Low Sucrose Levels Promote Extensive \textit{Streptococcus mutans}-Induced Dental Caries

SUZANNE M. MICHALEK, JERRY R. McGHEE,* TETSUO SHIOTA, AND DOUGLAS DEVENYNS

\textit{The Department of Microbiology* and The Institute of Dental Research, The University of Alabama in Birmingham, University Station, Birmingham, Alabama 35294}

Received for publication 22 December 1976

One-tenth percent sucrose significantly promotes dental caries induced by \textit{Streptococcus mutans} in young gnotobiotic rats. Maximum caries activity was observed in rats provided a purified diet containing 3% sucrose.

A direct relationship between dietary sucrose consumption and dental caries incidence has been shown in man (6) as well as in experimental animals, e.g., monkeys (1), rats (8, 13), and hamsters (5). Dental caries is an infectious disease in which the oral bacterium \textit{Streptococcus mutans} has been implicated as a principal etiological agent (4), although other oral bacterial species probably contribute to this disease (2). Expression of \textit{S. mutans} virulence correlates with its ability to utilize sucrose for: (i) the synthesis of soluble and insoluble glucans, which facilitate adherence to the tooth surface, and (ii) fermentation, which yields organic acids and subsequently demineralizes the tooth (2). Investigations have shown that carbohydrates, e.g., glucose, fructose, starch, and others, can be utilized by \textit{S. mutans}; however, pathogenesis, in the presence of these substances, is significantly less than that observed when sucrose serves as the substrate (3, 8, 12, 14). Other studies support the hypothesis that the frequency and amount of sucrose intake increases the incidence of tooth decay (6). We report here that as little as 0.1% dietary sucrose is an adequate substrate for the promotion of significant caries, and levels of 3 to 10% yield maximum caries activity in young gnotobiotic rats mono-infected with \textit{S. mutans}.

Young weanling gnotobiotic rats (20 days old) were used in this study. Rat pups were randomly divided into six groups; each group was maintained in a separate isolator. Animals in each isolator were provided (ad libitum) either diet no. 300, a purified diet without sucrose, cornstarch (67%) being the carbohydrate source (9, 11), or one of the following: diet no. 300.1, 301, 303, 305, or 310. The last five diets were the same as diet no. 300, except that 0.1, 1, 3, 5, or 10% sucrose, respectively, was substituted for an amount that equaled that of the cornstarch in diet no. 300. Individual weanling rats were challenged by dispensing, with the aid of a micropipette, 50 \( \mu \)l of an 18-h culture of \textit{S. mutans} 6715 that contained \( 5.6 \times 10^6 \) to \( 6.0 \times 10^6 \) colony-forming units into the oral cavity of each rat. Previous studies in this laboratory have shown this bacterial strain to be highly virulent (9). The day after challenge, and at weekly intervals thereafter, colonization of \textit{S. mutans} was confirmed by collecting and plating individual oral-swab samples on Mitis-Sali-
It is further apparent from Table 1 that greater amounts of plaque and colony-forming units in plaque correlated with the increasing amounts of sucrose fed to the mono-infected rats. Rats fed diet no. 300.1 had significantly more plaque and colony-forming units in plaque (P ≤ 0.01) than rats fed a sucrose-free diet. Between seven- and ninefold more S. mutans could be recovered from rats fed diets no. 301, 303, 305, and 310 than from rats receiving diet no. 300 (no sucrose). No other bacterial species were detected. No significant differences were observed in the mean body weights of any of the six dietary groups tested.

The foregoing results indicate that as little as 0.1% dietary sucrose provided sufficient substrate for the accumulation of S. mutans plaque in mono-infected rats. Furthermore, 1% or more sucrose promotes significant plaque accumulations and caries, whereas diets containing 3% sucrose or more yield a maximum caries incidence.

A review of available information suggest that sucrose is a primary substrate for the promotion of dental caries (1, 5, 6, 8, 13). This hypothesis receives support from our investigation, since we observed significant dental caries experience in gnotobiotic rats provided with low levels of this substrate. From our studies it would appear that relatively small amounts of sucrose are more than adequate for the promotion of dental caries. We further suggest that the experimental use of artificially high levels of sucrose is not necessary and indeed would favor the parasite by allowing colonization and pathogenesis under insensitive conditions.

We wish to thank T. Ikeda and J. Navia for helpful suggestions and thorough review of this work, C. Sims for editorial advice, and J. Morris for typing this manuscript. This work was supported by Public Health Service grants DE-04217-02 and DE-02670 from the National Institute of Dental Research and CA-13148 from the National Cancer Institute. It was also supported by contract 82491-01 from the National Institute of Dental Research.

### Table 1. Mean caries and plaque scores and numbers of S. mutans CFU in gnotobiotic rats fed either a diet with no sucrose or diets with increasing amounts of sucrose

<table>
<thead>
<tr>
<th>Group (diet no.)</th>
<th>Mean enamel caries scores (%)</th>
<th>Mean no. of CFU per mandible (×10⁶)</th>
<th>Mean smooth-surface plaque score (%)</th>
<th>Mean body wt (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Buccal</td>
<td>Sulcal</td>
<td>Proximal</td>
<td>Total</td>
</tr>
<tr>
<td>300</td>
<td>22.4</td>
<td>60.2</td>
<td>2.6</td>
<td>30.6</td>
</tr>
<tr>
<td>300.1</td>
<td>46.9</td>
<td>72.7</td>
<td>25.6</td>
<td>54.1</td>
</tr>
<tr>
<td>301</td>
<td>90.1</td>
<td>93.4</td>
<td>92.3</td>
<td>91.7</td>
</tr>
<tr>
<td>303</td>
<td>98.8</td>
<td>96.4</td>
<td>101.3</td>
<td>98.2</td>
</tr>
<tr>
<td>305</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>310</td>
<td>(25.8 ± 0.7)</td>
<td>(22.0 ± 0.5)</td>
<td>(7.8 ± 0.1)</td>
<td>(55.6 ± 0.5)</td>
</tr>
</tbody>
</table>

* Values obtained from 50 rats per group. CFU, Colony-forming units.

* Evaluated by the method of Keyes (7).

* Values represent the mean ± standard error.

various agar and on blood agar with brain heart infusion broth base (Difco Laboratories, Inc., Detroit, Mich.).

Twenty-five days after challenge with S. mutans, all rats (45 days old) were removed from isolators and sacrificed. Mandibles from each rat were aseptically removed and then defleshed with a sterile scalpel. The right mandible from each animal was stained with safranin and scored for the level of plaque by the method previously described (10). The left mandible, in 3 ml of sterile phosphate buffer (0.067 M, pH 7.2), was sonically disrupted (Branson Sonifier cell disruptor, Branson Instruments Co., Plainview, N.Y.) for 15 s at a setting of 30. After dilution in the same buffer, portions of the solution were plated on Mitis-Salivarius agar and on blood agar with brain heart infusion broth base. Plates were incubated for 24 h at 37°C in an atmosphere of 95% nitrogen and 5% carbon dioxide. The total number of S. mutans colony-forming units per mandible was determined. Subsequent to plaque and microbial analyses, both mandibles from each animal were stained and scored for caries by the procedure of Keyes (7).

Animals that received dietary sucrose exhibited a higher incidence of caries commensurate with the increase in sucrose consumption (Fig. 1). Diet no. 301 promoted caries development that slightly exceeded 90% of the maximum enamel caries development observed in this system (Table 1). Animals provided diets containing 3 to 10% sucrose demonstrated, on all molar surfaces, maximum caries induction by S. mutans 6715 (Fig. 1). These results (Table 1) also demonstrate that as little as 0.1% dietary sucrose is sufficient for the promotion of 50% of the total enamel caries-inducing potential of this strain of S. mutans in this system. Furthermore, this caries activity (with diet no. 300.1) was significantly higher (P ≤ 0.01) than that obtained in animals provided a diet with no added sucrose (no. 300).
LITERATURE CITED


