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EFFECT OF CARIES PREVENTIVE MEASURES IN CHILDREN HIGHLY INFECTED WITH THE BACTERIUM *STREPTOCOCCUS MUTANS*

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Summary—The number of *Strep. mutans* in saliva samples was examined in 101 13–14-year-old children, 53 in a control and 48 in the test group. All in the test group with 2.5×10^5 *Strep. mutans* per ml saliva were treated with 1 per cent chlorhexidine gel, once a day, for 14 days when the number of *Strep. mutans* was greatly reduced. Saliva samples were then examined in the test group every 4th month and all children with *Strep. mutans* levels above 2.5×10^5 were treated. A few selected children had fissure sealants applied to the occlusal surfaces. After 3 years, the mean number of new carious lesions was 9.6 in the control group and 4.2 in the test group. In the children with 10^6 *Strep. mutans* at the start of the study, the corresponding figures were 20.8 compared with 3.9. Thus a reduction in caries activity can be achieved by controlled antimicrobial treatment.

INTRODUCTION

Streptococcus mutans plays an important role in the initial development of dental caries (reviewed by Hamada and Slade, 1980). As subjects highly infected with *Strep. mutans* develop more caries than those with a low level of this microorganism (Klock and Krasse, 1978; Loesche and Straffon, 1979; Köhler, Pettersson and Bratthall, 1981) the prevalence of *Strep. mutans* can be used to select individuals for prophylactic and antimicrobial treatment. Attempts have been made to control the caries activity in children by weekly topical application of vancomycin and a reduction in the number of *Strep. mutans* and caries increment was observed (De Paola, Jordan and Soparkar, 1977; Jordan and De Paola, 1977). In children highly infected with *Strep. mutans*, a significant caries reduction was obtained by means of non-specific preventive measures (Klock and Krasse, 1978). By the use of chlorhexidine, *Strep. mutans* can be suppressed in dental plaque and saliva (Emilson and Fornell, 1976; Emilson, Krasse and Westergren, 1976; Schiött, Briner and Löe, 1976) and treatment with chlorhexidine gel can cause a long-lasting reduction of *Strep. mutans* in man (Emilson, 1978a, 1981). We have investigated the effect of repeated treatment with chlorhexidine gel on caries activity in teenagers using *Strep. mutans* as an indicator.

MATERIALS AND METHODS

Subjects

All children in 4 classes of the 7th grade in an elementary school of the town of Mölndal, Sweden, participated. Altogether there were 101 children, 55 girls and 46 boys, aged 13–14 years. The annual treatment was completed by one of the authors just before the start of the study. All carious lesions had been restored and defective fillings adjusted. The treatment also included oral hygiene instructions, dietary advice, careful tooth-cleaning and topical application of a fluoride varnish (Duraphat®, Woelm, Eshwege, W. Germany). The children were informed about the role of *Strep. mutans* and lactobacilli in the development of dental caries.

Clinical examination

The study started in the spring of 1977 and lasted for 3 years. At the baseline examination and after 1, 2 and 3 years, the children were examined clinically and radiographically. Plaque and gingivitis were examined in a manner identical to that described by Axelsson and Lindhe (1975) and were expressed as the percentage of erupted permanent tooth surfaces harbouring plaque and the percentage of inflamed units in relation to the total number of gingival units.

Dental caries was recorded after careful professional dental cleaning. Criteria described by Gustafsson *et al.* (1954) were used to identify carious lesions. In addition to macroscopic defects (clinical caries), caries without defect (incipient smooth-surface lesions, initial caries) was diagnosed on visible (buccal and lingual) surfaces. Caries without defect was defined as demineralized spots of enamel that had lost their normal translucence and assumed a chalk-like consistency. X-ray detectable caries was diagnosed as well-defined areas of the approximal surfaces in the X-ray film which could not be verified as cavities on inspection with a mirror and probe. At each examination, four posterior bite-wing radiographs were available. Clinical as well as radiographic examination was performed by two dentists working independently. Their individual observations were compared and a joint diagnostic decision was made. At the clinical examination, one of the dentists did not know the group affiliation of the children and at the radiographic analyses neither of the examiners knew to which group the children belonged.

Enlargements of existing lesions were also registered according to special criteria. Enlargement was registered if (1) clinically-registered incipient carious lesions had developed into a macroscopic defect and

(2) radiographically-registered carious lesions had increased in size from one stage to one of the following: (a) more than half the enamel thickness, (b) into the dentine and (c) more than half the thickness of dentine. Caries was recorded as approximal, buccolingual and occlusal caries and recurrent caries. Unerupted and extracted permanent teeth were also noted.

Saliva collection and bacteriological examination

At each sampling, the children were examined with regard to the number of *Strep. mutans* and lactobacilli per ml saliva. Samples were collected between 0800 and 1000 h. Saliva was collected in a cup for 5 min after stimulation by paraffin-wax chewing. One millilitre of saliva was transferred to a bottle containing 6 ml VMG II transport medium (Möller, 1966), transported to the laboratory and cultivated within 3 h.

For the quantitative estimation of *Strep. mutans* and lactobacilli, a micromethod was used (Westergren and Krasse, 1978). The samples were dispersed on a Whirlimixer (Fison Scientific, Loughborough, England) for 60 s and further diluted in 10-fold steps in 0.05 M phosphate buffer (pH 7.3). Duplicate samples of 25 μl of the appropriate dilutions were placed on the surface of mitis-salivarius bacitracin (MSB) agar (Gold, Jordan and van Houte, 1973), which is selective for *Strep. mutans*, and in Rogosa selective lactobacillus (SL) agar (Difco). The MSB agar plates were incubated for 48 h at 37 C in an atmosphere of 95 per cent N_2 and 5 per cent CO_2 and the Rogosa SL agar plates were incubated aerobically for 72 h at 37 C. Counts were made of colonies with morphological characteristics of *Strep. mutans* (Krasse, 1966; Carlsson, 1967; Edwardsson, 1970) on the MSB agar and of colonies exhibiting the typical morphology of lactobacilli on Rogosa SL agar. Different colonies on MSB were isolated and biochemically tested according to the scheme of Shklair and Keene (1974). Duplicate samples were taken at baseline with an interval on one week and the mean numbers of colony-forming units (c.f.u.) of *Strep. mutans* and lactobacilli were calculated. At subsequent collections, only single saliva samples were taken. The children were informed about the results of the bacteriological examinations.

Design of the experiment and treatment procedures

Two of the 4 classes were randomly assigned to either a control (53 children) or a test (48 children) group. Based on the number of *Strep. mutans* per ml saliva at the start of the study, the children in each group were divided into 3 subgroups (Table 1). Six and four children were lost in the control and test groups, respectively, during the 3 years owing to their having moved to other towns.

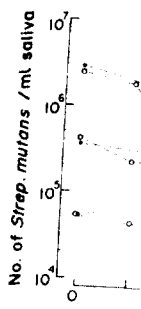
In the test group, the children with a average of $>2.5 \times 10^5$ *Strep. mutans* per ml saliva were selected for treatment, in order to eliminate or reduce the population of *Strep. mutans* to low values. Written consent was obtained from the parents of each of these children after giving them individual information about the study. After careful tooth cleaning, a gel (pH 7.2) containing 1 per cent chlorhexidine digluconate (ICI Ltd, England), 2 per cent methyl-cellulose and flavouring agents was applied to the teeth for 5 min by means of individually-designed applicators formed of thermoplastic polysterol (Drufo®) over models made from alginate impressions of the jaws. After instruction, this treatment was then performed at home once a day for 14 days. New saliva samples were taken 2–3 days after completed gel treatment, in order to determine whether a satisfactory drop in the *Strep. mutans* count had been obtained. When the number of *Strep. mutans* was reduced below 2.5×10^5 c.f.u. per ml saliva in the test children, fissure sealing of all unfilled sound occlusal surfaces of premolars and molars were carried out, in order to avoid the occlusal fissures acting as a source of *Strep. mutans* re-infection. The mixed sealant (Concise Enamel Bond System®, 3M Colo., U.S.A.) was applied but, only on this occasion, at the start of the study.

Saliva samples were then taken every 4th month, during the 3 years, from all children in the test group. The children who had $>2.5 \times 10^5$ *Strep. mutans* per ml saliva on each sampling occasion were treated with chlorhexidine gel at home for 14 days and the effect on the *Strep. mutans* population was checked by taking new saliva samples. The salivary *Strep. mutans* level in the control children was determined at 6-month intervals. All children were reminded at each sampling time of the importance of good dietary and oral hygiene habits. Throughout the study, all chil-

Table 1. Distribution of children during the 3-year study in the control (C) and test (T) groups according to the *Strep. mutans* level in saliva at the start of the study

Strep. mutans per ml saliva	Year 0		1		2		3	
	C	T	C	T	C	T	C	T
$<2.5 \times 10^5$	27	22	27	22	26	22	26	21
$\geq 2.5 \times 10^5$ – $<10^6$	15	16	15	16	14	16	13	14
$\geq 10^6$	11	10	11	10	9	9	8	9
Total	53	48	53	48	49	47	47	44
Median $\times 10^5$	2.5	2.2						
Range $\times 10^5$	ND–132.0	ND–61.3						

ND, not detected ($<10^3$ c.f.u./ml saliva).



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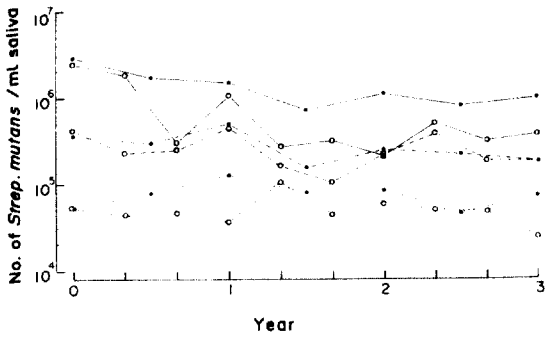

Fig. 1. Log median number of *Strep. mutans* per ml saliva in the control group sampled every 6th month (●) and in the test group sampled every 4th month (○) in children with *Strep. mutans* counts of $< 2.5 \times 10^5$ (. . .), $\geq 2.5 \times 10^5$ $< 10^6$ (---) and $\geq 10^6$ ( ) at the start of the study.

dren rinsed with an 0.2 per cent sodium fluoride solution, once a fortnight, during the school terms.

Statistical analysis

Differences in the clinical and bacteriological variables were analysed using Student's *t*-test. Because of the great variability of the microbial data, these values were first transformed to $\log^{10}$, in order to stabilize the variance.

RESULTS

At the start of the study, there were no statistically significant differences between the control and test subgroups in the number of *Strep. mutans* per ml of saliva (Fig. 1). The pre-experimental caries data are presented in Table 2. There were no statistically significant differences between the control and test groups in the number of decayed and filled surfaces or surfaces at risk.

The number of chlorhexidine treatments per child performed during the 3 years is shown in Table 3. In the first year, 34 children were treated. The number of children needing chlorhexidine treatment decreased with time. During the whole period, 9 children were untreated, 6 were treated once, 9 were treated 2–3 times and 20 4–9 times.

A total of 162 chlorhexidine treatments were performed during the 3-year experimental period in the test group and the effects on *Strep. mutans* and lactobacillus counts are shown in Table 4. The median value of the *Strep. mutans* amount decreased from 7.5×10^5 c.f.u./ml before treatment to 0.2×10^5 a few days after. No effect of the chlorhexidine treatment could be seen on the lactobacillus count in saliva.

Table 3. Distribution of children according to the number of chlorhexidine treatments during the 3 years of the experiment

No. of treatments	Year 1	2	3	0–3
0	14	22	16	9
1	12	10	15	6
2–3	22	15	13	9
4–6				13
7–9				7
Total	48	47	44	44

Table 4. Number of *Strep. mutans* and lactobacilli in saliva ($\times 10^5$) before and after all chlorhexidine treatments ($n = 162$), when all treatments during the 3-year experimental period are summarized

	Before	After
Strep. mutans		
Median	7.5	0.2
Range	2.5–210.0	ND–2.4
Mean ± SD	16.1 ± 26.4	0.4 ± 0.6
Lactobacilli		
Median	0.1	0.1
Range	ND–19.2	ND–8.8
Mean ± SD	0.9 ± 2.4	0.7 ± 1.5

ND, not detected ($< 10^3$ c.f.u./ml saliva).

Table 2. Number of decayed and filled, extracted and unerupted tooth surfaces in the control ($n = 53$) and test ($n = 48$) groups at the start of the study. Mean and standard deviation are given

Tooth surfaces	C	T	*p*
Decayed			
Approximal*	3.5 ± 6.8	4.9 ± 3.7	NS
Bucc–Ling†	5.8 ± 6.6	6.2 ± 5.3	NS
Filled	11.9 ± 7.1	13.8 ± 9.3	NS
Extracted	3.3 ± 6.5	2.6 ± 4.9	NS
Unerupted	4.4 ± 12.2	3.8 ± 8.7	NS
Surfaces at risk	99.1 ± 17.8	96.7 ± 14.9	NS

* Caries diagnosed radiographically.
† Incipient smooth surface lesions on buccal and lingual surfaces.
NS, non-significant.

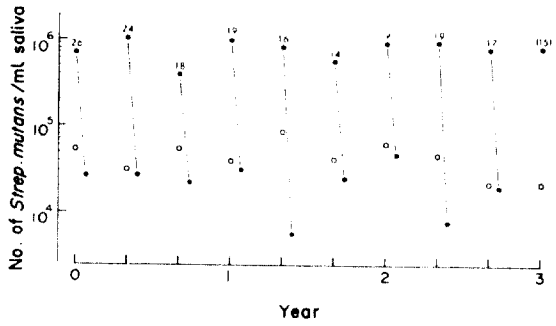

Fig. 2. Log median number of *Strep. mutans* per ml saliva in the test group at each sampling. Results are presented for children before and after chlorhexidine treatment (●, number of children denoted) and for untreated children (○).

The *Strep mutans* population was clearly reduced in the test children with $>10^6$ c.f.u. per ml saliva at the start of the study (Fig. 1). These highly infected children were on average treated on 6 of the 9 possible occasions. The effect on *Strep. mutans* in the other groups was less obvious. The children with low values at the start of the study were on average treated 1.5 times, and those in the middle group 5 times. In the control groups, there were small variations in the *Strep. mutans* population among the children. The effect of the chlorhexidine regimen on the number of *Strep. mutans* in those children treated in each period is shown in Fig. 2. After each treatment, the *Strep. mutans* population was reduced to below the threshold level of 2.5×10^5 c.f.u. ml but increased again in many children after treatment. At the start of the study 26 children were treated but during the 3-year period the number of children who needed treatment decreased. On one occasion, only 9 children had to be treated. At the end of the study, the median value of the *Strep. mutans* count in children of the highly infected group was 0.37×10^6 c.f.u./ml saliva compared to 2.4×10^6 c.f.u./ml at the start of the study (Fig. 1). In the control group, the corresponding values were 1.0 and 2.9×10^6.

The mean number of lactobacilli was examined in saliva among children in the control and test groups who had different salivary numbers of *Strep. mutans* at the start of the study. A tendency towards higher lactobacillus counts with an increased *Strep. mutans* population was observed, but there were no statistically significant differences between the control and test groups at the baseline or during the study in the lactobacillus population in the three *Strep. mutans* classes.

Table 5 shows the mean number of decayed tooth surfaces and Table 6 the number of enlargements of existing carious lesions in relation to the *Strep. mutans* count at the start of the study, for children who completed 1, 2 and 3 years, respectively and in Figs 3 and 4 for those who completed the whole experiment. There was a statistically significant difference between the control and test groups in the total number of new carious lesions ($p < 0.001$) and enlargements ($p < 0.05$) during the 3 years. The difference between the two groups is most obvious in the children with $>10^6$ *Strep. mutans* per ml saliva at the start of the study. In the control group, these children showed a mean number of 20.8 new decayed surfaces, whereas the children in the test group had 3.9 new decayed surfaces. This difference is highly significant ($p < 0.001$). The best treatment effect was seen on the bucco-lingual surfaces, where only 0.6 new carious lesions developed during the 3 years, compared to 7.4 in the control group. A similar numerical difference was seen for approximal surfaces. In the test group, children with more than 2.5×10^5 *Strep. mutans* per ml saliva at the start of the study developed no occlusal lesions during the first 2 years of the trial, probably as a consequence of the fissure sealing. The difference in the total number of enlarged carious lesions in the highest *Strep. mutans* class (9.4 enlargements in the control group and 2.3 in the test group) approached statistical significance ($p < 0.10$), and the difference in the middle *Strep. mutans* class (3.9 versus 1.1) was significant ($p < 0.05$).

Plaque and gingival conditions were examined annually, about 3 months after the last chlorhexidine treatment. No significant differences were observed between the control and test groups.

DISCUSSION

Our findings clearly show that caries activity can be controlled in children with high *Strep. mutans* counts by the use of chlorhexidine. The effect was especially pronounced in those children who initially had high numbers of *Strep. mutans*, in whom caries reduction of about 80 per cent was achieved compared to children in the control group (Fig. 3).

In addition to the chlorhexidine treatment, some of the children in the test group had their fissures sealed at the start of the study. This measure seemed to have

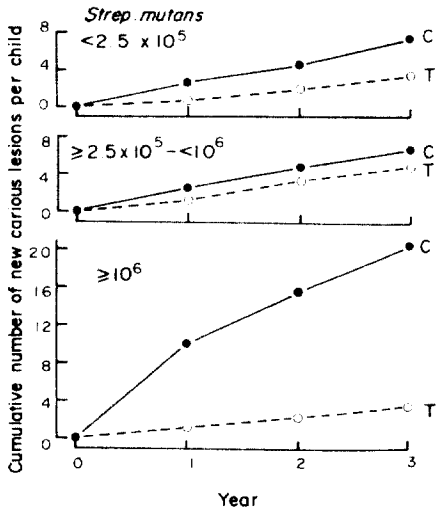

Fig. 3. Cumulative mean number of new carious lesions per child in the control (●) and test (○) groups in relation to the number of *Strep. mutans* at the start of the study. Only children who completed the 3-year experimental study are included.

Table 5. Number of new clinical and radiological carious lesions (mean ± SD) in relation to the number of *Strep. mutans* per ml saliva at the start of the study. Results are presented for children who completed 1, 2 and 3 years of the study, respectively, and the whole experimental period (0–3)

		1		2		3		0–3	
Strep. mutans	Surfaces	C	T	C	T	C	T	C	T
$<2.5 \times 10^5$	Total	2.4 ± 2.9	1.1 ± 1.6**	1.9 ± 2.0	1.2 ± 1.3	2.9 ± 4.4	1.6 ± 1.9	7.4 ± 7.2	3.6 ± 2.9
	Approximal	0.9 ± 1.5	0.6 ± 1.2	0.7 ± 0.9	0.8 ± 1.1	1.7 ± 2.1	1.2 ± 1.6	3.4 ± 3.3	2.4 ± 2.5
	Bucc–Ling	0.7 ± 1.0	0.3 ± 0.6	1.2 ± 1.4	0.4 ± 0.7	0.6 ± 1.1	0.1 ± 0.5	2.5 ± 2.0	0.7 ± 0.9
	Occlusal	0.6 ± 1.1	0.2 ± 0.5	0	0	0.2 ± 0.5	0	0.7 ± 1.1	0.2 ± 0.5
	Recurrent	0.3 ± 0.9	0.1 ± 0.2	0	0	0.5 ± 1.8	0.2 ± 0.4	0.8 ± 2.6	0.2 ± 0.4
$\geq 2.5 \times 10^5 - <10^6$	Total	2.6 ± 2.4	1.6 ± 2.3	2.2 ± 2.1	2.3 ± 2.5	2.1 ± 2.7	1.4 ± 2.9	7.1 ± 5.2	5.2 ± 6.9
	Approximal	1.3 ± 1.6	0.9 ± 1.5	0.9 ± 1.0	1.1 ± 1.5	0.9 ± 1.4	1.1 ± 1.9	3.0 ± 2.9	2.9 ± 4.5
	Bucc–Ling	0.8 ± 1.4	0.8 ± 1.4	1.3 ± 1.5	1.1 ± 1.5	1.0 ± 1.2	0.3 ± 0.7	3.3 ± 2.6	2.1 ± 2.8
	Occlusal	0.3 ± 0.6	0	0	0	0.2 ± 0.6	0	0.5 ± 0.8	0.1 ± 0.5
	Recurrent	0.3 ± 0.6	0	0.1 ± 0.3	0.1 ± 0.3	0	0	0.2 ± 0.6	0.1 ± 0.3
$\geq 10^6$	Total	8.8 ± 8.7	1.7 ± 1.7***	5.1 ± 3.4	0.9 ± 1.4****	4.9 ± 3.2	1.6 ± 1.7***	20.8 ± 9.9	3.9 ± 3.7****
	Approximal	3.4 ± 5.4	1.2 ± 1.0	1.8 ± 1.6	0.7 ± 1.1	2.9 ± 2.0	1.2 ± 1.7	9.3 ± 7.0	3.0 ± 3.6**
	Bucc–Ling	2.9 ± 3.4	0.4 ± 0.7**	2.7 ± 1.9	0.2 ± 0.4****	1.6 ± 2.1	0	7.4 ± 3.0	0.6 ± 1.0****
	Occlusal	1.7 ± 1.7	0	0.2 ± 0.4	0	0.3 ± 0.7	0.2 ± 0.4	2.6 ± 2.0	0.2 ± 0.4****
	Recurrent	0.8 ± 1.0	0.1 ± 0.3**	0.4 ± 0.7	0	0.1 ± 0.4	0.1 ± 0.3	1.5 ± 1.7	0.1 ± 0.3**
ND–$>10^6$	Total	3.8 ± 5.2	1.4 ± 1.9**	2.6 ± 2.6	1.5 ± 1.9***	3.0 ± 3.9	1.5 ± 2.2***	9.6 ± 8.8	4.2 ± 4.6****

Significance of differences between control and test groups. $^{**}p < 0.05$, $^{***}p < 0.01$, $^{****}p < 0.001$.

Table 6. Number of enlarged carious lesions (mean ± SD), in relation to the number of *Strep. mutans* per ml saliva at the start of the study. Results are presented for children who completed 1, 2 and 3 years of the study respectively and the whole experimental period (0–3)

Strep. mutans		1 C	1 T	2 C	2 T	3 C	3 T	0–3 C	0–3 T
$<2.5 \times 10^5$	Total	1.1 ± 3.4	0.8 ± 1.4	0.5 ± 1.0	0.6 ± 1.3	1.0 ± 1.6	1.0 ± 1.1	2.6 ± 5.6	2.3 ± 3.2
	Approximal	0.7 ± 1.8	0.8 ± 1.4	0.5 ± 1.0	0.6 ± 1.3	0.9 ± 1.4	1.0 ± 1.1	2.1 ± 3.9	2.3 ± 3.2
	Bucc–Ling	0.4 ± 1.7	0	0	0	0.1 ± 0.3	0	0.5 ± 2.0	0
$\geq 2.5 \times 10^5 - <10^6$	Total	1.2 ± 1.7	0.3 ± 0.5**	1.1 ± 1.8	0.3 ± 0.5*	1.2 ± 1.4	0.7 ± 1.0	3.8 ± 4.1	1.1 ± 1.5**
	Approximal	1.1 ± 1.8	0.3 ± 0.5*	1.1 ± 1.8	0.3 ± 0.5*	0.9 ± 1.1	0.6 ± 1.0	3.3 ± 3.9	1.1 ± 1.5**
	Bucc–Ling	0.1 ± 0.3	0	0.1 ± 0.3	0	0.4 ± 0.8	0.1 ± 0.3	0.5 ± 1.1	0.1 ± 0.3**
$\geq 10^6$	Total	2.1 ± 2.8	1.0 ± 1.1	3.0 ± 3.4	0.2 ± 0.4**	3.6 ± 3.8	1.2 ± 1.6*	9.4 ± 10.0	2.3 ± 2.7*
	Approximal	1.7 ± 2.4	1.0 ± 1.1	2.8 ± 3.2	0.2 ± 0.4**	3.1 ± 3.2	1.2 ± 1.6	8.1 ± 8.5	2.3 ± 2.7*
	Bucc–Ling	0.4 ± 0.7	0	0.2 ± 0.4	0	0.5 ± 0.8	0	1.3 ± 1.8	0
ND–$>10^6$	Total	1.3 ± 2.9	0.7 ± 1.1	1.1 ± 2.0	0.4 ± 0.9	1.5 ± 2.2	0.9 ± 1.2	4.1 ± 6.6	2.0 ± 2.7**

Significance of differences between control and test groups $*p < 0.10$, $**p < 0.05$.

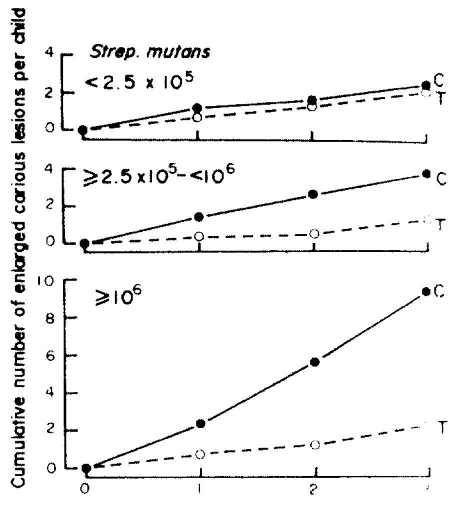

Fig. 4. Cumulative mean number of enlarged carious lesions per child in the control (●) and test (○) groups in relation to the number of *Strep. mutans* at the start of the study. Only children who completed the 3-year experimental period are included.

only marginal influence on caries activity. Between the test and control group, there was a mean difference of 0.8 occlusal cavities for the 3 years, compared to the total mean difference of 5.4 decayed surfaces (Table 5).

Children in the control group with $>2.5 \times 10^5$ *Strep. mutans* per ml saliva did not receive treatment with a placebo gel. It is known, however, that such treatment has a very limited effect on the *Strep. mutans* population (Emilson, 1981).

There was a tendency for a drop in the lactobacillus count to occur especially among children in the control group. Thus, changes in the dietary habits of the groups cannot explain the differences in caries activity between the groups.

As the difference in treatment between the control and test group seems to have only a minor effect on caries development, it is reasonable to ascribe the low caries activity among the test children to chlorhexidine treatment. *Strep. mutans* was used for selection of the children for treatment and for control of the treatment results. Consequently, the effect of the chlorhexidine treatment using *Strep. mutans* as an indicator must be the main reason why such a significant caries-inhibiting effect was obtained.

In most untreated children, the number of *Strep. mutans* was stable throughout the study. However, one of the children in the control group had a value of 2.48×10^5 *Strep. mutans* per ml saliva at the start of the study, i.e. a value which was just below the threshold level. This boy showed considerably higher values of *Strep. mutans* on all the following sampling ocassions and was the child who developed most caries during the study (36 new carious lesions and 28 enlargements), thus contributing markedly to the mean number of new lesions in the control group. If he had been placed in the control group with *Strep. mutans* values between $>2.5 \times 10^5$ and $<10^6$, the dif-

Table 6. Number of enlarged carious lesions (mean ± SD), in relation to the number of *Strep. mutans* per ml saliva at the start of the study. Results are presented for children who completed 1, 2 and 3 years of the study respectively and the whole experimental period (0–3)

Strep. mutans		1 C	1 T	2 C	2 T	3 C	3 T	0–3 C	0–3 T
$< 2.5 \pm 10^5$	Total	1.1 ± 3.4	0.8 ± 1.4	0.5 ± 1.0	0.6 ± 1.3	1.0 ± 1.6	1.0 ± 1.1	2.6 ± 5.6	2.3 ± 3.2
	Approximal	0.7 ± 1.8	0.8 ± 1.4	0.5 ± 1.0	0.6 ± 1.3	0.9 ± 1.4	1.0 ± 1.1	2.1 ± 3.9	2.3 ± 3.2
	Bucc-Ling	0.4 ± 1.7	0	0	0	0.1 ± 0.3	0	0.5 ± 2.0	0
$\geq 2.5 \times 10^5 - < 10^6$	Total	1.2 ± 1.7	0.3 ± 0.5**	1.1 ± 1.8	0.3 ± 0.5*	1.2 ± 1.4	0.7 ± 1.0	3.8 ± 4.1	1.1 ± 1.5**
	Approximal	1.1 ± 1.8	0.3 ± 0.5*	1.1 ± 1.8	0.3 ± 0.5*	0.9 ± 1.1	0.6 ± 1.0	3.3 ± 3.9	1.1 ± 1.5**
	Bucc-Ling	0.1 ± 0.3	0	0.1 ± 0.3	0	0.4 ± 0.8	0.1 ± 0.3	0.5 ± 1.1	0.1 ± 0.3**
$\geq 10^6$	Total	2.1 ± 2.8	1.0 ± 1.1	3.0 ± 3.4	0.2 ± 0.4**	3.6 ± 3.8	1.2 ± 1.6*	9.4 ± 10.0	2.3 ± 2.7*
	Approximal	1.7 ± 2.4	1.0 ± 1.1	2.8 ± 3.2	0.2 ± 0.4**	3.1 ± 3.2	1.2 ± 1.6	8.1 ± 8.5	2.3 ± 2.7*
	Bucc Ling	0.4 ± 0.7	0	0.2 ± 0.4	0	0.5 ± 0.8	0	1.3 ± 1.8	0
ND–$> 10^6$	Total	1.3 ± 2.9	0.7 ± 1.1	1.1 ± 2.0	0.4 ± 0.9	1.5 ± 2.2	0.9 ± 1.2	4.1 ± 6.6	2.0 ± 2.7**

Significance of differences between control and test groups $*p < 0.10$, $**p < 0.05$.

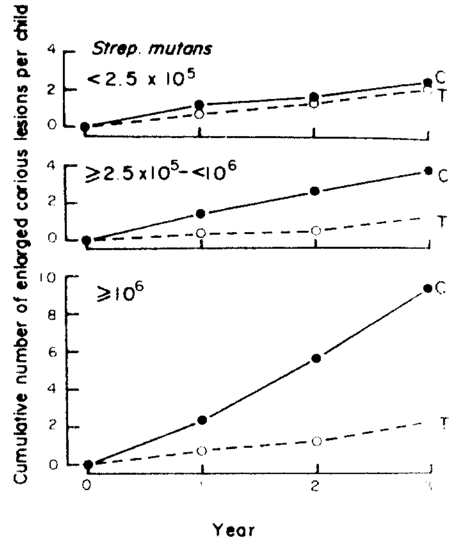

Fig. 4. Cumulative mean number of enlarged carious lesions per child in the control (●) and test (○) groups in relation to the number of *Strep. mutans* at the start of the study. Only children who completed the 3-year experimental period are included.

only marginal influence on caries activity. Between the test and control group, there was a mean difference of 0.8 occlusal cavities for the 3 years, compared to the total mean difference of 5.4 decayed surfaces (Table 5).

Children in the control group with $> 2.5 \times 10^5$ *Strep. mutans* per ml saliva did not receive treatment with a placebo gel. It is known, however, that such treatment has a very limited effect on the *Strep. mutans* population (Emilson, 1981).

There was a tendency for a drop in the lactobacillus count to occur especially among children in the control group. Thus, changes in the dietary habits of the groups cannot explain the differences in caries activity between the groups.

As the difference in treatment between the control and test group seems to have only a minor effect on caries development, it is reasonable to ascribe the low caries activity among the test children to chlorhexidine treatment. *Strep. mutans* was used for selection of the children for treatment and for control of the treatment results. Consequently, the effect of the chlorhexidine treatment using *Strep. mutans* as an indicator must be the main reason why such a significant caries-inhibiting effect was obtained.

In most untreated children, the number of *Strep. mutans* was stable throughout the study. However, one of the children in the control group had a value of 2.48×10^5 *Strep. mutans* per ml saliva at the start of the study, i.e. a value which was just below the threshold level. This boy showed considerably higher values of *Strep. mutans* on all the following sampling ocassions and was the child who developed most caries during the study (36 new carious lesions and 28 enlargements), thus contributing markedly to the mean number of new lesions in the control group. If he had been placed in the control group with *Strep. mutans* values between $> 2.5 \times 10^5$ and $< 10^6$, the dif-

ference compared to the corresponding test group in new carious lesions would have been statistically significant ($p < 0.05$). Furthermore, the gradient of new decayed surfaces from the low *Strep. mutans* group to the groups with higher numbers would have been more obvious. This illustrates what might happen in an individual case when a borderline value from a baseline examination is used for grouping the subjects. In the test group, this influence on the ensuing caries activity was eliminated, as the children were frequently examined for *Strep. mutans* and treated according to need.

The difference in caries activity was mainly due to the new lesions which developed on approximal and bucco-lingual surfaces. The chlorhexidine treatment also had an effect on the enlargement of existing caries lesions which was unexpected because growth of the lesion is generally associated with the lactobacilli (Ikeda, Sandham and Bradley, 1973; Edwardsson, 1974) and no significant drop in the lactobacillus count in saliva was observed in the test group. One explanation might be that the chlorhexidine treatment had an effect on the bacteria in the deeper parts of the lesion. Chlorhexidine has a broad antimicrobial spectrum and is effective against most oral microorganisms (Emilson, 1977b), but we only monitored the effect on *Strep. mutans* and lactobacilli.

No significant differences between the control and test group were observed with regard to plaque and gingival conditions. This might partly be explained by the fact that the indices for these conditions were recorded about 3 months after the chlorhexidine treatment. Thus, a short-term effect of the treatment cannot be excluded.

After treatment with chlorhexidine gel for 14 days, it takes about 3–4 months for the number of *Strep. mutans* to return to pre-treatment levels (Emilson, 1977a, 1981). An interval of 4 months was therefore allowed between the samplings. There was great variation among the treated children; some had to be treated on every occasion; others were treated only once or a few times during the 3 years of the experiment. However, the number of subjects needing treatment on each occasion decreased during the study. This variation in treatment need may be at least partly explained by variations in the sucrose intake. In hamsters, the low proportion of *Strep. mutans* in plaque achieved by chlorhexidine treatment persists when they are given a sucrose-free diet (Emilson and Westergren, 1979).

Reduction in the number of *Strep mutans* for a period of 3–4 months (Fig. 1) and the postulated effect of chlorhexidine treatment on other bacteria may have given early enamel lesions a chance to re-mineralize and retard the development of other lesions. Such a period of rest, repeated a few times a year, is apparently of great importance in children exposed to a heavy cariogenic challenge. This might in turn explain why the effect of chlorhexidine treatment was especially pronounced in children with one million or more *Strep. mutans* per ml saliva at the start of the study. Furthermore, these children, about 20 per cent of the control group, needed most treatment. In the Swedish School Dental Service it is well recognized that a small proportion of children take up a large part of the treatment resources (Sellman, Syrrist and Gustafsson, 1957). As shown here, such children can be selected at the age of 13–14 yrs on the basis of the *Strep. mutans* count. By antimicrobial treatment, properly controlled, a remarkable reduction of the caries activity can be achieved. The results illustrate the value of such treatment when dietary counselling or other preventive efforts have failed to suppress and keep the *Strep. mutans* population low.

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