NEISSERIA IN EARLY STAGE OF DENTAL PLAQUE

BY

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ABSTRACT

Neisseria in the early stage of dental plaque was studied. Two hundred seventeen strains of Neisseria were isolated from the 12-hour plaque of 9 subjects by using lactose-agar. The isolated strains were divided into 5 major groups with biological characteristics. One hundred twenty-one strains (90%) produced glycogen-like polysaccharide from sucrose. They were divided into 3 groups. Groups 1 and 2 were identified as N. mucosa and N. sicca, respectively. There were 105 strains of N. sicca, being the most predominant of the species. The number of strains not producing polysaccharide was 96 (44%). Groups 4 and 5 were Branhamella and N. subflava. The absorption spectra of the ethanol extracts of the pigment in each group were similar and this result supported propriety of the classification by biological characteristics. The adhesiveness of Neisseria to the glass plate was examined. Approximately one-half of N. sicca showed an adhesion capacity.

INTRODUCTION

The dental plaque-forming process has been defined as the deposition of pellicles initially which may be composed of salivary mucoid and on which bacteria is adsorbed and mature. Accordingly, the initially adsorbed bacteria or bacterial species may have an important relation to the subsequent development of plaque.

Howell et al. reported that the aerobic gram-positive cocci and Neisseria were predominant in the early stage of the plaque and anaerobic bacteria proliferated subsequently. Ritz also described that Neisseria and Rotheia were predominant in the one-day plaque and occupied about 10% of the plaque bacteria while in the 9-day plaque, Neisseria and Rotheia decreased to 2% and the anaerobic bacteria proliferated. He also reported that the rate of plaque formation over a 5-day period might depend in part on the initial level of the aerobic organisms such as Neisseria. Takazoe showed that gram-negative aerobic bacteria which contained glycogen-like polysaccharide was adhering to the tooth. Thereafter, Ichinokawa isolated the Neisseria from the young dental plaque and showed that Neisseria sicca, which produce glycogen-like polysaccharide, possesses adhesive capacity in vitro.

In this experiment, Neisseria was isolated from the 12-hour plaque of the smooth surface of the upper incisor and the distribution of the species and the relationship to the developing of dental plaque were examined.

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Received for publication, June 15, 1978.
Materials and Methods

Isolation of Neisseria. Nine subjects with clinically healthy incisor and gingiva were selected and cleaned thoroughly by brushing. After 12 hours, the plaque samples were obtained from the labial surface of the upper incisors by scrubbing with sterilized cotton swabs. The cotton swabs were put into 5 ml of sterilized saline solution and shaken vigorously with the Thermostirrer. The suspension was serially diluted by 10-fold dilution. Each diluent, 0.05 ml in quantity, was inoculated onto the brain heart infusion agar supplemented with 1% lactose (L-BHI) by using a sterilized bent glass rod and incubated aerobically at 37°C.

Twenty-five colonies which did not ferment lactose were isolated at random and inoculated on L-BHI and purified.

Identification of Neisseria. The isolated Neisseria was identified as follows: Gram-negative cocci, not fermenting lactose, produced catalase and oxidase. Incidentally, the catalase and oxidase activities were tested as follows: For the catalase test, a drop of 3% hydrogen peroxide solution placed on a glass slide and the organisms on BHI were emulsified and then bubbling was observed. For the oxidase test, a 1% dimethyl p-phenylene diamine hydrochloride solution was flooded on the 2-day plate culture and the color change of the colony was observed.

The following characterizations were used for the identification of Neisseria species.

1) Fermentation of carbohydrate: The organism was inoculated on BHI agar containing carbohydrate (1%) and methyl red (0.02%). Glucose, maltose, fructose, and sucrose were used for the sugar fermentation test.

2) Reduction of nitrate and nitrite: BHI broth added 0.5% potassium nitrate or 0.5% potassium nitrite was used as the test medium. Alpha-naphthol solution and sulphanil solution were used as reagents.

3) Hemolysis: Horse blood agar was used for the hemolysis test.

4) Adhesion on glass tube and glass plate: BHI broth supplemented with 5% sucrose was used. One loop of the 1-day broth culture was inoculated on the above medium and incubated for 3 days. After incubation the medium was removed by decantation and the glass tube was washed with distilled water and the adhesive mass of organisms was stained with gram stain. A return circulation apparatus shown in Fig. 1 was used for testing the adhesive test for the organisms. The Neisseria culture was incubated in the apparatus for 12 hours and observed for the adhesiveness of the organism to the glass plate placed in the tube.

5) Polysaccharide production of Neisseria: Polysaccharide production was ob-

Fig. 1. Return Circulation Apparatus for Adhesive Test
served on the sucrose-supplemented HI agar. Iodine solution was flooded on the 24-hour culture and the brown color appearance was observed.

6) Spectrophotometric determination of pigment produced by Neisseria: Two strains were selected from each group at random and the color substances were extracted from the culture and identified by the colorimetric method described by Berger. The method was as follows: Selected strains were inoculated in 150 ml of BH-glucose media in 500 ml Erlenmeyer flask and incubated aerobically for 6 days at 37°C. The cells were obtained by centrifugation and washed twice with physiologic saline and again with 70% acetone. The washed cells were suspended in 4 ml of methanol, warmed at 90°C for 4 minutes and then kept overnight at 4°C. The color of the supernatant solution was calculated by the absorbance between 250–500 nm. spectrophotometrically.

7) Adhesion of plaque on tooth surface: The adhesiveness of the plaque bacteria to the surface of the test teeth was detected by gargling with neutral red solution 24 and 48 hours after brushing.

Results

Two hundred seventeen strains of Neisseria were isolated from the early stage of dental plaque of the upper incisor surface of nine subjects. The authors were able to divide the 217 isolated strains into 6 major groups by their biological characteristics as mentioned above (Table 1).

Of the 217 isolated strains of Neisseria,

Table 1. Biological Characteristics of Isolated Organisms

<table>
<thead>
<tr>
<th>Group</th>
<th>Polysaccharide from sucrose</th>
<th>NO₃ reduction</th>
<th>NO₂ reduction</th>
<th>Adherent</th>
<th>Hemolysis</th>
<th>Acid from Glucose</th>
<th>Maltose</th>
<th>Fructose</th>
<th>Sucrose</th>
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<tr>
<td>1</td>
<td>+</td>
<td>+</td>
<td>+</td>
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<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>6</td>
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<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
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</tr>
</tbody>
</table>

N. mucosa
N. sicca
Braahamella (N. catarhalis)
N. subflava
121 (56%) produced glycogen-like polysaccharide from sucrose. They were divided into 3 groups by the reduction of nitrate and/or nitrite. Group 1 reduced both of them, Group 2 reduced nitrite but not nitrate and Group 3 reduced neither of them. However, all of them fermented the carbohydrates tested. From these results, Group 1 was identified as N. mucosa and Group 2 as N. sicca. Unfortunately Group 3 could not be identified.

The number of strains belonging to Group 1 (N. mucosa), Group 2 (N. sicca) and Group 3 (unidentified group) were 4, 106, and 11, respectively.

The strains not producing polysaccharide numbered 96 (44%) out of the 217 isolated strains and were divided into 5 major groups (Groups 4 to 6). The strains belonging to Group 4 reduced nitrate and nitrite and fermented none of the sugar tested, being 12 in number. They were identified as Branhamella. The strains belonging to Group 5 reduced nitrite but not nitrate and were identified as N. subflava. They showed, however, variable characteristics in sugar fermentation and were divided into 4 subgroups. Eighty-two strains fell into this group. The remaining 2 strains belonging to Group 6 could not be identified, both reducing neither nitrate nor nitrite and fermenting no sugar.

The absorption spectra of the ethanol-extracted pigment produced by the representative strains of each group are shown in Figure 2.

Group 1 had peaks at 280, 370 and 425 nm (Fig. 2). Group 2 had a peak at 412 nm. The spectrum of Group 3 was similar to Group 1, and this indicated that the strains of Group 3 may be identified as N. mucosa, although they did not reduce nitrate or nitrite. Group 4 had peaks at 280, 350, 400 and 440 nm. All of the sub-

groups of Group 5 showed the same pattern of spectrum, having peaks at 280, 340, 356, 380, 405 and 425 nm. These results emphasize that the strains of Group 5 are of the same species. Group 6 had a spectrum pattern different from the other groups (Fig. 2).

The individual differences in the Neisseria species in the early plaque are shown
in Table 2.
As shown in the table, N. subflava was the most predominant species in 3 subjects (TH, ON, HA). On the other hand, N. sicca was the predominant species in the other 6 subjects.

In the case of the former 3 subjects, deposition of plaque on the incisor surface was very small in amount at 24 and 48 hours after brushing. In contrast, in the latter 6 subjects the plaque was highly developed at 24 hours after brushing. These results indicate that N. sicca has some relation to the deposition and development of early plaque at least on the smooth surface.

Adsorption of Neisseria onto the glass surface was carried out in vitro by using the apparatus shown in Figure 1. The twenty-four-hour broth culture was circulated in the apparatus for 8 hours. In the case of polysaccharide-producing strains, 42 of 90 strains (48%) of N. sicca and 8 of 11 strains (73%) of Branhamella showed adsorption on to the glass surface. On the other hand, 10 of 82 strains (12%) of N. subflava, which do not produce polysaccharide were found to adsorb on to the glass surface. Almost the same results were obtained by the experiment using the test
Table 2. Individual Difference of Neisseria

<table>
<thead>
<tr>
<th></th>
<th>Total</th>
<th>TH</th>
<th>ON</th>
<th>HA</th>
<th>MA</th>
<th>NA</th>
<th>OI</th>
<th>TO</th>
<th>TG</th>
<th>LS</th>
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<tbody>
<tr>
<td>N. mucosa</td>
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<td>1</td>
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<td>16</td>
<td>15</td>
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<td>Group 3</td>
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<td>2</td>
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<td>21</td>
<td>7</td>
<td>6</td>
<td>3</td>
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<tr>
<td>Branhamella</td>
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<td>10</td>
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<td>Group 6</td>
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</tr>
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Table 3. Characteristics of Broth Culture

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<th>Total</th>
<th>Adherence to glass plate</th>
</tr>
</thead>
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<td></td>
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<td>Inside</td>
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<tr>
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<td>0</td>
</tr>
<tr>
<td>N. sicca</td>
<td>90</td>
<td>42</td>
</tr>
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<td>11</td>
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discussion

It has been well known that pellicles form on the tooth surface initially and the Neisseria species as well as the streptococci and Rothia species deposit on the pellicles.

By the report of Takao^{8)}, the gram-stained preparation of the early plaque showed that there were many bacteria other than streptococci and some of them were shown to have polysaccharide which was stained by iodine solution and identified as Neisseria.

In this experiment, Neisseriae were isolated from the 12-hour plaque on the surface of the upper incisor. Two hundred and seventeen strains of Neisseria were isolated from 9 subjects and identified according to Bergey's manual (8th edition). Furthermore, pigment was extracted from the representative strains by the method of Berger^{9)}, and the absorption spectrum was measured for ascertaining the species.

As a result, N. sicca, which produce polysaccharide, and N. subflava, which do not produce polysaccharide, were detected as the predominant species, and they accounted for 86% of the Neisseriae in the plaque.

The adsorbing capacity of Neisseria on to the glass surface was investigated on 200 isolated strains.

Sixty-five (32.5%) of 200 strains were found to have the capacity to adsorb onto the glass surface in vitro. Among these, 42 strains were N. sicca, 8 were Branhamella catarrhalis, 10 were N. subflava and 5 were unidentified Neisseria. These results were a little different from the report by Ichinokawa^{10}) in which N. sicca was the only species having the adsorbing capacity. This may due to the different experimental methods.

Eight of 11 strains of Branhamella were found to adsorb onto the glass surface, al-
though Brachamella does not produce polysaccharide. Since Brachamella has been reported to produce a capsule, it may have some relation to adsorption. However, the capsular material could not be found in the experiment.

The isolated Brachamella strains were only 11 out of 217 strains and 10 were isolated from one subject (ON). From these results, it is rather difficult to believe that Brachamella has some factors causing deposition of plaque.

The relation between the individual distribution of Neisseria species and the development of dental plaque was as follows:

In the case of 3 subjects (TH, ON, HA), the development of the dental plaque was so slow that only a slight deposition of the plaque was observed at 48 hours after brushing, the most predominant species being N. subflava, with little or no N. sicca. In contrast, N. sicca was the most predominant in the other 6 subjects, the dental plaque depositing heavily after 24 hours.

References