INFECTION AND IMMUNITY, Jan. 1983, p. 43–49
0019-9567/83/010043-07$02.00/0

Vol. 39, No. 1

Non-Cariogenicity of the Disaccharide Palatinose in Experimental Dental Caries of Rats

TAKASHI OOSHIMA,[1]* AKIRA IZUMITANI,[1] SHIZUO SOBUE,[1] NOBUO OKAHASHI,[2] AND SHIGEYUKI HAMADA[2]

Department of Pedodontics, Osaka University Dental School, 4-3-48, Nakanoshima, Kita-ku, Osaka,[1] and Department of Dental Research, The National Institute of Health, 2-10-35, Kamiosaki, Shinagawa-ku, Tokyo,[2] Japan

Received 10 May 1982/Accepted 13 September 1982

The caries-inducing activity of palatinose (isomaltulose, α-D-glucopyranosyl-1,6-fructose) was examined in in vitro and in vivo experiments, comparing it with other carbohydrates. When *Streptococcus mutans* was successively subcultured in a broth medium containing 1% palatinose, the strains belonging to serotype *a*, *d*, or *g* did not ferment palatinose, whereas the strains belonging to serotype *b*, *c*, *e*, or *f* did ferment palatinose. Furthermore, palatinose significantly inhibited the synthesis of insoluble glucan from sucrose by *S. mutans*. Specific-pathogen-free rats which had been infected with *S. mutans* 6715 and fed a diet containing 56% palatinose did not develop significant dental caries. However, rats infected similarily, but fed a diet containing sucrose, glucose, fructose, or a glucose-fructose mixture manifested significant caries when compared with the noninfected, sucrose-fed control rats. Furthermore, it was found that replacement of half of the sucrose content with palatinose resulted in decreased caries development compared with caries development in rats fed the sucrose diet.

Dental caries is a most prevalent disease among civilized human populations. Evidence to support the importance of sucrose in the pathogenesis of dental caries has been obtained from epidemiological investigations with human subjects. The Vipeholm study revealed that the development of dental caries was related to the frequency of intake of sugar-containing foodstuffs, especially sweet between-meal snacks (13). Furthermore, it has been found that the deliberate restriction of sucrose from foodstuffs results in the reduction of dental caries (17). Epidemiological surveys by Sognnaes (32), Takeuchi (33), and many others provided further circumstantial evidence indicating the caries-inducing properties of sucrose; these surveys clearly suggested the relationship between sugar consumption and caries incidence before, during, and after World War II.

Streptococcus mutans is implicated in the development of dental caries as a prime pathogenic microorganism in both humans and experimental animals (15). *S. mutans* produces extracellular glucosyltransferase (GTase), which catalyzes the synthesis of extracellular water-soluble and -insoluble glucan from sucrose. Glucan synthesis promotes the adherence of *S. mutans* to solid surfaces, including those of teeth. Furthermore, *S. mutans* produces large quantities of acids from many kinds of sugars, the acids leading to localized decalcification of enamel surfaces.

Also, when compared with other mono- and disaccharides, sucrose has been shown to promote the highest level of dental caries in experimental animal model systems (4, 8, 9, 12, 30). Therefore, it has been suggested that one major method of controlling dental caries is to restrict sucrose consumption. Many investigators have examined the possibility of substituting non-cariogenic sugars or sugar alcohols for sucrose (3, 19, 24, 35).

Palatinose (isomaltulose) is a disaccharide, α-D-glucopyranosyl-1,6-fructose, which has been found in honey and sugar cane extract (29, 31). Recently, Shimizu and Nakajima (personal communication) devised an industrial method to convert sucrose into palatinose by the use of a glucosyltransferase derived from a strain of *Protaminobacter rubrum*. The palatinose prepared thus is more than 99.8% pure. Palatinose possesses stable chemical and physical properties and is degraded by intestinal palatinases (glucosidases) to produce free glucose and fructose (2, 5). Palatinose is about half as sweet as sucrose. The preliminary experiments revealed that certain strains of *S. mutans* did not ferment palatinose, and that palatinose inhibited the production of insoluble glucan from sucrose by *S. mutans* GTase (K. Ohta and I. Takazoe, 1980,

Shikwa Gakuho 80:1696–1697, abstract [in Japanese]). The purpose of the present investigation is to examine the effect of palatinose on the development of sucrose-induced dental caries in rats infected with *S. mutans*.

MATERIALS AND METHODS

Saccharides. A highly purified preparation of palatinose (99.8% pure) was kindly supplied by Mitsui Seito Co. (Tokyo). Other saccharides, including sucrose, glucose, fructose, and cornstarch, were reagent-grade products.

Microorganisms. Seven strains of *S. mutans*, representing the seven serotypes *a* to *g*, were employed in this study: E49 (serotype *a*), BHT (*b*), MT8148 (*c*), B13 (*d*), MT703R (*e*), OMZ175 (*f*), and 6715 (*g*). Stock cultures of each strain were maintained at 4°C on brain heart infusion agar (Difco Laboratories) slants. Other bacterial strains were arbitrarily selected from the culture collections of our departments.

Utilization of palatinose by *S. mutans*. Seven strains of *S. mutans* were cultured at 37°C for 20 h in brain heart infusion broth, centrifuged, and washed with phosphate-buffered saline (0.005 M, pH 7.0). The collected cells were suspended again in phosphate-buffered saline, and then the original suspension was diluted with phosphate-buffered saline 200 times, adjusting the cell concentration to an optical density at 550 nm of 0.15. The original cell suspension (3 ml) thus prepared was added to 12 ml of 5% sugar solution. The pH was adjusted to 7.0 with NaOH solution, and the pH of the reaction mixture was periodically measured with a combination electrode (GST155C) connected to a HM5B pH meter (Toa Denpa Co., Tokyo).

Strains of *S. mutans* representing the seven serotypes were cultured at 37°C for 48 h in phenol red broth (Difco) containing 1% palatinose. Each strain was subcultured every 48 h, and the growth of the strain and the color change of the medium were examined. A color change of phenol red broth medium from red to yellow indicates positive fermentation of palatinose by a test strain.

Effect of palatinose on insoluble glucan synthesis from sucrose. Crude GTase used in this experiment was prepared from a culture supernatant of *S. mutans* strain MT8148 (*c*), B13 (*d*), and 6715 (*g*) grown overnight in brain heart infusion broth as described previously (16). The GTase preparation produced water-insoluble glucan exclusively, and no detectable fructosyltransferase activity was demonstrated as determined by the incorporation of radioactivity into ethanol-insoluble polysaccharides from [^{14}C]fructose-labeled sucrose. The effect of palatinose on insoluble glucan synthesis from sucrose by GTase was determined as follows. The standard reaction mixture consisted of 1% (final concentration) sucrose, 100 μl of GTase preparation, and 0 to 5% palatinose in a total volume of 1.0 ml of potassium phosphate buffer (0.05 M, pH 6.8). The reaction mixture was incubated for 2 h at 37°C, and glucan synthesis was terminated by heating the mixture at 100°C for 5 min. The heat-treated samples were then centrifuged, and the precipitate was washed with 50% ethanol twice and 95% ethanol twice. The precipitate was then dissolved with 1 N NaOH. The total carbohydrate content of the precipitate was assayed by the phenol-sulfric acid method (6). Finally, the inhibitory effect of palatinose on the adherence of newly synthesized insoluble glucan from sucrose was assessed by the method of Hamada and Torii (16).

Caries induction in rats. Specific-pathogen-free Sprague-Dawley rats were purchased from CLEA Japan (Osaka, Japan) and were raised essentially under the conditions described previously (14). Rat pups were randomly divided into 12 groups, A to L (10 pups per group). Group A was a noninfected control; the rats were fed caries-inducing diet 2000 containing 56% sucrose (23). Group B was a positive control; the rats were infected with *S. mutans* 6715 (serotype *g*) and fed diet 2000 throughout the experimental period. Rats of the following groups were infected with *S. mutans* and then immediately afterward fed a modified diet containing different sugars in place of the sucrose in diet 2000: group C, 56% palatinose; group D, 56% glucose; group E, 56% fructose; group F, 56% cornstarch; group G, 28% glucose plus 28% fructose; group H, 28% sucrose plus 28% palatinose; group I, 28% sucrose plus 28% glucose; group J, 28% sucrose plus 28% fructose; group K, 28% sucrose plus 28% cornstarch; group L, 28% sucrose plus 14% glucose plus 14% fructose. All sugars incorporated into the diets were finely pulverized and passed through a 100-mesh sieve.

As summarized in Fig. 1, weanling rats (15 days old) were given both an ordinary powdered diet MF (Oriental Yeast Co., Osaka) containing tetracycline (4 mg/g diet) and deionized water containing penicillin G (4,000 U/ml of water) ad libitum to make it easy for the inoculated organisms to establish in the rat oral cavity. After depression with antibiotics, the animals of group B to L were infected on 5 consecutive days with *S. mutans* 6715 as reported previously (26), and all of the animals were fed diets containing the various sugars, as described above, throughout the duration of the experiment. Oral swabs were periodically taken from all animals for bacteriological surveys by using a cotton applicator which was then shaken in a test tube containing 1 ml of sterile saline. Suspended samples were then streaked on mitis salivarius agar containing streptomycin (500 μg/ml, final concentration). The plates were incubated at 37°C for 2 days, and the number of colonies recovered was determined. Implantation or absence of *S. mutans* was confirmed by the method of Keyes and Fitzgerald (22).

At the end of the experiment, the animals were sacrificed under ether anesthesia, and the jaws were removed. The jaws were then stained with erythrocin, and the deposition of dental plaque on the tooth surfaces of molars was evaluated by the procedure of Regolati and Hotz (27).

The carious lesions were scored on the unsectioned and sectioned jaws by the methods of Keyes (20, 21).

RESULTS

Resting cell suspensions of *S. mutans* 6715 produced acid from sucrose or glucose, and the pH of the reaction mixtures lowered markedly within several minutes (Fig. 2). However, palatinose and cornstarch were not utilized by the strain, and no significant acid production was demonstrated. Similar pH curves were obtained

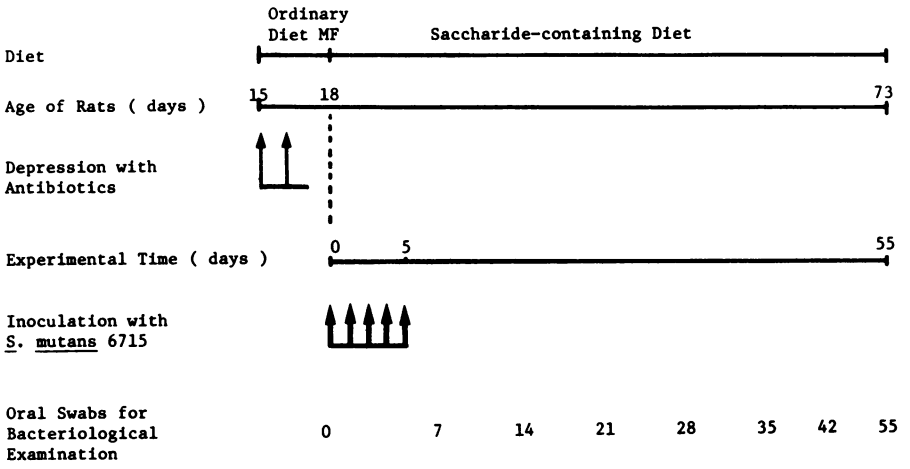

FIG. 1. Experimental design for examining the cariogenicity of palatinose and other sugars in specific-pathogen-free Sprague-Dawley rats.

with other *S. mutans* strains (data not shown). We also examined the fermentability of palatinose by various strains of *S. mutans*. When inoculated into phenol red broth containing 1% palatinose, brain heart infusion broth cultures of *S. mutans*, regardless of serotype, did not ferment palatinose. This does not mean that the cultures were dead, for they did grow when subcultured in brain heart infusion broth. *S. mutans* serotypes *a*, *d*, and *g* could not ferment palatinose even when these organisms were repeatedly subcultured in phenol red broth containing 1% palatinose. However, organisms belonging to serotype *b*, *c*, *e*, or *f* did ferment palatinose when subcultured two or three times in the broth medium containing 1% palatinose. Several oral streptococcal species, i.e., *Streptococcus sanguis*, *S. salivarius*, *S. mitior*, and *S. milleri*, did not ferment palatinose, but Lancefield group A, C, and G streptococcal strains did ferment palatinose (data not shown).

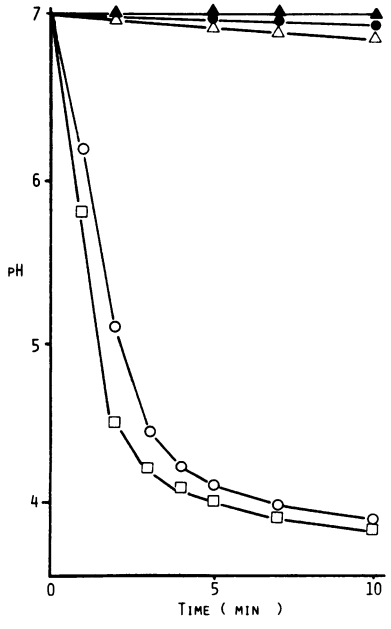

FIG. 2. Acid production of *S. mutans* from various sugars. Cell suspensions of *S. mutans* 6715 were prepared and mixed with 5% sugar solution as described in the text. Symbols: ▲, water; ○, sucrose; □, glucose; △, palatinose; ●, cornstarch.

It was found that GTase prepared from *S. mutans* 6715, MT8148, and B13 did not synthesize insoluble glucan from palatinose, whereas such GTase did synthesize insoluble glucan from sucrose (data not shown). Furthermore, the amount of insoluble glucan synthesized from sucrose by the GTase decreased markedly when increasing amounts of palatinose were added to the reaction mixture containing 1% sucrose (Table 1).

Adherence to a glass surface of the insoluble glucan synthesized from sucrose was also inhibited significantly in the presence of palatinose (data not shown).

The effect of palatinose and several other saccharides on rat dental caries induced by *S. mutans* 6715 (serotype *g*) was determined. *S. mutans* was easily established in the oral cavity of rats fed sucrose-containing diets. However, palatinose or cornstarch diet without sucrose barely supported the establishment of *S. mutans* (Table 2). In the rats fed glucose, fructose, or a mixture of glucose and fructose (group D, E, or G, respectively), the recovery of inoculated *S. mutans* was very low at the time of the first bacteriological examination after the infection.

TABLE 1. Inhibitory effect of palatinose on insoluble glucan synthesis by extracellular GTase of *S. mutans*[a]

GTase from *S. mutans* strain:	Insoluble glucan synthesis (% of control)[b] with final concn of palatinose:						
	0	0.1	0.3	0.5	1	3	5
6715	100 (420 μg/ml)	82	48	40	26	11	7
MT8148	100 (520 μg/ml)	65	61	49	36	11	5
B13	100 (460 μg/ml)	91	84	70	48	18	10

[a] The indicated levels of palatinose were incubated with crude GTase and 1% sucrose in the standard assay system. Insoluble glucan synthesized was measured by phenol-sulfuric acid method. The control assay was carried out without palatinose. The amount of insoluble glucan formed is shown within parentheses.
[b] Percentage of control (0% palatinose) = 100.

The level of recovery became higher gradually and reached the highest level at the end of experiment (Table 2). No streptomycin-resistant streptococci were detected in any of the uninfected rats throughout the experiment.

Noninfected control rats which were fed a high sucrose diet (group A) developed baseline plaque formation and dental caries, whereas *S. mutans*-infected rats manifested a notable accumulation of dental plaque and dental caries (Table 3). When sucrose, the major component in diet 2000, was totally replaced with palatinose (group C), the degree of plaque formation and caries development was decreased to the baseline level. On the other hand, the caries-inducing ability of glucose (group D), fructose (group E), or the mixture of glucose and fructose (group G) was less than half of that of sucrose (group B). Plaque indexes of group D, E, and G decreased concomitantly with the decrease of caries scores of these groups. Replacement of half of the sucrose content in diet 2000 with glucose (group I) resulted in increased caries development, whereas similar replacement with fructose (group J) somewhat inhibited caries development. Of interest is that the caries score of rats fed the diet containing the mixture of sucrose and palatinose (group H) was markedly lower than that of the rats fed diet 2000 (sucrose only; group B). Also, the plaque index of group H rats was less than that of group B. Although it is not a sweetening saccharide, cornstarch strongly inhibited caries-inducing activity (group F). Complete replacement of sucrose in diet 2000 with cornstarch resulted in negligible caries and insignificant plaque formation.

To embody the severity of sulcal lesions, the jaws were sectioned, and carious lesions of the pits and fissures of the rat molars were scored by the procedures of Keyes (21) (Table 4). The results were essentially similar to those on unsectioned jaws. However, the caries score of the animals fed palatinose diet (group C) was lower than that of the noninfected rats (group A).

No significant signs or symptoms of unwanted reactions in rats were observed during the experiment among the various groups, except for the cornstarch-consuming groups (F and K); the weight gains of these rats were significantly lower than in other groups.

DISCUSSION

Previous studies demonstrated that diets containing high levels of sucrose have a strong caries-conductive potential in experimental rodents infected with *S. mutans* (1, 8, 12, 34, 36). The aim of the present study was to examine the effect of palatinose on in vitro acid production

TABLE 2. Recovery of inoculated *S. mutans* 6715 from rat tooth surfaces

Rat group	Saccharide in diet	Recovery of *S. mutans*[a] on the following week after inoculation:							
		0	1	2	3	4	5	6	8
B	Sucrose 56%	0	3.7	4	4	4	4	4	4
C	Palatinose 56%	0	0.6	0.9	0.9	0.7	0.5	0.5	0.7
D	Glucose 56%	0	0.7	1.5	2.2	2.9	3.1	3.4	3.6
E	Fructose 56%	0	1.5	2.2	3.6	3.4	3.5	3.7	4
F	Cornstarch 56%	0	2.1	3.1	2.0	0.9	0.7	0.4	0.8
G	Glucose 28% + fructose 28%	0	0.5	1.9	2.2	2.4	3.5	3.7	4
H	Sucrose 28% + palatinose 28%	0	1.8	4	4	4	4	4	4
I	Sucrose 28% + glucose 28%	0	3.4	4	4	4	4	4	4
J	Sucrose 28% + fructose 28%	0	1.5	3.9	4	4	4	4	4
K	Sucrose 28% + cornstarch 28%	0	2.1	4	4	3.9	3.5	2.6	3.4
L	Sucrose 28% + glucose 14% + fructose 14%	0	3.5	4	4	3.9	4	4	4

[a] The grade of recovery was evaluated by the method of Keyes and Fitzgerald (22).

TABLE 3. Plaque deposition and caries development in specific-pathogen-free rats infected with *S. mutans* 6715

Rat group	Inoculation of *S. mutans* 6715	Saccharide in diet	Plaque index (mean ± SE)	Caries score[a] (mean ± SE)
A	−	Sucrose 56%	0.3 ± 0.0[b]	3.1 ± 0.4[b]
B	+	Sucrose 56%	1.2 ± 0.1	45.6 ± 4.7
C	+	Palatinose 56%	0.4 ± 0.0[b]	3.5 ± 0.6[b]
D	+	Glucose 56%	0.5 ± 0.0[b]	22.9 ± 2.3[c]
E	+	Fructose 56%	0.4 ± 0.0[b]	20.5 ± 2.2[c]
F	+	Cornstarch 56%	0.4 ± 0.0[b]	1.7 ± 0.1[b]
G	+	Glucose 28% + fructose 28%	0.5 ± 0.1[b]	18.2 ± 1.7[b]
H	+	Sucrose 28% + palatinose 28%	0.6 ± 0.1[b]	17.2 ± 2.5[b]
I	+	Sucrose 28% + glucose 28%	1.1 ± 0.1	53.7 ± 5.1
J	+	Sucrose 28% + fructose 28%	0.7 ± 0.0[c]	35.4 ± 4.0
K	+	Sucrose 28% + cornstarch 28%	0.5 ± 0.0[b]	8.6 ± 0.6[b]
L	+	Sucrose 28% + glucose 14% + fructose 14%	0.8 ± 0.1[c]	35.2 ± 4.3

[a] Caries scores were determined by the method of Keyes (20). Statistical analyses (*t*-tests) were carried out between group B and the other groups.
[b] $P < 0.001$.
[c] $P < 0.01$.

by oral streptococci and on in vivo experimental dental caries in specific-pathogen-free rats inoculated with *S. mutans*. Most of the oral streptococci could not produce significant amounts of acid from palatinose in vitro, although certain serotypes of *S. mutans* became adapted and fermented palatinose after being repeatedly subcultured in palatinose-containing broth. The findings suggest that such an adaptation could take place in certain bacterial species of the oral microbial flora, possibly resulting in the utilization of palatinose by alternative metabolic pathways. Furthermore, palatinose inhibited insoluble glucan synthesis from sucrose by extracellular GTase of *S. mutans* (Table 1). These results suggest the possibility that palatinose might be not only non-cariogenic but also anticariogenic. Animal experiments were therefore performed to examine the possibility.

Organisms of *S. mutans* 6715 inoculated into the rat oral cavity were readily established when a sucrose diet was given, but the recovery of the organisms from rats fed a palatinose or cornstarch diet was usually low throughout the experiment (Table 2). These results indicate that sucrose facilitates the implantation of *S. mutans* in the rat oral cavity; in contrast, the establishment of inoculated organisms was not significant in rats fed palatinose or cornstarch. At the end of the experiment, a notable plaque deposition occurred in animals fed a sucrose diet, whereas less plaque accumulation was observed in rats

TABLE 4. Caries score of sulcal lesions in specific-pathogen-free rats infected with *S. mutans* 6715

Rat group	Caries score[a] (mean ± SE)			
	E	Ds	Dm	Dx
A	25.5 ± 1.8[b]	11.1 ± 2.0[b]	2.3 ± 1.1[b]	0.3 ± 0.2[b]
B	50.7 ± 1.7	43.9 ± 2.2	25.1 ± 3.0	12.3 ± 2.3
C	16.9 ± 2.6[b]	7.0 ± 1.8[b]	0.7 ± 0.4[b]	0
D	41.9 ± 1.9[c]	31.3 ± 2.2[d]	22.6 ± 4.1	10.5 ± 1.9
E	41.5 ± 3.1[c]	28.2 ± 3.5[d]	12.9 ± 2.9[c]	7.9 ± 2.4
F	6.6 ± 0.7[b]	0.4 ± 0.3[b]	0	0
G	36.9 ± 2.3[d]	23.4 ± 2.9[b]	8.8 ± 1.9[d]	4.6 ± 1.4[c]
H	40.9 ± 2.0[d]	25.1 ± 3.6[d]	7.4 ± 2.5[d]	1.8 ± 0.9[d]
I	53.8 ± 1.3	47.0 ± 2.0	31.4 ± 2.4	22.2 ± 2.7[c]
J	50.1 ± 2.2	39.5 ± 3.2	21.0 ± 3.0	9.6 ± 1.9
K	28.5 ± 2.5[b]	7.6 ± 1.8[b]	0.5 ± 0.3[b]	0
L	45.5 ± 1.5	36.1 ± 2.7	15.9 ± 3.1	7.7 ± 2.2

[a] Caries scores were determined by the method of Keyes (21). Statistical analyses (*t*-tests) were carried out between group B and the other groups.
[b] $P < 0.001$.
[c] $P < 0.05$.
[d] $P < 0.01$.

fed diets containing sugars other than sucrose. The caries incidence of animals fed a palatinose diet was very low and was comparable to, or even less than, that of the noninfected control rats fed sucrose (Table 2).

Fitzgerald and Fitzgerald (7) reported the anticaries potential of 2-dexoy-D-glucose in hamsters infected with *S. mutans* and reared with diet 2000. This glucose derivative is known to be rapidly phosphorylated in competition with a number of hexoses (28). As a result, it inhibits the acid production and growth of *S. mutans*. In the present study, the lack of a suitable substrate for acid formation may explain the low caries activity in the rats on the palatinose diet. The cornstarch diet was also not conducive to dental caries.

Green and Hartles (10) and Huxley (18) examined the effect of diets containing various percentages of sucrose and cornstarch on dental caries in rats. They found that the animals fed a cornstarch-only diet had the least dental caries, and that there were no significant differences in body weight gains between the animals consuming sucrose and cornstarch diets. It was therefore concluded that cornstarch was least cariogenic. However, in the present experiment weight gains of animals fed cornstarch-containing diets (groups F and K) were lower than those of the groups fed mono- or disaccharides. This might indicate that the seemingly low caries-inducing activity of cornstarch is simply attributable to the reduced frequency of intake of the diet. Therefore, it is premature to conclude from the present study that cornstarch is non-cariogenic or cariostatic.

Dietary glucose, fructose, and the mixture of glucose and fructose did support the colonization of *S. mutans*, but the degree of implantation of this organism was not as great as in the case of sucrose. The organisms inoculated might persist to grow in the areas which afford protection from the adverse conditions in the mouth (i.e., pits and fissures). The number of organisms increased gradually as dental caries were induced in the protected areas, and a high level of recovery similar to that produced by sucrose was reached. The successful implantation of *S. mutans* in the mouths of the rats fed these diets may be attributable to the high dose of inoculated organisms (a total of about 6.0×10^{10} colony-forming units), for Van Houte et al. (37) demonstrated that the satisfactory colonization of *S. mutans* in the absence of sucrose required a high cell inoculum. The caries scores of rats fed these monosaccharides or the mixture were found to be nearly half of that of the positive control rats fed sucrose. Comparison of the cariogenicity of sucrose, glucose, and the mixture of glucose and fructose has been performed by several investigators (8, 9, 11, 12), and various results have been reported. The data reported here are essentially consistent with the findings of Frostell et al. (8). Recently, Birkhed et al. (3) reported that the differences in the caries scores between animals fed sucrose and invert sugar (equal amounts of fructose and glucose) are statistically significant when sucrose is completely substituted with invert sugar. In the present study, similar results were obtained when sucrose was totally replaced with the equal mixture of glucose and fructose. However, when half of the sucrose was substituted with the mixture of glucose and fructose, the caries score reached a level similar to those of animals fed sucrose-containing diets.

Ikeda et al. (19) reported that a sucrose-free coupling sugar preparation promoted significantly less caries than did sucrose in either gnotobiotic rats or conventional rats. However, the preparation was composed of glucose, fructose, maltose, and several oligosaccharides, saccharides which served as a substrate for acid production by *S. mutans*. Furthermore, the inhibition rate of the coupling sugar preparation on insoluble glucan synthesis was only 39.7%, whereas that of palatinose was 74% in our experimental system.

As shown above, the caries-inducing activity of palatinose was extremely low. Moreover, even when palatinose was substituted for half the sucrose in a diet, the caries score was decreased notably. We also got similar results with our experimental conditions when rats fed sucrose or palatinose diet were infected with *S. mutans* MT8148R (serotype *c*) (unpublished data). The results indicate that palatinose is not only non-cariogenic, but also has an inhibitory effect on the development of rats dental caries induced by *S. mutans* and sucrose. Van der Hoeven (35) reported that the cariogenicity of palatinit, an equimolar mixture of the disaccharide alcohols (α-D-glucopyranosyl-1,6-sorbitol and α-D-glucopyranosyl-1,6-mannitol) which is manufactured from palatinose (29), was low when tested in conventional rats. However, weight gains of the rats were lower on the palatinit diet than that on the sucrose diet. Side effects such as diarrhea have been observed in caries experiments with diets containing certain sucrose substitutes, especially sugar alcohols (24, 25). In our experiment, animals fed a palatinose diet did not suffer from diarrhea; as a consequence, the weight gains of these animals were comparable to those of animals fed sucrose, glucose, or fructose diets. Furthermore, palatinose did not cause any side effects detectable by visual inspection of the rats throughout the experiment. Also, palatinose can be obtained at a reasonable price, since it can be

manufactured from sucrose on an industrial scale (J. Shimizu and Y. Nakajima, personal communication). The findings reported here strongly suggest that partial replacement of sucrose with palatinose might turn out to be advantageous in preventing dental caries.

ACKNOWLEDGMENTS

We thank Dawn E. Diehnelt for correcting the English version of the manuscript.

This work was supported by grant 870117 from the Ministry of Education, Science and Culture of Japan.

LITERATURE CITED

1. **Amsbaugh, S. M., and W. H. Bowen.** 1981. The influence of carbohydrates on the cariogenicity of tested diets in rats, p. 309–317. Proceedings: Symposium on Animal Models in Cariology (a special supplement to Microbiology Abstracts). Information Retrieval, Inc., Washington, D.C.
2. **Auricchio, S., A. Rubino, R. Tosi, G. Semenza, M. Landolt, H. Kistler, and A. Prader.** 1963. Disaccharidase activities in human intestinal mucosa. Enzymol. Biol. Clin. **3**:193–208.
3. **Birkhed, D., V. Topitsoglou, S. Edwardsson, and G. Frostell.** 1981. Cariogenicity of invert sugar in long-term rat experiments. Caries Res. **15**:302–307.
4. **Campbell, R. G., and D. D. Zinner.** 1969. Effect of certain dietary sugars on hamster caries. J. Nutrit. **100**:11–20.
5. **Dahlqvist, A.** 1961. Hydrolysis of palatinose (isomaltulose) by pig intestinal glycosidases. Acta Chem. Scand. **15**:808–816.
6. **Dubois, M., K. A. Gilles, J. K. Hamilton, P. A. Rebers, and F. Smith.** 1956. Colorimetric method for determination of sugars and related substances. Anal. Chem. **28**:350–356.
7. **Fitzgerald, R. J., and D. B. Fitzgerald.** 1977. Inhibition of caries in hamsters by 2-deoxy-D-glucose. J. Dent. Res. **56**:1431.
8. **Frostell, G., P. H. Keyes, and R. H. Larson.** 1967. Effect of various sugars and sugar substitutes on dental caries in hamsters and rats. J. Nutrit. **93**:65–76.
9. **Green, R. M., and R. L. Hartles.** 1969. The effect of diets containing different mono- and disaccharides on the incidence of dental caries in the Albino rat. Arch. Oral Biol. **14**:235–241.
10. **Green, R. M., and R. L. Hartles.** 1970. The effects of diets containing varying percentages of sucrose and maize starch on caries in the Albino rat. Caries Res. **4**:188–192.
11. **Greenby, T. H., and J. B. Hutchinson.** 1969. The effects of diets containing sucrose, glucose or fructose on experimental dental caries in two strains of rats. Arch. Oral Biol. **14**:373–380.
12. **Guggenheim, B., K. G. König, E. Herzog, and H. R. Mühlemann.** 1966. The cariogenicity of different dietary carbohydrates tested on rats in relative gnotobiosis with a streptococcus producing extracellular polysaccharide. Helv. Odontol. Acta **10**:101–113.
13. **Gustafsson, B. E., C. E. Quensel, L. S. Lanke, C. Lundquist, H. Grahnen, B. E. Bonow, and B. Krasse.** 1954. The effect of different levels of carbohydrate intake on caries activity in 436 individuals observed for five years. Acta Odontol. Scand. **11**:232–363.
14. **Hamada, S., T. Ooshima, M. Torii, H. Imanishi, N. Masuda, S. Sobue, and S. Kotani.** 1978. Dental caries induction in experimental animals by clinical strains of *Streptococcus mutans* isolated from Japanese children. Microbiol. Immunol. **22**:301–314.
15. **Hamada, S., and H. D. Slade.** 1980. Biology, immunology and cariogenicity of *Streptococcus mutans*. Microbiol. Rev. **44**:331–384.
16. **Hamada, S., and M. Torii.** 1978. Effect of sucrose in culture media on the location of glucosyltransferase of *Streptococcus mutans* and cell adherence of glass surfaces. Infect. Immun. **20**:592–599.
17. **Harris, R.** 1963. Biology of the children of Hopewood House, Bowral, Australia. 4. Observation on dental caries experience extending over five years (1957–1961). J. Dent. Res. **42**:1387–1399.
18. **Huxley, H. G.** 1977. The cariogenicity of dietary sucrose at various levels in two strains of rat under unrestricted and controlled-frequency feeding conditions. Caries Res. **11**:237–242.
19. **Ikeda, T., T. Shiota, J. R. McGhee, S. Otaka, S. M. Michalek, K. Ochiai, M. Hirasawa, and K. Sugimoto.** 1978. Virulence of *Streptococcus mutans*: comparison of the effects of a coupling sugar and sucrose on certain metabolic activities and cariogenicity. Infect. Immun. **19**:477–480.
20. **Keyes, P. H.** 1944. A method of recording and scoring gross carious lesions in the molar teeth of Syrian hamsters. J. Dent. Res. **23**:439–444.
21. **Keyes, P. H.** 1958. Dental caries in the molar teeth of rats. II. A method for diagnosing and scoring several types of lesions simultaneously. J. Dent. Res. **37**:1088–1099.
22. **Keyes, P. H., and R. J. Fitzgerald.** 1962. Dental caries in the Syrian hamsters. IX. Arch. Oral Biol. **7**:267–278.
23. **Keyes, P. H., and H. V. Jordan.** 1964. Periodontal lesions in the Syrian hamster. III. Findings related to an infectious and transmissible component. Arch. Oral Biol. **9**:377–400.
24. **Mäkinen, K. K.** 1972. The role of sucrose and other sugars in the development of dental caries: a review. Int. Dent. J. **22**:363–386.
25. **Mühlemann, H. R., R. Schmid, T. Noguchi, T. Imfeld, and R. S. Hirsh.** 1977. Some dental effects of xylitol under laboratory and *In vivo* conditions. Caries Res. **11**:263–276.
26. **Ooshima, T., S. Sobue, S. Hamada, and S. Kotani.** 1981. Susceptibility of rats, hamsters and mice to carious infection by *Streptococcus mutans* serotype *c* and *d* organisms. J. Dent. Res. **60**:855–859.
27. **Regolati, B., and P. Hotz.** 1972. Cariostatic effect of glycerophosphate. Helv. Odontol. Acta **16**:13–18.
28. **Schachtele, C. F.** 1975. Glucose transport in *Streptococcus mutans*: preparation of cytoplasmic membranes and characteristics of phosphotransferase activity. J. Dent. Res. **54**:330–338.
29. **Schiweck, H.** 1980. Palatinit-Herstellung, technologische Eigenschaften und Analytik palatinithaltiger Lebensmittel. Alimenta **19**:5–16.
30. **Shaw, J. H., I. Krumins, and R. J. Gibbons.** 1967. Comparison of sucrose, lactose, maltose and glucose in the causation of experimental oral disease. Arch. Oral Biol. **12**:755–768.
31. **Siddiqui, I. R., and B. Fukgala.** 1967. Isolation and characterization of oligosaccharides from honey. I. Disaccharides. J. Apicultural Res. **6**:139–145.
32. **Sognnaes, R. F.** 1948. Analysis of wartime reduction of dental caries in European children. Am. J. Dis. Child. **75**:792–821.
33. **Takeuchi, M.** 1960. Epidemiological study on relation between dental caries incidence and sugar consumption. Bull. Tokyo Dent. Coll. **1**:58–70.
34. **Tanzer, J. M.** 1979. Essential dependence of smooth surface caries on, and argumentation of fissure caries by, sucrose and *Streptococcus mutans* infection. Infect. Immun. **25**:526–531.
35. **Van der Hoeven, J. S.** 1980. Cariogenicity of disaccharide alcohols in rats. Caries Res. **14**:61–66.
36. **Van Houte, J., and V. N. Upeslacis.** 1976. Studies of the mechanism of sucrose-associated colonization of *Streptococcus mutans* on teeth of conventional rats. J. Dent. Res. **55**:216–222.
37. **Van Houte, J., V. N. Upeslacis, H. V. Jordan, Z. Skobe, and D. B. Green.** 1976. Role of sucrose in colonization of *Streptococcus mutans* in conventional Sprague-Dawley rats. J. Dent. Res. **55**:202–215.