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# THE EFFECT OF CARIES-PREVENTIVE MEASURES IN MOTHERS ON DENTAL CARIES AND THE ORAL PRESENCE OF THE BACTERIA *STREPTOCOCCUS MUTANS* AND LACTOBACILLI IN THEIR CHILDREN

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**Summary**—Seventy-seven first-time mothers were selected on the basis of high salivary counts of *Strep. mutans* [$> 10^5$ c.f.u. (colony forming units) per ml saliva]; 40 mothers were in the control group and 37 in the test group. Their infants were 3–8 months of age at the start of the study. A prophylactic programme for the test mothers, aiming at a reduction of *Strep. mutans*, was repeated at intervals of 2–4 months as and when necessary until their children were 3 years old. The test mothers as a group showed approx. 10-fold fewer *Strep. mutans* during the test period. At the age of 3 years, 70 per cent of the children in the control group carried *Strep. mutans*, compared with 41 per cent in the test group ($p < 0.01$). Fifty-two per cent of the children who carried *Strep. mutans* had caries at this age, compared to 3 per cent of the children without this organism. The time when *Strep. mutans* was first detected in the children seemed to influence subsequent development of caries because 77 per cent of the children who carried *Strep. mutans* at the age of 15 months had caries at the age of 3 years. Approximately 40 per cent of the children in both the control and the test group had detectable lactobacilli in their saliva at 3 years. In general, the children in the control group had more lactobacilli. No significant difference in frequency of intake of sucrose-containing foods and beverages was revealed from the dietary records but there was a tendency towards less frequent intake of sucrose in the control children with no detectable *Strep. mutans*, compared to those with *Strep. mutans*.

## INTRODUCTION

*Streptococcus mutans* plays a key role in the initiation of human enamel caries (reviewed by van Houte, 1980). Preventive measures aimed at a reduction of salivary *Strep. mutans* result in decrease in caries incidence (Zickert, Emilson and Krasse, 1982). Köhler, Bratthall and Krasse (1983) demonstrated that the initial establishment of *Strep. mutans* in infants could be prevented or delayed by caries-preventive measures in mothers carrying a high number of this organism in their saliva. These infants have now reached the age of 3 years and we have investigated them again.

## MATERIALS AND METHODS

A total of 77 first-time-mothers and their infants, alternately assigned to a control group ($n = 40$) and a test group ($n = 37$), completed a clinical trial (Köhler *et al.*, 1983). During the study, four subjects from the original test group elected not to participate further. The mothers were selected on the basis of high salivary *Strep. mutans* counts [$\geq 10^6$ c.f.u. (colony forming units) per ml saliva], when their infants were 3–8 months old. A preventive programme was instituted, aiming at a reduction of *Strep. mutans* in saliva in order to prevent or delay early establishment of this organism in the infants (Köhler *et al.*, 1982; Köhler *et al.*, 1983). The children were sampled for the presence of *Strep. mutans* at 4-month intervals, starting at the age of 15 months. The mothers were also sampled at this time. Examination of the children 3 years of age included salivary sampling and recording of dental caries and dietary habits. In addition to the children in the control and the test groups, 24 children whose mothers carried $< 3 \times 10^5$ *Strep. mutans* per ml saliva at selection (median = 104,000 c.f.u. per ml saliva), were examined for the presence of *Strep. mutans* (Köhler *et al.*, 1983). Twenty-two of these children were examined for the presence of caries at the age of 3 years.

### *Sample collection and microbiological analysis*

*Mothers.* One millilitre of paraffin-stimulated saliva was collected and transferred to VMG II transport medium (Möller, 1966). The samples were cultured on mitis salivarius bacitracin (MSB) agar selective for *Strep. mutans* (Gold, Jordan and van Houte, 1973) and on a pre-dried surface of Rogosa selective lactobacillus (SL) agar (Difco 0480) (Rogosa, Mitchell and Wiseman, 1951), using the micropipette method described by Westergren and Krasse (1978). The number of *Strep. mutans* c.f.u. and lactobacilli c.f.u. per ml saliva was estimated.

*Children.* Depending on the child's ability to cooperate, 0.05–1.0 ml of paraffin wax stimulated saliva was collected and transferred to 1 ml of reduced transport fluid (RTF) (Syed and Loesche, 1972). RTF does not contain charcoal particles and facilitates the identification of colonies when the samples are cultured undiluted. The samples were cultured direct and from $10^1$ and $10^2$ dilutions on MSB-agar and Rogosa SL agar. The numbers of *Strep. mutans* c.f.u. and lactobacilli c.f.u. per ml saliva were estimated by the micropipette method (Westergren and Krasse,

1978). Children unable to deliver a conventional saliva sample were sampled by a simplified technique, described by Köhler and Bratthall (1979), for estimation of salivary *Strep. mutans*. Analyses for the presence of lactobacilli were not performed in these children. Colonies with both typical and atypical morphology for *Strep. mutans* were isolated for identification and serotyping by immunofluorescent identification (Bratthall, 1972). The antisera were pooled and adsorbed in such a way that serotypes *c, e, f* or *d, g* were identified.

*Clinical examination.* Clinical examination was performed by two dentists working independently. Their individual observations were compared and a joint diagnostic decision was made. Neither dentist was aware of whether the child carried *Strep. mutans* or not, and one did not know the group affiliation of the children. Clinical caries was recorded according to the criteria described by Koch (1967) namely cavities in which a probe would enter and anatomical fissures in which the probe stuck on slight pressure.

In view of the young age of the children, the accuracy of the examination depended on the ability of the child to cooperate. Only clinical caries will therefore be reported and the children are classified in two groups; (1) children with clinical caries and (2) children with no detectable clinical caries.

*Dietary habits.* The mothers were instructed to write a 3-day dietary record (3 DR) for their children. The mothers were also interviewed on the frequency of intake of sucrose-containing foods and beverages such as sweets, biscuits, soft drinks, sweet soups and ice-cream. The frequency of intake was scored per week or per day. Each intake was given a point corresponding to its frequency, i.e. once a day gave seven points and once a week one point. The sum of the points for each child was divided by seven to calculate the intake of these sucrose-containing items per day. Consumption of fruit juice more than once a day and fruit yoghurt (8–10 per cent added sugar) was included. The 3 DR was analysed for (i) the number of intakes per day, (ii) the number of sucrose-containing meals and (iii) the number of sucrose-containing between-meal snacks.

*Statistical analysis.* Statistical significance was determined using the chi-square test and Student's *t*-test. A difference at the level of $p < 0.05$ was considered as significant.

## RESULTS

An approx. 10-fold lower salivary number of *Strep. mutans* and lactobacilli was achieved in the mothers over a 2.5 year period, that is until their infants were 3 years old (Table 1). The median, mean and range were calculated on the median of all samples collected from each mother after the initial sample. The effect on the establishment of *Strep. mutans* in their children (Fig. 1) was described in detail by Köhler *et al.* (1983). At the age of 3 years, 28 out of 40 children in the control group (70 per cent) and 15 out of 37 in the test group (41 per cent) harboured *Strep. mutans*. The difference between the two groups was significant ($p = 6.76$, $p < 0.01$; Table 2).

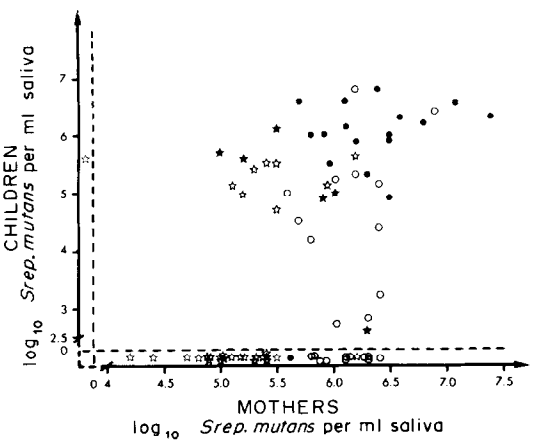


Fig. 1. Relationship between the salivary level of *Strep. mutans* in the mothers ($\log_{10}$ of the median of all samples collected during the experimental period) and in their 3-year-old children. Open circle, closed circle, control group; open star, closed star, test group. filled symbols indicate children with detectable caries.

A total of 23 children (30 per cent) had caries at the age of 3 years. Seventeen out of 40 in the control group (43 per cent) had caries, compared to 6 out of 37 in the test group (16 per cent; Table 3). The difference between the groups was significant ($p = 6.34$, $p < 0.02$). All children with caries but one had detectable levels of *Strep. mutans*, most of them high (Fig. 1). Thus 22 out of 43 children who carried *Strep. mutans* had caries (51 per cent) whereas only one out of 34 children with no detectable *Strep. mutans* had caries (3 per cent; Table 3). Table 3 also shows the distribution of children with caries in the control and test groups, in relation to the age when *Strep. mutans* was first detected in the child. Seventy-seven per cent of the children with detectable *Strep. mutans* at the age of 15 months developed caries compared to 33 per cent of the children who showed detectable *Strep. mutans* at the age of three years

Table 1. Salivary numbers of *Strep. mutans* and lactobacilli of the mothers in the control and test groups for the whole experimental period

| | c.f.u. *Strep. mutans* per ml saliva × $10^6$ | | | c.f.u. Lactobacilli per ml saliva × $10^3$ | | |
|---|---|---|---|---|---|---|
| | *n** | Median† | Range | *n* | Median | Range |
| Control | 40 | 1.4 | 0.5–25.0 | 40 | 69.5 | ND–20,000 |
| Test | 37 | 0.2 | ND‡–1.8 | 37 | 7.0 | ND–640 |

*Number of subjects. †Calculated on the median of all samples collected after the initial sample. ‡Not detected (<200 c.f.u.). c.f.u. = colony forming units.

Table 2. Salivary numbers of *Strep. mutans* and lactobacilli of the children in the control and test group at 3 years of age

| | c.f.u. *Strep. mutans* per ml saliva × $10^3$ | | | | | c.f.u. Lactobacilli per ml saliva × $10^3$ | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | *n** | No. of children with *Strep. mutans* | Median | Mean | Range | *n* | No. of children with lbc | Median | Mean | Range |
| Control | 40 | 28 (70%) | 152 | 956 | ND† 6300 | 37 | 15 (41%) | ND | 23 | ND 426 |
| Test | 37 | 15‡ (41%) | ND | 120 | ND–1356 | 36 | 14 (39%) | ND | 2 | ND–29 |

*Number of subjects. †ND = not detected (< 20–200 c.f.u.). c.f.u. = colony forming units. ‡Difference between control and test group significant at $p < 0.01$ (chi-square test).

Table 3. Number of children with caries at the age of 3 years in relation to the infection of *Strep. mutans* and the age when *Strep. mutans* was first detected

| | Number of children | | Age of detection of *Strep. mutans* (months) | | | |
|---|---|---|---|---|---|---|
| | With caries examined | With caries non *Strep. mutans* infected | 15 | 19–31 | 36 | Total at 36 months |
| | | | No. of children with caries/no. of *Strep. mutans* infected children | | | |
| Control | 17/40 (43)* | 1/12 (8) | 7/9 (78) | 7/15 (47) | 2/4 (50) | 16/28 (57) |
| Test | 6/37 (16)† | 0/22 (0) | 3/4 (75) | 3/9 (33) | 0/2 (0) | 6/15 (40) |
| Total | 23/77 (30) | 1/34 (3) | 10/13 (77) | 10/24 (42) | 2/6 (33) | 22/43 (51) |

*Percentage of children with caries. †Difference between control and test groups significant at $p < 0.02$ (chi-square test).

(Table 3). In general, the children with caries had more *Strep. mutans* at the age of 3 years (Table 4, Fig. 1).

The mothers, who had a low level of *Strep. mutans* at the initial selection, also generally had a low level at the examination when their children were 3 years of age (median = 217,000 c.f.u. per ml saliva). Five of their children were infected with *Strep. mutans* at this age (23 per cent) and four of these infected children had detectable caries (18 per cent of all children in this group). The percentage of children with caries is thus approx. the same as in the test group (Table 3).

Serotypes *c, e, f* were the most prevalent serotypes and were carried by 95 per cent of the infected children. Thirty-three per cent harboured serotype *d* or *g*, usually in combination with serotypes *c, e, f*. No difference in distribution of serotypes was found between children with caries and those with no detectable caries (Table 5).

Lactobacilli were detected in saliva in approx. 40 per cent of the children in both groups (Table 2). Children in the control group generally carried more lactobacilli than children in the test group. Children with caries also had more than children with no detectable caries. Thus, 13 out of 15 children with > 4000 c.f.u. per ml had caries, compared to 2 out of 13 children with < 4000 c.f.u. per ml. Five children with no detectable lactobacilli developed caries.

No difference in consumption of certain sucrose-containing foods and beverages between the control and test groups was revealed by the dietary history. A tendency towards a lower frequency of intake was

Table 4. Salivary numbers of *Strep. mutans* and lactobacilli in children at the age of 3 years with caries and those with no detectable caries

| | c.f.u. *Strep. mutans* per ml saliva × $10^3$ | | | | c.f.u. Lactobacilli per ml saliva × $10^3$ | | | |
|---|---|---|---|---|---|---|---|---|
| | *n** | Median | Mean | Range | *n* | Median | Mean | Range |
| Caries | 23 | 1000 | 1350 | ND–6240 | 21 | 4.7 | 40.4 | ND–426 |
| No caries | 54 | ND† | 216 | ND–6300 | 52 | ND | 1.5 | ND–64 |

*Number of subjects. †ND = not detected (< 200 c.f.u.). c.f.u. = colony forming units.

Table 5. Distribution of serotypes *c, e, f* and *d, g* among children with caries and those with no detectable caries

| | | Serotype | | |
|---|---|---|---|---|
| | *n** | *c, e, f* | *d, g* | *c, e, f* and *d, g* |
| Caries | 22 | 15 | 1 | 6 |
| No detectable caries | 21 | 14 | 1 | 6 |
| Total | 43 | 29 | 2 | 12 |

*Number of *Strep. mutans*-infected infants.

Table 6. The mean frequency of intake per day fo certain sucrose-containing foods and beverages in relation to infection with *Strep. mutans* and detection of caries at the age of 3 years

| | Control group | | | Test group | | |
|---|---|---|---|---|---|---|
| | No. of children | Intake frequency ± SD | Range | No. of children | Intake frequency | Range |
| Total | 40 | 2.3 ± 1.1 | 0.4 ± 4.7 | 37 | 2.1 ± 1.0 | 0.1 ± 5.7 |
| Non-infected | 12 | 1.9 ± 1.1 | 0.4 ± 3.8 | 22 | 2.2 ± 0.8 | 0.9 ± 3.7 |
| *Strep. mutans*-infected | 28 | NS* 2.5 ± 1.1 NS | 0.6 ± 4.7 | 15 | 2.1 ± 1.3 | 0.1 ± 5.7 |
| *Strep. mutans*-infected + caries | 16 | 2.6 ± 1.2 | 0.6 ± 4.7 | 6 | 1.7 ± 0.5 | 1.2 ± 2.3 |

*NS = not statistically significant.

found in the non-infected subjects in the control group, compared to the *Strep. mutans*-infected children in the same group (Table 6). The same tendency was found when the 3 DR were analysed for the number of intakes per day and the number of between-meal snacks with sucrose-containing foods and beverages. Thus, the non-infected control subjects had 4.8 (SD ± 0.5) intakes per day compared to 5.6 (SD ± 1.3 ) for the *Strep. mutans*-infected control subjects; the number of sucrose-containing between-meal snacks was 1.1 (SD ± 0.7) and 1.9 (SD ± 1.4), respectively.

## DISCUSSION

Only three per cent of children who did not have detectable *Strep. mutans* at the age of 3 years developed caries compared to 51 per cent of the *Strep. mutans*-infected children. The results support the view that *Strep. mutans* plays an important role in the development of caries in man. However, in view of the multifactorial nature of dental caries, disease is not the inevitable outcome of infection. Nevertheless, the fact that 77 per cent of the children who were infected early with *Strep. mutans* developed caries, compared to 33 per cent when *Strep. mutans* was first detected at 3 years of age, illustrates a higher caries risk after early colonization of the mouth. The influence of the age of infection with *Strep. mutans* on subsequent development of caries has been demonstrated in animal studies (Schuster, Morse and Birksen, 1978) and in man (Alaluusua and Renkonen, 1983).

No significant differences in sucrose consumption could be found between children within the groups or between the test and control groups, in spite of varying levels of *Strep. mutans* infection and caries experience. In the control group, however, a tendency towards a higher and more frequent consumption of sucrose was found in the *Strep. mutans*-infected children with caries, compared to those who were not infected and had no detectable caries. The lack of significant differences in sucrose consumption might be due to difficulties in obtaining accurate information on dietary habits. Another possibility is that if the child is colonized by *Strep. mutans*, an ordinary Swedish diet contains enough sucrose to support both the *Strep. mutans* infection and the production of caries. However, other factors than sucrose intake, e.g. the composition of saliva and exposure to fluoride, could have modified the outcome of the *Strep. mutans* infection. None of the children had been given fluoride supplements. A group of children (4 in both the control and the test group) had, however, grown up with approx. 2 parts/$10^6$ fluoride in the water supply. Four of these children carried high numbers of *Strep. mutans* (3 with $>10^5$ c.f.u. and 1 with $6 \times 10^6$ c.f.u. per ml saliva) but had no detectable caries, an observation consistent with earlier findings from fluoride areas (de Stoppelaar, van Houte and Backer Dirks, 1969). It is interesting that the child with caries but with no detectable *Strep. mutans* had no detectable lactobacilli either but had a high and frequent sucrose intake. The lesions were located in the tooth fissures and other acid-producing organisms than *Strep. mutans* and lactobacilli had probably caused them. In animals, fissure caries can develop after a high sucrose challenge without a *Strep. mutans* flora (Tanzer, 1979).

Lactobacilli were found in 40 per cent of the children and more were found in children with caries. In several children no lactobacilli at all were detected but caries was still present. Thus, the role of lactobacilli is uncertain, as the presence of this organism was not studied longitudinally. In general, lactobacilli are associated with the presence and progression of carious lesions (van Houte *et al.*, 1972; Ikeda, Sandham and Bradley, 1973, Edwardsson, 1974), but certain strains of lactobacilli can produce dental caries in animals (Fitzgerald *et al.*, 1980). In some human studies, lactobacilli have been associated with early enamel lesions (Loesche and Straffon, 1979; van Houte, Aasenden and Peebles, 1981).

Lactobacilli were present in equal numbers of control and test children and there was no correlation between the presence of lactobacilli in the children and high salivary levels of lactobacilli in their mothers. It is generally assumed that a high lactobacillus count is associated with a high carbohydrate intake and with an increased risk of developing caries (Jay, 1974; Krasse and Edwardsson, 1965; Krasse, 1978; Crossner, 1981). A high level of lactobacilli in a mother might therefore reflect caries-conductive dietary habits of the family. Thus, the presence of lactobacilli in the child does not seem to be related to the salivary source in the mother, as was found with *Strep. mutans*, but rather to the presence of carious lesions in the child. This conclusion is supported by the high lactobacillus counts in children with caries.

The results support the infectious and transmissible nature of dental caries. This implies that a caries preventive programme should include measures to control the infection.

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