Effect of Level of Glucan Synthesis and Lactic Acid Formation on Caries Development in Golden Syrian Hamsters[1]

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ABSTRACT. **The objective of this study was to determine if strains of *Streptococcus mutans* differing in ability to synthesize glucan and form lactic acid could induce different levels of caries in hamsters. To depress the indigenous microflora, penicillin was given in the drinking water and tetracycline was incorporated into the diet. After five days, the hamsters were distributed among five groups and placed into flexible plastic isolators. Isolators were used to decrease the chances for cross-contamination. Four of the five groups were infected daily for 14 days with the following strains of *S. mutans:* OMZ-175, FORD, 107B and TEA. The fifth group served as an uninfected control. Animals were killed after seven weeks and caries was scored. Significant correlations of caries scores were obtained with levels of acid production but not with insoluble glucan production.**

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INTRODUCTION

Streptococcus mutans is believed to be the prime etiologic agent of coronal caries in both humans (Gibbons and Van Houte 1975a) and animals (Fitzgerald 1968). It has been suggested that the cariogenicity (virulence) of *S. mutans* is due to the ability of the organism to adhere to the tooth surface, to then colonize or aggregate by synthesizing water insoluble glucans, and to produce lactic acid by catabolizing fermentable carbohydrates to demineralize the enamel of teeth. The concept that the initiation of dental caries is associated with the development of sticky (insoluble) glucans has been proposed (de Stoppelaar et al. 1971). They reported that a mutant of *S. mutans,* which was unable to synthesize insoluble glucan, was no longer cariogenic in germ-free rats and that caries activity was greatly reduced in hamsters. Mao and Rosen (1980) have also shown that *S. mutans* defective in glucan synthesis causes no buccal-lingual caries in gnotobiotic rats. The importance of the glucans in the etiology of dental caries has been reviewed by a number of authors (Newbrun 1972, Gibbons and Van Houte 1975). There is little doubt that the insoluble glucan synthesized from sucrose by *S. mutans* plays a significant role in caries activity.

A second virulence factor characteristic of *S. mutans* is its ability to produce lactic acid. Some investigators (Jordan 1965, Drucker and Melville 1968) found no significant differences between cariogenic and noncariogenic streptococci regarding either the amount of lactic acid or other types of fermentation acids produced. However, others (Hillman 1978, Mao and Rosen 1980) isolated several mutants of *S. mutans* that made less lactic acid than the wild-type strains and had lower caries activity in test animals (Mao and Rosen 1980, Johnson et al. 1978). The above data support the importance of lactic acid in the etiology of dental caries.

Previous studies dealing with the cariogenicity of *S. mutans* have evaluated glucan synthesis or lactic acid production relative to caries activity. Except for proxi-

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mal caries, a recent study (Rosen et al. 1985) found that various strains of *S. mutans* were cariogenic in gnotobiotic rats regardless of the amounts of insoluble glucan they produced. Proximal caries was related to the amount of acid produced but not to the levels of glucan. Sulcal caries was not related to the amount of acid or insoluble glucan. This lack of correlation between levels of insoluble glucan and lactic acid with sulcal caries may be related to the animal model chosen. Rats are prone to develop carious lesions in sulci, in addition to the lesions affecting smooth surfaces of their teeth. The production of decay in occlusal pit and fissures is less dependent upon adherent microbial masses, because these sites provide stagnant areas where food can become impacted and bacteria can proliferate in a protected environment (Gibbons 1972). The hamster may be a more suitable model for determining the role of glucan production in the causation of dental caries. Hamsters are known to be developers of heavy plaque made up largely of cariogenic streptococci; rats generally develop minimal amounts of visible plaque containing several organisms (Krasse and Edwardson 1966). The fissures in the molar teeth of hamsters are wider and do not extend buccalingually as in the rat. When hamsters are infected with an appropriate cariogenic organism and fed a caries-promoting diet, carious lesions are visible after 35 to 42 days (Johansen 1954, Keyes 1959).

The purpose of this study was to infect golden syrian hamsters with strains of *S. mutans* that produce varying amounts of lactic acid and insoluble glucan to determine if there is a correlation between levels of glucan and lactic acid with caries experience.

MATERIALS AND METHODS

Strains of *S. mutans,* including data on their ability to synthesize insoluble glucan and form lactic acid, were supplied by I. L. Shklair, Dental Research, Great Lakes Naval Training Center, Illinois. Quadruplicate cultures of each organism were grown for 48 h in 5 mL of a chemically-defined medium containing 5% sucrose. Glucan was determined by using the total carbohydrate, phenol-sulfuric acid procedure (Osborne et al. 1976). Lactic acid was assayed by using a gas chromatograph (Osborne et al. 1976); DNA was determined by the diphenylamine procedure (Ashwell 1957). The amounts of lactic acid and insoluble glucan were expressed as moles of lactic acid per mg of DNA and mg of glucose equivalents per mg of DNA (Osborne et al. 1976).

One hundred 21-day-old female weanling golden syrian hamsters (Harlan, Indianapolis, Indiana) were received and placed in cages. There were 20 cages with five animals in each cage. The animals were fed Diet MIT 305 and demineralized water *ad libitum.* The following day, 4000 μ per mL of penicillin G was incorporated into the animals drinking water; 4 mg per g tetracycline was added to the MIT 305 diet (Teklad, Madison, Wisconsin) to depress indigenous *S. mutans* (Ooshima et al. 1981).

After five days, antibiotic therapy was discontinued. The hamsters were weighed and randomly distributed into five groups and placed into sterile, flexible, plastic isolators. Each isolator contained four cages with five animals per cage. This was done to decrease the chance of cross contamination. The hamsters received Diet 2000 (56% sucrose) (Ziegler Brothers, Gardners, Pennsylvania) and demineralized water *ad libitum.*

Four of the five groups were inoculated daily for 14 d with the following strains of *S. mutans:* OMZ 175, FORD, 107B and TEA. Their serotypes respectively are: c, d, b and d (Shklair and Keene 1974). Ford and 107B were isolated from naval recruits; TEA was obtained from J. Navia, Dental Research Institute, Birmingham, Alabama. All strains exist as freeze-dried cultures at Great Lakes, Illinois. These strains of *S. mutans* were selected based on their ability to produce variable levels of insoluble glucan and lactic acid (Table 1). The fifth group served as an uninfected control. For inoculation, the organisms were cultured anaerobically in Todd-Hewitt broth (DIFCO Laboratories, Detroit, Michigan) for 24 h at 37° C anaerobically. The bacteria were suspended in the broth and found to contain 6.5×10^5 colony-forming units. Animals were inoculated into the mouth by dipping sterile cotton swabs into the culture; the remainder of the culture was added to the drinking water.

Samples of the oral flora were taken from the oral cavity prior to antibiotic therapy, post-antibiotic therapy, after implantation of the specific *S. mutans,* and at 7, 11, 15, 21, 36 and 48 d thereafter. This was done to compare re-isolates from the original strain used for infection.

A sterile cotton swab was inserted into the oral cavity of the hamster and along the buccal surface of the molar teeth. The sample was then streaked into Mitis-Salivarius Bacitracin Agar (MSB) (Gold et al. 1973) and incubated for 24 h in an anaerobic chamber at 37° C. Aerobic incubation was then carried out for an additional 24 h.

Streptococcus mutans was recovered on MSB prior to antibiotic treatment. Further recovery of *S. mutans* did not occur until two weeks after antibiotic treatment ceased. Verification of serotypes was made by biochemical tests (Shklair and Keene 1974). After seven weeks, the animals were killed by carbon dioxide inhalation. Evaluation of the caries was made by the method of Keyes (1944).

The data were analyzed with an analysis of variance to determine if there was a difference in the mean caries score of the five groups. The Newman-Keuls multiple range test was used to determine if the difference in caries scores were significant. A simple correlation analysis was performed to determine the relationship between amount of acid and caries and between insoluble glucan and caries. The level of significance for all statistical tests was set at $P < 0.05$.

RESULTS

Recovery of the serotypes from infected hamsters was not consistent. Serotype b dominated in recoveries from all groups except OMZ-175 which was infected with serotype c. Only serotype c was recovered from these animals. Both serotypes b and d were recovered from hamsters infected with serotype d. *Streptococcus mutans*

TABLE 1

Insoluble glucan, lactic acid and mean (±*SE*) *caries scores for golden Syrian hamsters infected with* Streptococcus mutans

Group	Insoluble glucan (mg glucan/mg DNA)	Lactic acid (moles lactic acid/mg DNA)	Mean caries score (±SE) (total severity)*
Uninfected control	—————	—————	28.6 (±3.7)
107B	0.43	1.4	32.8 (±7.8)
OMZ-175	4.2	1.9	37.0 (±3.1)
TEA	47.6	2.1	37.3 (±3.9)
FORD	40.1	2.7	51.9 (±5.8)

*Values within vertical lines are not significantly different ($P > 0.05$)

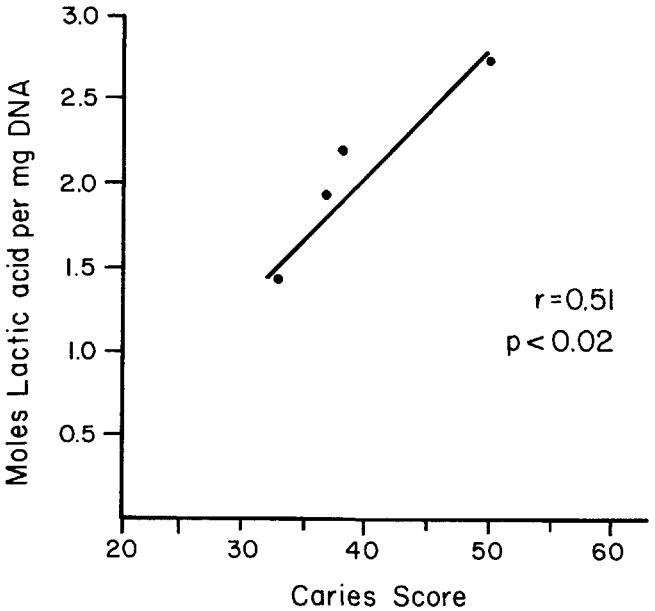

FIGURE 1. Correlation of lactic acid production per mg DNA with caries scores.

was not recovered from the uninfected control until the last recovery day. Serotype b was recovered at that time.

The mean caries scores and standard errors for the infected groups and for the control group are listed in Table 1. The control group had the lowest caries score (28.6 ±3.71), but was not significantly different from the three other groups (107B, OMZ-175, TEA) which had scores ranging from 32.8 (±7.8) to 37.3 (±3.9). The group infected with the FORD strain had a score of 51.9 (±5.83) which was significantly ($P < 0.05$) higher than the other groups. The correlation analysis showed that lactic acid production is significantly ($P < 0.02$) related to caries score (Fig. 1). There was no significant correlation between insoluble glucan and caries score. However, hamsters infected with the Ford strain, which had the highest level of insoluble glucan, also had the highest caries score.

DISCUSSION

The traits of insoluble glucan and lactate following animal passage are stable according to previous studies in germ-free rats (Rosen et al. 1985). Stability studies were not done in the present study because of the uncertainty of recovering the same organism. According to our recovery data, indigenous *S. mutans* strains did emerge since serotype b appeared in the uninfected control and other groups infected with serotype d. This could explain the high level of caries in the uninfected group. It is not known how the Ford strain caused a significant increase in caries. It could have interacted with the indigenous flora or, most likely, was able to become established in dental plaque and cause caries due to its ability to produce high levels of lactic acid.

The correlation analyses showed that lactic acid production may be a critical virulence factor in causing caries in hamsters. This supports other studies which show acid production to be an important factor (Mao and Rosen 1980, Rosen et al. 1985, Hillman 1978, Johnson et al. 1978).

The establishment of insoluble glucan as a correlative virulence factor is not very clear. This study shows that only one strain of *S. mutans* (FORD) causes a high level of caries and produces a high level of insoluble glucan. However, no significant correlation of insoluble glucan levels with caries scores could be shown with all strains as was the case for acid production. Other studies have shown that some level of insoluble glucan is needed (Mao and Rosen 1980, de Stoppelar et al. 1971, Newbrun 1972, Gibbons and Van Houte 1975b). However, we are not aware of studies that have shown a significant correlation of levels of insoluble glucan with caries development. It is possible that a certain threshold of insoluble glucan is needed for caries to occur. Additional levels of insoluble glucan may have little or no effect on increasing the severity of dental caries.

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