Effects of repeated scaling and root planing and/or controlled oral hygiene on the periodontal attachment level and pocket depths in beagle dogs

II. Bacteriological findings

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A study was performed to evaluate the effect of initial and/or repeated scaling with or without controlled oral hygiene on the level of periodontal attachment and pocket depth in beagle dogs. The clinical results of this three-year longitudinal study have been reported previously (Morrison et al. 1979). The purpose of the present report is to present the microbiological results of subgingival plaque samples obtained from selected sites at the conclusion of the study.

Eight beagle dogs with moderately developed periodontitis were divided into experimental and control animals. The experimental group received a thorough scaling and root planing at the start of the experiment following which the animals were subjected to daily toothbrushing and rubber cup and pumice prophylaxis every second week for 36 months. Four control dogs were not subjected to any oral hygiene procedures for the entire period of the study. The teeth of two quadrants in each animal of the experimental and control group were scaled and root planed every six months.

After three years subgingival plaques from the mesial aspect of the fourth premolar in each of the quadrants of each animal were collected by sterile curettes, processed anaerobically, and cultured in an anaerobic glove box. Significantly lower total viable colony forming units (CFU) as well as significantly lower anaerobe/aerobe ratios were found in the subgingival plaques of the experimental animals. The total CFU of *Bacteroides asaccharolyticus* was 25 times lower, and the proportion of CFU of this organism was nine times lower in the experimental sites when compared with the control sites. Repeated scaling every six months also lowered the total CFU and the proportion of *Bacteroides asaccharolyticus* in the subgingival plaques of the experimental as well as the control animals. The data suggest that the level of *Bacteroides asaccharolyticus* as key organisms as well as the anaerobe/aerobe ratio are valuable microbiological parameters in evaluating the efficacy of periodontal therapy.

Introduction

Accumulation of microbial plaque on healthy dento-gingival surfaces is followed by gingival inflammation (Löe, Theilade & Jensen 1965). The longitudinal studies in beagle dogs have shown that chronic gingi-

vitis may develop into periodontitis (Lindhe, Hamp & Löe 1975). Microbial organisms might be associated as etiologic agent in the development of the disease process. Bacteriological studies (Syed, Svanberg & Svanberg 1980, 1981) on the predominant flora of supra- and subgingival plaques of beagle dogs have been published recently. These studies suggest that subgingival plaques associated with gingivitis and periodontitis are composed predominantly of asaccharolytic Gram-negative anaerobic bacteria. Gram-positive organisms comprise only a minor proportion of the cultivable flora. In humans, ultrastructural (Listgarten 1976) and cultural studies (Slots 1977, Tanner et al. 1979, Darwish, Hyppa & Socransky 1978) document the close association between Gram-negative anaerobic microorganisms and deep periodontal pockets. Furthermore, recent studies (Spiegel, Minah & Krywolap 1979, Tanner et al. 1979, Loesche et al. 1981) have associated asaccharolytic strains of black pigmented *Bacteroides* with sites of severe periodontitis exhibiting 5 mm or more of pocket depth.

In order to evaluate the effect of initial and/or repeated subgingival scaling with or without controlled oral hygiene on the level of periodontal attachment and pocket depth, a clinical study in beagle dogs was conducted for 3 years (Morrison et al. 1979). At the conclusion of this study plaque samples from selected sites in the experimental and control animals were subjected to microbiological analysis using anaerobic culturing procedures. The results of this comparative study are presented in this report.

Material and Methods

Clinical Design
Eight healthy beagle dogs, 3½ to 6 years of age with moderate naturally developed periodontal disease were divided into experimental and control animals. In the experi-
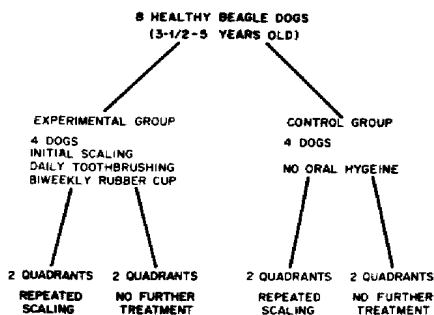

Fig. 1. Experimental design of the performed treatment regimes.

mental group, the teeth of four dogs received a thorough scaling and root planing at the start of the experiment. Thereafter, the experimental dogs were subjected to daily tooth brushing and a rubber cup and pumice prophylaxis every two weeks for 36 months. The remaining four control dogs were *not* subjected to any oral hygiene measures for the entire period of the study. The teeth of two quadrants diagonally opposed in each animal of the experimental and control groups were scaled and root planed every six months (Fig. 1).

At baseline and after 1, 6, 12, 24, and 36 months, clinical parameters for plaque deposits, calculus, gingival health and periodontal attachment levels, and pocket depths were recorded by the same examiner. The clinical findings have been reported previously (Morrison et al. 1979).

Sample Collection and Bacteriological Procedures
Prior to the 36 months clinical examination, microbiological samples were collected from all the dogs which ranged in age from six to nine years at that time. A total of 12 sites in the experimental dogs and 14 sites in the control dogs were sampled. Each sample was collected from the mesial aspect of the maxillary or mandibular fourth pre-

molar. Supragingival plaque was removed from the site by a sterile curette. Following this, a microbiological sample of subgingival plaque was removed separately with a sterile curette. All the specimens were removed by the same investigator. Each plaque sample was immediately placed in a reduced transport fluid (RTF) without EDTA (Syed & Loesche 1972), dispersed for 20 seconds by a Kontes Sonifier, serially diluted in RTF, and plated on an enriched Trypticase soy blood agar (ETSA, Syed 1980) by a Spiral Plater (Spiral Systems Marketing Ltd. Bethesda, Maryland). Aerobic plates were incubated in the field, and anaerobic plates were transported to the laboratory in Gaspack jars (BBL Microbiology Systems, Cockeysville, Maryland), incubated in the anaerobic glove box (Aranki et al. 1969) (Coy Manufacturing Comp., Ann Arbor, Michigan) for six to eight days following which total colony forming units (CFU) and differential counts were determined.

For each sample, at least 150 to 200 CFU were enumerated, differential counts were made where possible, and representative colonies were picked using a stereomicroscope equipped with a zoom lens and an illuminator. The organisms were characterized using taxonomic criteria described earlier (Syed et al. 1980).

The colonies on aerobic ETSA plates were enumerated and anaerobe to aerobe ratios were calculated. ETSA with 5 % sucrose was used to enumerate extracellular polysaccharide forming bacteria, if present. ETSA with .002 % chlorhexidine was used to enumerate chlorhexidine resistant microorganisms including *Bacteroides ochraceus*, (*Capnocytophaga*, Leadbetter et al. 1979), *Streptococcus* species, and gliding organisms. *Actinomyces viscosus*, *Actinomyces naeslundii*, and gelatinase producing organisms were recovered from Gelatin Metronidazole Cadmium (GMC) agar (Kornman & Loesche 1978). Bile esculin agar was used

to determine whether enterococci and other enteric or fecal organisms were present in the samples. In the experimental dogs, the plaque samples were collected three days after the cessation of the tooth brushing and oral hygiene procedures. The same protocol was followed for culturing the samples of the control animals except that there was a delay of three to twelve hours between the collection and the culturing due to the fact that these samples were not processed in the field.

For statistical analysis the Wilcoxon rank sum and Fisher's exact test for independent samples and the Wilcoxon signed rank test for related samples in the same animals were used at $\alpha = 0.05$ (Hollander & Wolfe 1973, Fleiss 1973).

Results

The results of the various clinical parameters measured at the plaque sample collection site are shown in Table 1.

The experimental animals were completely free of gingival inflammation, with no increase in pocket depth or loss of attachment over the previous three years. In the

Table 1

Frequency distributions of clinical parameters at the sites of plaque collection (mesial aspect of maxillary and mandibular fourth premolar)

		Experimental N = 12	Controls N = 14
Gingival Index Scores	0	12	0
	1	0	9
	2	0	5
Pocket depths in mm	1–3	12	3
	4–8	0	11
Loss of attach- ment in mm	0	12	4
	1–3	0	3
	≥ 4	0	7

Table 2

Prevalence of organisms in subgingival plaque of beagle dogs

Organism	Prevalence in experimental and control dogs	Significance ($\alpha = 0.05$) experimental vs. control dogs †
S. sanguis	0*	--
S. mutans	0	--
S. faecalis	0	--
Veillonella sp.	0	--
A. viscosus	+	NS
A. naeslundii	+	NS
Actinomyces sp.	+	NS
Streptococcus sp.	+ + +	--
F. nucleatum	+	NS
B. ochraceus (Capnocytophaga)	+ + +	NS
Other gliding organisms	+ + +	--

*   Undetected + occasional + + + in great numbers

† Fisher's exact test

-- not applicable

control animals the plaque collection sites had gingival inflammation, periodontal pocket depths of 4 to 8 mm, and in 50 % of the sites the loss of 4 mm or more of periodontal attachment (Table 1).

Analysis of the composition of the microbiota showed that the flora was qualitatively similar in both healthy and diseased sites. Some bacterial species were either not found or undetectable in these sites (Table 2). *Actinomyces* species were more frequently detected in control animals. Organisms resistant to chlorhexidine such as gliding organisms and streptococci were found in the plaque of both groups of animals. *Bacteroides asaccharolyticus* which exhibited catalase activity was found in the plaque samples of both groups. When the viable CFU were compared, the median of total CFU in the experimental animal was 10 to 20 times lower than the median of total CFU in the control group (Table 3). A wide range of total CFU was seen in the control sites which received repeated scaling every six months but were not subjected to oral hygiene procedures.

Table 3

Comparison of total viable counts (CFU) and anaerobe/aerobe ratio of subgingival plaque samples from experimental and control dogs

	Experimental Oral hygiene		Controls No oral hygiene		Level of significance p <	
	A. Initial scaling	B. Repeated scaling	C. No scaling	D. Repeated scaling	A vs. C	B vs. D
Total viable CFU × 10⁵ Median (range)	7.5 (0.1–27.9)	5.2 (0.1–57.5)	70.7 (4.6–370.7)	106.8 (1.2–406.0)	0.05	0.05
Anaerobe/aerobe Ratio Median (range)	1.0 (0.6–2.6)	2.6 (1.3–40.3)	15.7 (1.6–50.0)	3.8 (0.3–50.0)	0.05	N.S.

N.S. = not significant

The anaerobe to aerobe ratio (total organisms grown under anaerobic condition/total organisms grown aerobically) in the scaled and unscaled sites in the oral hygiene group was 1.0 and 2.6 respectively (Table 3). The median anaerobe to aerobe ratio for the control sites was 2 to 15 times higher than in the experimental sites. There was a trend of a lower anaerobe to aerobe ratio in the control sites when the sites had been scaled repeatedly every six months.

The median of total CFU of *Bacteroides asaccharolyticus* was 25 times lower in the experimental sites than in the control sites (Table 4). In those control sites which were never subjected to oral hygiene or scaling procedures the median of *Bacteroides asaccharolyticus* CFU was higher when compared to the control sites which were subjected to repeated scaling every six months.

The comparison of the proportion of CFU showed that the median of this organism in the experimental dogs was ninefold lower than in the control sites (Table 4). In the experimental sites which had repeatedly been scaled the proportions of *Bacteroides asaccharolyticus* were lower than in the sites which were not scaled repeatedly.

Discussion

This study has demonstrated that following an initial scaling and root planing in the beagle dog periodontal attachment levels were essentially maintained by controlled oral hygiene. This was documented not only by analyzing all the sites of the experimental and control animals (Morrison et al. 1979) but also by analyzing the selected sites for bacteriological sampling in the present study. Although only relatively few sites were analyzed bacteriologically, the present information may be considered representative for the previously published clinical data (Morrison et al. 1979, Lang et al. 1979).

Since the microbial flora appears to vary from site to site within the same mouth, the results of this study should be interpreted with the perspective that only one predetermined site per quadrant in each dog was sampled.

The quantitative differences in total CFU and in the composition of the subgingival microflora such as proportional changes in *Bacteroides asaccharolyticus* and anaerobe to aerobe ratio appeared to be an interesting result of the periodontal treatment. The

Table 4

Comparison of *Bacteroides asaccharolyticus* counts and proportions in the subgingival plaque of experimental and control dogs

	Experimental Oral hygiene		Controls No oral hygiene		Level of significance $p <$	
	A. Initial scaling	B. Repeated scaling	C. No scaling	D. Repeated scaling	A vs. C	B vs. D
Black-pigmented *Bacteroides* CFU × 10⁵ Median (range)	1.0 (0.0–13.4)	0.4 (0.0–22.4)	25.6 (0.7–185.5)	14.4 (0.4–239.7)	0.05	0.05
Bacteroides asaccharolyticus % of total CFU Median (range)	5.7 (9.0–72.9)	3.8 (0.0–56.5)	45.7 (14.9–84.2)	32.9 (13.5–74.0)	0.05	0.05

cross sectional studies in the beagle dog and in humans (Syed et al. 1980, 1981, Loesche et al. 1981, Spiegel et al. 1979) also indicated a close association between high proportions of Gram-negative anaerobic species such as *Bacteroides asaccharolyticus* and periodontal tissue pathology. Although no cause and effect relationship has been established between these organisms and periodontal lesions, the results of this and other studies strongly suggest that these organisms might be contributing in the disease process. Several possible pathogenic mechanisms have been proposed. Noxious waste products such as organic acids, H_2S and NH_4 (Macdonald & Gibbons 1962), or endotoxin may be released into the underlying connective tissue (Schwartz, Stinson & Parker 1972). Furthermore, there is evidence of antigenic stimulation of peripheral blood lymphocytes by this organism (Baker et al. 1976, Lang & Smith 1977). Even though *Bacteroides asaccharolyticus* may not be the only pathogenic organism of the subgingival periodontal flora, it may represent one of the key organisms in the microflora with pathogenic potential. The fact that *Bacteroides asaccharolyticus* was also found in low proportions (3.8–5.7 %) in healthy sites provides evidence for the opportunistic nature of the periodontal plaque infection. Obviously, a threshold level of the proportion of *Bacteroides asaccharolyticus* is needed for the continuous loss of periodontal attachment. In the present study this level may have been approximately at 15 % since no site in the control animals had less than 13.5 % *Bacteroides asaccharolyticus*.

Scaling every six months did not influence the levels of these indicator organisms *(Bacteroides asaccharolyticus)* or other bacteriological parameters (total CFU, anaerobe/aerobe ratio) when controlled oral hygiene was maintained. In the control animals there was a trend for the total CFU

and the proportions of *Bacteroides asaccharolyticus* to be lower in the quadrants which were repeatedly scaled every six months, indicating that the microbial ecosystem may significantly be changed by repeated scaling procedures (Listgarten, Lindhe & Helldén 1978).

In the present study, the major components of the total subgingival cultivable flora of the control sites were qualitatively very similar to those of the experimental sites. It was composed of anaerobes and facultative organisms which were weakly fermentative or non-fermentative (except for the streptococci and actinomycetes). This is not meant to suggest that there were no differences between the two groups. The total colony forming units, the anaerobe/aerobe ratio, and the number and proportions of *Bacteroides asaccharolyticus* were significantly lower in the plaques of the experimental dogs when compared to the controls documenting the important effect of the treatment rendered.

Since this is a cross-sectional study, the cause and effect relationship of specific organisms in periodontal disease cannot be established. However, the data strongly suggest that the level of *Bacteroides asaccharolyticus* and the anaerobe/aerobe ratio should be monitored in evaluating the oral health status and efficacy of periodontal treatment.

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