anaerobic bacteria. *Am. J. clin. Nutr.* **25**, 1829.
- Brown J. P., Brown R. J., Hyde B. C. & Bakner C. M. (1978). Gut microflora interactions with two experimental polymeric food additives in the rat. *Fd Cosmet. Toxicol.* **16**, 307.
- Dubach U. C., Lejune R., Forgo I. & Bueckert A. (1974). Untersuchungen über den Einfluss von Xylit auf die Stulflora. *Dtsch. med. Wschr.* **98**, 1960.
- Förster H. (1978). Tolerance in the human: adults and children. In *Xylitol*. Edited by J. N. Counsell. p. 43. Applied Science Publishers, Barking, Essex.
- Joint FAO/WHO Expert Committee on Food Additives (1983). Toxicological Evaluation of Certain Food Additives. *WHO Fd Add. Ser.* no. 18, p. 161.
- Krishnan H., James H. M., Bais R., Rofe A. M., Edwards J. B. & Conyers R. A. J. (1980a). Some biochemical studies on the adaptation associated with xylitol ingestion in rats. *Aust. J. exp. Biol. med. Sci.* **58**, 627.
- Krishnan H., Wilkinson I., Joyce L., Rofe A. M., Bais R., Conyers R. A. J. & Edwards J. B. (1980b). The effects of dietary xylitol on the ability of rat caecal flora to metabolise xylitol. *Aust. J. exp. Biol. med. Sci.* **58**, 639.
- Leegwater D. C., de Groot A. P. & van Kalmthout-Kuyper M. (1974). The aetiology of caecal enlargement in the rat. *Fd Cosmet. Toxicol.* **12**, 687.
- Mäkinen K. K. (1978). Biochemical principles of the use of xylitol in medicine and nutrition with special consideration of dental aspects. *Experientia Suppl.* **30**, p. 1.
- Salminen E., Salminen S., Bridges J. W. & Marks V. (1983). *Developments in The Science and Practise of Toxicology*. Edited by A. W. Hayes, T. Schnell & T. Miya. p. 333. Elsevier, Amsterdam.
- Salminen S., Salminen E. & Marks V. (1982). The effects of xylitol on the secretion of insulin and gastric inhibitory polypeptide in man and rats. *Diabetologia* **22**, 480.
- Varo P., Westermarck-Rosendahl C., Hyvönen L. & Koivistoinen P. (1979). The baking behavior of different sugars and sugar alcohols as determined by high pressure liquid chromatography. *Lebensm. Wissens. Technol.* **12**, 153.
- Wekell M. M., Hartman W. J. & Dong F. M. (1980). Incidence of increased numbers of Clostridium perfringens in the intestinal tracts of rats fed xylitol. *J. Nutr.* **110**, 2103.