MORPHOLOGICAL SURVEY OF EXPERIMENTALLY PRODUCED HUMAN DENTAL PLAQUE AFTER EXPOSURE TO A SUBSTITUTION SWEETNER, 70% COUPLING SUGAR C*

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Abstract: After treatment with a substitution sweetener, Coupling sugar C, made in Japan, and a conventional sweetener, sucrose, the dental plaque formed on removable enamel slabs which had been placed on a device placed in the human oral cavity was subjected to the morphological survey by transmission electronmicroscopy. The deep layer of dental plaque formed on the 6th day after starting the treatment was used. There was no appreciable morphological difference in the dental plaque on the enamel slabs between 70% C-S. C treatment and 70% sucrose treatment.

Introduction

To prevent the formation of dental cavities, a number of food products marketed in Japan use C-S. C (Coupling sugar C) instead of sugar1). Previously, I reported2,3) on the morphological difference observed in the deep layer of the dental plaque formed on the enamel slabs mounted on an improved device of Koulourides et al.4) following the treatment with 3% C-S. C and 3% sucrose. Some candies and nougats on the market, however, contain as much as 70% sugar1). Therefore, this study was done with 70% C-S. C and 70% sucrose solutions, otherwise under exactly the same experimental conditions as those of the previous study3) employing the enamel slabs without the covering mesh.

Materials and Methods

Volunteer: A male 19 years old with 4 filled teeth. He had no cavities. Oral device: As shown in Fig. 1. Test materials: As shown in Fig. 2, 3 enamel slabs free from any cavities were prepared from a thirr molar tooth extracted from a patient. They were attached to a removable rack with wax. Test solutions: Physiological saline as control, 70% sucrose solution, and 70% C-S. C solution. Conditions for application of test solutions: The enamel slabs were removed from the oral rack placed in the oral cavity of the volunteer 4 times per day to undergo the following treatment. Namely, they were removed from the rack at 10, 12, 14, and 16 hours to undergo a treatment with either one of the three test solutions for a period of 30 minutes at 37°C, and returned to the in vivo oral rack. The same procedures were repeated for 6 consecutive days. Preparation of samples: The enamel slabs were fixed with 2% glutaraldehyde for a period of 4-5
hours immediately after completion of the treatment. Then they were fixed over night with 1% osmic acid, embedded in Epon followed by the preparation of ground sections of 50μ in thickness for microradiography. The ground sections were decalcified with 0.5% chromium sulfate for 1 week, and embedded again in Epon for the preparation of ultrathin sections. Then they were stained with uranylacetate followed by lead citrate for electronmicroscopic observation (HS-8) of the deep layer of dental plaque.

Results

General matters: The enamel slabs were kept in the oral cavity of the volunteer approximately for 21 hours per day, and in an incubator regulated at 37°C for 3 hours (about 2 hours in the test solution and 1 hour for meals and brushing the teeth). No abnormality was recognized in the oral cavity of the volunteer throughout the period of the experiment. Plaque formation was recognized macroscopically from the 5th day after starting the experiment.

Photomicroscopic findings: The thickness of dental plaque observed on the ground sections was less than 45μ regardless of the treatment. In all cases, subsurface decalcification was absent in the microradiograms.

Electron microscopic findings:

1. Physiological saline treatment: Granular particles (cuticle-like substance) were practically absent on the surface of enamel slabs. The microorganisms observed in the dental plaque were mostly coccoid with smooth cell walls having numerous short fimbriae. Small granular particles were found extensively in the intercellular space of the microorganisms. These granules, moreover, were found to gather on the surface of the enamel slabs. (Fig. 3).

2. 70% sucrose treatment: The surface of the enamel slab was covered with a thin layer of granular particles. Microorganisms found in the dental plaque were mostly coccoid with smooth cell walls. The surface of the cell walls was covered with numerous fimbriae stretching out perpendicularly. In the intercellular space there were several layers of a belt of amorphous mucoid substance. The periphery of this belt was strongly electron-opaque with numerous deep wrinkles. The inside of the belt was filled with numerous short fibers. Electron-opaque matter resembling deformed bacterial cells was also found sporadically in the inside of the belt. Bacterial cells and small granular particles were found between the layers of overlapping belts, but there was no
fibrous matter or small vacuoles. (Fig. 4 and 6).

3. 70% C-S. C treatment: A thin layer of granular particles was found on the surface of the enamel slabs. The microorganisms found in the dental plaque were mostly coccoid with smooth cell walls. As in the case of 70% sucrose treatment, the formation of numerous fimbriae was observed surrounding the cell walls. The intercellular space had several layers of amorphous mucoid belts like those observed with 70% sucrose treatment. The periphery of these belts was electron-opaque with numerous deep wrinkles. Contained in the belt were numerous short fibers with a small number of electron-opaque substances and vacuoles. In the intercellular space, though fibrous matter was practically absent, a small number of vacuoles were observed with minute granular particles adjacent to the thin layer of granules on the surface of the enamel slabs. (Fig. 5).

Table 1 shows a comparison of the above findings.

<table>
<thead>
<tr>
<th>Test solutions</th>
<th>NaCl</th>
<th>sucrose</th>
<th>Coupling sugar C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Density (%)</td>
<td>0.9</td>
<td>70</td>
<td>70</td>
</tr>
<tr>
<td>granular particles on the enamel slab cells</td>
<td>-</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>form - coccoid</td>
<td>#</td>
<td>#</td>
<td>+</td>
</tr>
<tr>
<td>- rod-like</td>
<td>-</td>
<td>~ ±</td>
<td>~ ±</td>
</tr>
<tr>
<td>division - streptococcus</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>- others</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>wrinkled cell wall</td>
<td>-</td>
<td>-</td>
<td>~ ±</td>
</tr>
<tr>
<td>fimbria</td>
<td>+</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>intercellular substances</td>
<td>-</td>
<td>++</td>
<td>+</td>
</tr>
<tr>
<td>amorphous mucoid belt</td>
<td>-</td>
<td>#</td>
<td>#</td>
</tr>
<tr>
<td>vacuoles</td>
<td>±</td>
<td>-</td>
<td>~ ±</td>
</tr>
<tr>
<td>small granules</td>
<td>#</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>fibrous substance</td>
<td>±</td>
<td>~ ±</td>
<td>-</td>
</tr>
<tr>
<td>marks</td>
<td>-</td>
<td>±</td>
<td>+</td>
</tr>
<tr>
<td>degrees</td>
<td>absent</td>
<td>slight</td>
<td>light</td>
</tr>
</tbody>
</table>

Discussion

Amorphous mucoid belts:

Amorphous mucoid belts did not exist in the dental plaque of physiological saline treatment. As in the case of epithelium, which is usually not present in the mature plaque5-7), the absence of amorphous mucoid belts in the dental plaque of physiological saline treatment might probably be due to the digestion by the surrounding microorganisms or their enzymes during the period of experimentation. In contrast to the findings of previous experiments with 3% sucrose, several

Fig. 3-5 Electronmicrographs of 0.9% NaCl-(Fig. 3), 70% sucrose-(Fig. 4), and 70% Coupling sugar C-(Fig. 5) treatment. E: enamel slab side. S: small granule. A: amorphous mucoid belt. G: granular particle. Bar: 1.7 μ

Fig. 6 Amorphous mucoid belt. Arrow indicates unit membrane. F: fibrous substance. Bar: 0.25 μ
layers of amorphous mucoid belts appeared in the dental plaque of both 70 % sucrose treatment and 70 % C-S·C treatment. The influence of sucrose contained in C-S·C at a ratio of 12.5 % (1) might have appeared, when the concentration of C-S·C was as high as 70 % and when the treatment was continued for a considerable period of time.

The amorphous mucoid belts resembled the epithelium of the dental plaque of 2-7 days (3, 5, 9). This resemblance, however, was limited to the unit membrane on the periphery and the fibrous matter in the inside. Absence of epithelial organs such as nucleus, mitochondria, ER, desmosomes, etc. in the belt might suggest degeneration of the epithelium by cornification (8, 10). However, the calcification of internal organs following cornification (8, 11) was not seen. As for the fibrous matter in the inside of the belt, it was difficult to conclude at present whether or not it was the result of the influence of sucrose on the tonofilament.

At any rate, it is difficult to conclude, from the findings of the present experiment, that the amorphous mucoid belts are the epithelium.

The relationship of amorphous mucoid belts to the surrounding bacterial cells and intercellular substances might have some connection to the fact that epithelial cells absorb bacterial cells and carry them away (7, 8, 12-17). In comparison with the findings of experiments done with 3 % sugar solutions including physiological saline, the results of the present experiment demonstrated a larger number of amorphous mucoid belts with somewhat more marked fimbriae surrounding the bacterial cells, a number of intercellular substances other than the amorphous mucoid belts, and much fewer bacterial cells. Further studies will be made in the future to elucidate the reason why there was such difference.

On subsurface decalcification:
As in the previous report (3), subsurface decalcification was absent on all slabs. On the other hand, there are reports demonstrating in vivo the development of subsurface decalcification on enamel slabs shown by microradiography on the 5 th to 7 th day after starting sucrose treatment (18, 19). It was said that decalcification was absent when the teeth were brushed. The reason why it was absent on the enamel slabs of the present study might probably be due to the slight mechanical shaking when the enamel slabs were removed from the rack at each treatment with test solutions. There was no major difference in the findings of dental plaque and enamel slabs between the treatment with 70 % C-S·C and the treatment with 70 % sucrose. Thus it can be said that the influence of the two test solutions on enamel slabs was almost identical.

Acknowledgement

I wish to express my heartfelt thanks to the volunteer, Mr. H. Sakuno.

References

4) Koulourides, T., Bodden, R., Keller, S., Manson-Hing, L., Last Lastra, J. and Housh, T.: Cariogenicity of Nine sugars tested with an Intraoral Device in Man, Caries


