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The effects of sorbitol on the gastrointestinal microflora in rats

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Summary: The influence of dietary sorbitol on the quantity and quality of faecal microflora was studied in Wistar albino rats. The animals were gradually adapted to 20 % dietary sorbitol or sucrose and faecal samples were analyzed for the numbers of major bacteria. No major changes in the numbers of total aerobic or anaerobic bacteria, aerobic streptococci and yeasts were observed after sorbitol feeding but sucrose appeared to decrease the total aerobes and anaerobes in the faeces. However, sorbitol feeding caused a clear shift in the rat faecal microbial population from gram-negative to gram-positive bacteria. All animals were capable of adapting to 20 % dietary sorbitol or sucrose and withstood sorbitol treatment without problems.

Zusammenfassung: Der Einfluß von mit der Nahrung zugeführtem Sorbit auf Art und Umfang der fäkalen Mikroflora wurde bei Wistar-Ratten untersucht. Die Tiere wurden langsam an 20 % Sorbit bzw. Saccharose in der Nahrung adaptiert; Kotproben wurden dann auf die Keimzahlen an den hauptsächlichen Bakterien analysiert. Sorbit-Fütterung führte zu keinen größeren Änderungen der Zahl von Gesamt-Aerobiern und -Anaerobiern sowie aeroben Streptokokken und Hefen, wogegen Saccharose die Gesamtzahl an Aerobiern und Anaerobiern in den Faeces zu vermindern schien. Die Sorbit-Fütterung führte jedoch zu einer eindeutigen Verschiebung in der Mikroorganismenflora im Darm von gram-negativen zu gram-positiven Bakterien. Alle Tiere konnten problemlos an 20 % Sorbit bzw. Zucker im Futter gewöhnt werden.

Key words: sorbitol, sucrose, intestinal flora, faecal flora, adaptation, rats, microbes

Introduction

Sorbitol is a polyol extensively used as a sweetener in dietetic and diabetic foods. The development of sorbitol as a sugar substitute has been followed by extensive investigations of its safety. Since sorbitol is absorbed more slowly from the gastrointestinal tract than most other common carbohydrates, it may, under circumstanes of high dietary intake, achieve considerable concentrations in the lower bowel. The microbiological effects of sorbitol in the gastrointestinal tract have received relatively little detailed attention and therefore we decided to determine what effects, if any, dietary sorbitol exerts on the gastrointestinal microflora of the Wistar albino rat. Earlier studies of polyols in humans (2, 5) and

in laboratory animals (3, 4, 5, 6) have indicated possible species differences in response to sugar substitutes. However, because the experimental designs were not directly comparable it is not possible to ascertain from the literature whether this is a true species difference.

The present study was designed to observe the possible effects of sorbitol on the quantity and quality of intestinal microflora in rats.

Experimental

The animals used were inbred SPF-derived starins of male Wistar albino rats weithing 170–230 g (University of Surrey Animal Unit) housed at the University of Surrey animal facilities. They were fed *ad libitum* on Spratts' Powdered Laboratory Diet for Rodents (Spratts' Ltd., U.K.) supplemented with up to 20 % sorbitol or sucrose. Supplementation was completed by gradual adaptation, i.e. by replacement of the base diet gradually with sorbitol and sucrose. Food and water consumption were measured weekly.

Each group of animals consisted of at least five animals. Details of the conditions of animal housing and feeding patterns were as described earlier (1, 5).

Faecal samples were collected from all animals following each week of treatment as follows. Each rat was held over a beaker until a faecal specimen was produced, the specimen was then transferred with forceps to a tube containing deaerated Ringer's diluent. The fresh weight concentrations were determined by weight difference and the tubes transferred into an anaerobic glove box (Forma Scientific, Marietta, Ohio, USA).

Examinations of anaerobic bacteria were conducted in an anaerobic glove-box. All specimens and suspensions were mixed using a vortex mixer for 30 seconds and serial tenfold dilutions to 10^{-7} were prepared of each sample. Microbiological media were prepared, poured into petri dishes and stored in the anaerobic glove-box for at least three 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, 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 15–150, or closest to that range, were used to calculate the numbers of organisms or colony-forming units (CFU)/ml of original suspension. Based on the weight of the sample used, the results were calculated as number of bacteria/g faeces. The culturing methods used were identical to those described by us earlier (5).

For gram-staining standard loopfuls of freshly collected faecal specimens were suspended in water, lightly centrifuged, heat fixed and stained using the Bacto Gram-stain set (Difco Ltd., Poole, U.K.). 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. All results were analyzed using the t-test and the Kolmogroff-Smirnov test.

Results and Discussion

During the adaptation and treatment periods freshly voided faeces were collected from each animal at regular intervals. While significant differences between sorbitol-treated and control animals were seen for some components (Table 1) of the faecal flora at various times no clear trends in these differences were apparent. Adaptation to 20 % dietary sucrose appeared to decrease the numbers of total aerobes and total anaerobes inthe faeces of Wistar rats (Table 1). No differences in food consumption were observed between the groups, but sorbitol-treated rats and mice tended to have slightly higher water intakes during the adaptation period. At the end of the adaptation the food intake of Wistar rats varied from 21–28 g/day resulting in sorbitol-intakes between 4.0–5.5 g/day.

Gradual adaptation to sorbital 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 sorbitol about 50–65 % of the bacteria present in the faeces were gram-positive (Fig. 1). A similar shift has been observed in animals fed a 20 % xylitol diet (1). In sorbitol adapted rats the major changes in the types of bacteria involved a great 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. These changes were

Table 1. Summary of faecal culture data for Wistar albino rats gradually adapted to a diet containing 20 % sorbitol or 20 % sucrose and rats given a control diet. Each value is a mean ± SEM for five rats.

Week of treatment	0	2	4
% added sorbitol or sucrose	0	10	20
Diet/bacteria	Log colony forming units/gram faeces		
Yeasts			
Control	9.69 ± 0.24	9.73 ± 0.18	9.45 ± 0.33
Sorbitol	10.30 ± 0.30	9.70 ± 0.22	9.82 ± 0.20
Sucrose	9.93 ± 0.82	9.57 ± 0.56	9.40 ± 0.33
Aerobic streptococci			
Control	7.60 ± 0.21	7.53 ± 0.32	7.70 ± 0.27
Sorbitol	7.98 ± 0.19	7.05 ± 0.19	7.11 ± 0.11
Sucrose	7.72 ± 0.22	7.63 ± 0.30	7.52 ± 0.29
Total aerobes			
Control	9.71 ± 0.33	9.96 ± 0.10	9.30 ± 0.65
Sorbitol	9.10 ± 0.22	9.13 ± 0.43	10.50 ± 0.33
Sucrose	10.32 ± 0.10	9.27 ± 0.19	7.60*± 0.82
Total anaerobes			
Control	10.60 ± 0.11	10.70 ± 0.19	10.92 ± 0.10
Sorbitol	10.20 ± 0.22	10.85 ± 0.17	10.05 ± 0.25
Sucrose	9.93 ± 0.82	9.11 ± 0.42	8.02*± 0.18

* significantly different from controls ($p < 0.01$)

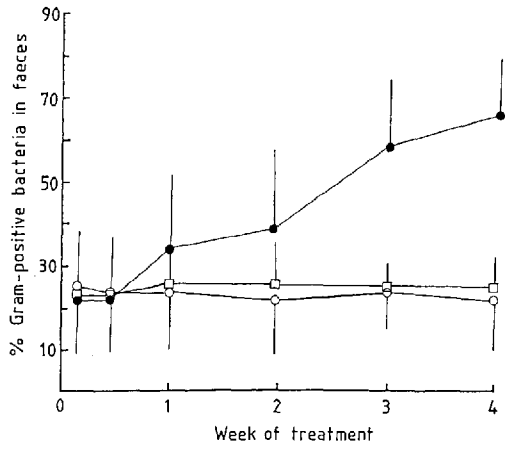

Fig. 1. The percentage of gram-positive bacteria in the faeces of rats receiving a control diet (○) or a diet designed to gradually adapt the rats to 20 % dietary sorbitol (●) or sucrose (□) with 5 % stepwise weekly increments. Each value is a mean ± SD for 10 rats. Significant differences from controls are indicated with an asterisk ($p < 0.01$).

similar to those described for xylitol (3, 4, 5, 7). The decrease in the numbers of total aerobes and anaerobes may be partly due to the decreased amount of carbohydrate substrate entering the large intestine. This is likely to occur since no differences in food consumption were observed and sucrose is absorbed from the small intestine.

Our results indicate that in the rat the bacteria are readily able to adapt to utilise sorbitol. Adaptation may occur either by selection of microorganisms capable of utilising sorbitol or possibly by induction of sorbitol dehydrogenases in the bacteria already present. During the period of sorbitol exposure qualitative changes in the population of faecal microorganisms occurred (Fig. 1) and these may be responsible for the adaptation.

In conclusion, oral administration of high doses of sorbitol to rats caused changes in faecal bacteria to a population capable of both surviving in environments containing high sorbitol concentrations. 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 to the tolerance of animals to diets containing high concentrations of sorbitol. Therefore these changes should be considered during the safety evaluation of sorbitol and other slowly absorbed carbohydrates.

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