Bacteriology of Human Gingivitis

L. V. H. MOORE, W. E. C. MOORE, E. P. CATO, R. M. SMIBERT, J. A. BURMEISTER*, A. M. BEST*, and R. R. RANNEY*

*Department of Anaerobic Microbiology, Virginia Polytechnic Institute and State University, Blacksburg, Virginia 24061; and *Department of Periodontics, School of Dentistry, Virginia Commonwealth University, Richmond, Virginia 23298*

The subgingival bacterial floras of naturally occurring gingivitis in adults and children were characterized and compared with the floras of other periodontal conditions previously studied. The composition of the gingivitis floras was found to be distinct from that of floras associated with health or with moderate, severe, or juvenile periodontitis. There were no major differences between the floras of naturally-occurring gingivitis and the floras of the human experimental gingivitis model. Data indicated that the flora of healthy sites within a mouth is influenced by the number of inflamed sites, which argues against independence of sites bacteriologically. Proportions of ten bacterial species increased in both gingivitis and periodontitis, as compared with health, in both adults and children. These species were found in both affected and unaffected sites of people with gingivitis. The numbers of five other cultivable species and the "large treponeme", which was not cultivated, increased in gingivitis and periodontitis of adults only.

Significant differences in non-spirochetal floras between children and adults were not found, although they were in the experimental gingivitis model studied previously. Cultivable spirochetes did differ between children and adults. Children had fewer samples positive for spirochetes, and children's positive samples contained greater proportions of T. socranskii *subsp.* paredis.

Some species that predominate in periodontitis, but which are absent from healthy gingivae, were found as a small percentage of the flora in gingivitis. This suggests that increased serum and blood in the gingival crevice encourage species that relate to periodontitis.

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Introduction.

The bacterial flora associated with gingivitis has not been as extensively characterized as have floras associated with periodontal health and periodontitis. Previous cross-sectional studies (Slots *et al.*, 1978; van Palenstein Helderman, 1975) reported that several species, predominately Gram-negative, and some of which differed between studies, were increased in number as compared with those in health. A more recent study (Savitt and Socransky, 1984) found the numbers of *Capnocytophaga gingivalis, Bacteroides intermedius, Eikenella corrodens,* and *Fusobacterium nucleatum* to be elevated compared with those in health; *C. gingivalis* was also elevated, compared with periodontitis states. In that study, both selective and non-selective media were used; identification was restricted to selected Gram-negative species. Isolation of spirochetes was not attempted in any of these previous bacteriological studies of naturally-occurring gingivitis.

The most detailed bacteriological data (Loesche and Syed, 1978; Moore *et al.*, 1982b, 1984b; Syed and Loesche, 1978) have been provided from study of the experimental gingivitis model of Löe *et al.* (1965), rather than from naturally-occurring gingivitis associated with plaque of indeterminate age. Among species found to increase in numbers in these studies, several were Gram-positive (actinomyces and streptococci), and there was a somewhat different array of Gram-negative species than had been reported in other studies. The floras in children with experimental gingivitis were found to differ significantly from those in adults (Moore *et al.*, 1984b).

The purpose of the present study was to determine the total cultivable flora associated with naturally-occurring gingivitis in adults and children, and to compare these floras with those previously found in the experimental gingivitis model; in health; and in juvenile, moderate, or severe periodontitis (Moore *et al.*, 1982a, 1982b, 1983, 1984a, 1984b, 1985).

Materials and methods.

Subjects.—Ten children, ages from 4 to 6, and 11 adults, ages from 24 to 34, were selected for bacteriological study to represent people with gingivitis. The Gingival Index (GI) (Löe and Silness, 1963) was used to measure severity of gingival inflammation at four sites per tooth. The amount of plaque was measured at the same sites by the Plaque Index (PII) of Silness and Löe (1964). The selection criterion for gingivitis was an average full-mouth GI equal to or exceeding 0.5 in the adults, and 0.4 in the children. Additionally, no subject had periodontitis (loss of periodontal attachment in the presence of probeable depth exceeding 3 mm), previous periodontal treatment other than scaling, a history of recent or chronic systemic illness, or treatment with antibiotics within the six weeks prior to bacteriological sampling.

Samples.—One or two sites with GI scores of 1 or 2 and one or two sites with GI scores of 0 were sampled from each subject. As described previously (Moore *et al.*, 1982b), each site was isolated with cotton rolls and dried with swabs, and the tooth surface coronal to the gingival margin was cleaned superficially with sterile toothpicks. The sample was taken with a nickel-plated Morse 00 scaler from the depth of the gingival crevice. Samples were transferred immediately to dilution medium containing fine glass beads under oxygen-free CO_2 for dispersion by vortex mixing, serial dilution, and bacteriological culture in roll tubes and on plates. Cultures were incubated for five days at 37° C, after which there was negligible increase in the number of colonies, and more of the colonies produced growth on subculture than did colonies incubated for seven or more days.

After the five days of incubation, 30 isolated colonies were sampled at random, 15 from pre-reduced D4 medium blood agar plates and 15 from pre-reduced anaerobically sterilized D4 medium roll tubes (Moore *et al.*, 1982b). The resulting cultures were characterized by electrophoretic patterns of soluble cellular proteins (Moore *et al.*, 1980), chromatographic analysis of acid products, and standard biochemical tests, as described previously (Holdeman *et al.*, 1977).

Spirochetes were isolated from OTI medium or RPI broth on peptone-fresh yeast extract-serum agar and identified by morphology, fermentation pattern, and biochemical reactions (Moore *et al.*, 1982b).

Data from previous studies.—Bacteriological data previ-

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ously obtained to represent the floras of other periodontal conditions were used for comparison with the gingivitis data generated in this study. Data representing the subgingival flora of periodontal health were obtained from samples of 27 sites with GI = 0 in 16 adults whose full-mouth GI was less than 0.5 and baseline samples of 29 sites with GI scores of 0 in four children who participated in the experimental gingivitis study (Moore *et al.*, 1984b). Corresponding data for other periodontal conditions were from 20 people with localized juvenile periodontitis (Moore *et al.*, 1985), 21 with early onset severe generalized periodontitis (Moore *et al.*, 1982b), 21 with adult periodontitis (Moore *et al.*, 1983), four adults (96 samples) in the experimental gingivitis model (Moore *et al.*, 1982a), and four children (102 samples) in the same model (Moore *et al.*, 1984b).

Data analysis.—The mean GI scores of three subject groups (adults and children with naturally-occurring gingivitis and healthy adult controls) were subjected to ANOVA, and between-group differences were tested by Tukey's HSD test. The percentages of the individual GI scores (0, 1, and 2) for each group were also compared by the Wilcoxon rank sum score and by ANOVA, and the same values for naturally-occurring gingivitis in children and adults combined were compared with those of the healthy controls by a Chi-square test. The compositions of the floras of different people, sites with different GI scores, and experimental gingivitis or naturally-occurring gingivitis were compared statistically by lambda analysis (Good, 1982; Moore *et al.*, 1982b). Because the flora composition of different sites within people is not independent (Moore *et al.*, 1984a), when more than two persons were to be compared, all samples (or sites of a given GI score) from each person were combined to represent the flora of that person.

Results.

Average full-mouth GI and PII scores for the adults and children with naturally-occurring gingivitis and for the healthy adult subjects are presented in Table 1. There were significant differences in GI among the three groups by ANOVA ($P < 0.0001$). Tukey's HSD test showed that GI was significantly greater in the adult than in the child naturally-occurring gingivitis groups, and greater in the children than in the healthy control subjects. Average PII also differed significantly among these three groups ($P < 0.0001$), but it was the children who had the highest plaque scores. The two naturally-occurring gingivitis groups together (adults and children) had higher GI and PII scores than did the healthy adult subjects ($P < 0.0001$). Non-parametric analyses of the proportions of the individual GI scores confirmed these results ($P < 0.0001$). Distributions of the percentages of sites with GI scores of 0, 1, or 2 in these populations are presented in Table 2. In all comparisons, it was clear that the healthy controls had the highest proportion of GI = 0 scores.

TABLE 1
FULL-MOUTH GINGIVAL AND PLAQUE INDICES IN NATURALLY-OCCURRING GINGIVITIS AND CONTROL GROUPS

Group	GI[a] (Mean ± S.E.M.)	PII[b] (Mean ± S.E.M.)
Naturally-occurring Gingivitis		
Adults	0.85 ± 0.10	0.79 ± 0.08
Children	0.58 ± 0.05	1.46 ± 0.10
Healthy Adults	0.36 ± 0.06	0.34 ± 0.04

[a]Löe and Silness, 1963.
[b]Silness and Löe, 1964.

TABLE 2
DISTRIBUTION OF THE PERCENTAGES OF SITES WITH GINGIVAL INDEX SCORES OF 0, 1, OR 2 IN THE NATURALLY-OCCURRING GINGIVITIS AND HEALTHY ADULT POPULATIONS STUDIED

Group (No. of Subjects)	GI Score[a] (Mean ± S. E. M.) % with GI = 0	% with GI = 1	% with GI = 2
Naturally-occurring Gingivitis			
Adults (11)	30.7 ± 4.8	59.3 ± 2.7	10.0 ± 3.7
Children (10)	47.0 ± 4.8	48.2 ± 5.2	4.8 ± 1.5
Healthy Adults (16)	68.5 ± 3.7	29.3 ± 3.4	2.2 ± 0.5

[a]Löe and Silness, 1963.

TABLE 3
DIFFERENCES BETWEEN NATURALLY-OCCURRING GINGIVITIS AND EXPERIMENTAL GINGIVITIS AS ANALYZED (BY PERSON) BY LAMBDA ANALYSIS[a] ($P = 0.99$ when all 21 persons with naturally-occurring gingivitis were compared with all eight persons with experimental gingivitis[b])

Score	Child Natural *vs.* Experimental	Adult Natural *vs.* Experimental
GI 0:	(10) *vs.* (4); P = 0.99	(9) *vs.* (4); P = 0.88
GI 1:	(7) *vs.* (4); P = 0.07	(7) *vs.* (4); P = 0.59
GI 2:	(6) *vs.* (4); P = 0.51	(8) *vs.* (4); P = 0.52
All:	(10) *vs.* (4); P = 0.98	(11) *vs.* (4); P = 0.94

Comparison	Child Natural	Child Experimental
GI 0 *vs.* GI 1	(10) *vs.* (7); P = 0.12	(4) *vs.* (4); P = 0.70
GI 0 *vs.* GI 2	(10) *vs.* (6); P = 0.53	(4) *vs.* (4); P = 0.03*
GI 1 *vs.* GI 2	(7) *vs.* (6); P = 0.14	(4) *vs.* (4); P = 0.45
	Adult Natural	**Adult Experimental**
GI 0 *vs.* GI 1	(9) *vs.* (7); P = 0.61	(4) *vs.* (4); P = 0.42
GI 0 *vs.* GI 2	(9) *vs.* (8); P = 0.47	(4) *vs.* (4); P = 0.10
GI 1 *vs.* GI 2	(7) *vs.* (8); P = 0.08	(4) *vs.* (4); P = 0.42

Score	Natural Child *vs.* Adult	Experimental Child *vs.* Adult
GI 0	(10) *vs.* (9); P = 0.85	(4) *vs.* (4); P = 0.03*
GI 1	(7) *vs.* (9); P = 0.02*	(4) *vs.* (4); P = 0.07
GI 2	(6) *vs.* (8); P = 0.73	(4) *vs.* (4); P = 0.03*
All	(10) *vs.* (11); P = 0.85	(4) *vs.* (4); P = 0.03*

[a]Numbers in parentheses = number of persons represented; asterisk (*) indicates statistical significance.
[b]All experimental gingivitis data are from Moore *et al.* (1982b, 1984b).

The subgingival floras of the 21 children and adults with naturally-occurring gingivitis differed significantly ($P = < 0.001$ for each comparison) from those of the healthy control subjects, those with localized juvenile periodontitis (Moore *et al.*, 1985), early onset severe generalized periodontitis (Moore *et al.*, 1982a), and adult periodontitis (Moore *et al.*, 1983). Therefore, the compositions of floras associated with gingivitis were distinct from those associated with health or with periodontitis.

Results of tests for differences between the floras of naturally-occurring and experimental gingivitis (Moore *et al.*, 1984b, 1982a) are shown in Table 3. There were no major differences in the compositions of these floras in the adults or children separately or combined ($P = 0.94$ to 0.99). However, in the experimental gingivitis model in children, the floras of sites with GI = 0 differed from those of GI = 2, but this difference was not seen in naturally-occurring gingivitis. There were no significant differences in the floras of sites with different GI scores when all samples from sites with the same score in each

person (for children and adults) were combined (GI 0 *vs.* 1, P = 0.38; GI 0 *vs.* 2, P = 0.23; GI 1 *vs.* 2, P = 0.39). Nevertheless, the floras of naturally-occurring gingivitis subjects (all sites in each person combined) differed significantly from the floras of healthy sites in healthy control subjects (all sites in each person combined) (P = <0.001), and sites with GI scores of 0, 1, or 2 in naturally-occurring gingivitis subjects differed from GI = 0 sites in healthy controls (P = 0.03, 0.002, and 0.001, respectively). Strikingly, the floras of the children and adults with naturally-occurring gingivitis did not differ in this study (P = 0.85), whereas they were significantly different (P = 0.03) in the experimental gingivitis model (Moore *et al.*, 1984b, 1985).

With one exception, the treponeme and mycoplasma data, based on selective media or selective isolation methods, showed the same types of relationships between the subject groups as did the non-treponemal flora. There was no significant difference between the naturally-occurring gingivitis and experimental gingivitis treponemal floras: four children with experimental gingivitis *vs.* 10 with naturally-occurring gingivitis, P = 0.67; four adults with experimental gingivitis *vs.* 11 with naturally-occurring gingivitis, P = 0.36; or all eight experimental subjects *vs.* all 21 natural gingivitis subjects, P = 0.88. However, the treponeme flora of children with naturally-occurring gingivitis was significantly different (P = 0.04) from that of adults with naturally-occurring gingivitis. A major difference appeared to be greater proportions of *T. socranskii* subsp. *paredis* in children than in adults (Table 4). The adult and child naturally-occurring gingivitis treponemal flora was different from that of healthy sites in the control subjects and from affected sites in subjects with juvenile or severe periodontitis (P <0.001 for each comparison), but not from affected sites in subjects with moderate periodontitis (P = 0.06).

Species associated with gingivitis are listed in Table 4. Table 5 lists species that represent at least 0.7% of the flora in any population and that are associated with health, that increased less than 1% in gingivitis over health or in periodontitis over health or gingivitis, or that were isolated only from periodontitis. In Tables 4 and 5, the figures for the % of the isolates of each taxon in the healthy children are derived from only four children. These were the subjects in the experimental gingivitis study (Moore *et al.*, 1984b) and represent 29 baseline samples from sites with a GI score of 0. Because the variation of flora composition is high among people (Moore *et al.*, 1984a), these data may not be truly representative of the periodontal flora in healthy children.

The six species that first appeared in both child and adult gingivitis and increased further (in incidence, for treponemal species, or as percent of isolates for other species) in at least one periodontitis group were *Eubacterium brachy, Eubacterium* D11, *Peptostreptococcus anaerobius, Treponema pectinovorum*, and treponemal species D and F (Table 4). *Eubacterium nodatum, Fusobacterium alocis, Haemophilus actinomycetemcomitans*, and *Treponema denticola* were not isolated from samples from healthy persons or children with naturally-occurring gingivitis, but were present in samples from adults with naturally-occurring gingivitis and increased further in periodontitis. The "large treponeme" was not isolated but was seen microscopically in samples from adult subjects with naturally-occurring gingivitis. The "large treponeme" was seen even more frequently in samples from subjects with periodontitis, particularly severe (generalized) and juvenile (localized) periodontitis. Five species were present in health but showed increases in both gingivitis and periodontitis. Sixteen species increased in naturally-occurring gingivitis but showed little or no increase in samples from subjects with periodontitis (Table 4).

Nine species were especially associated with the healthy flora (Table 5). The concentrations of these species did not increase, and usually decreased, in gingivitis and periodontitis. The concentration or incidence of 19 taxa increased less than 1% in gingivitis *vs.* health and in periodontitis *vs.* gingivitis. The concentration or incidence of 17 taxa did not increase by 1% in gingivitis as compared with health but showed at least a 1% increase in samples from periodontitis as compared with health (Table 5).

The significance of taxa that are present as a small percent of the total population is open to question. However, except for the healthy children (only four subjects), from 853 to 1711 non-treponemal isolates from 27 to 53 samples from 10 to 21 subjects in each population were analyzed. We therefore believe that the low numbers observed in gingivitis may be significant, particularly when no isolates were found in health and a substantial increase was noted in periodontitis.

Discussion.

A major finding of this study was that the subgingival flora of gingivitis differs from both that of health and that of periodontitis. These findings are consistent with significant changes in the flora through a sequence of health to gingivitis to periodontitis, with the majority of predominant species of destructive periodontitis already present in the gingivitis state. Whether a critical number of certain combinations of these species must be reached in order for gingivitis to progress to periodontitis, or whether changes in host defense or response factors occur that permit destruction of attachment by bacterial species and quantities that are already present, remain open questions.

Certain species (*e.g., Bacteroides gingivalis, Eubacterium nodatum*, and *Eubacterium timidum*) that were numerous in periodontitis samples were not present or did not increase appreciably in gingivitis as compared with health. This may indicate that these species have relatively more importance as tissue-destructive agents, or, alternatively, that they increase in numbers subsequent to attachment loss rather than as its cause.

Although nomenclature and criteria for identification of bacterial species have changed considerably in the past 10 years, comparison of our results with those previously reported is possible in most cases. The observed increase of *F. nucleatum* in gingivitis as compared with health has been reported previously from studies of gingivitis in adults (Savitt and Socransky, 1984; Slots *et al.*, 1978; van Palenstein Helderman, 1975), as have increased numbers of veillonellae (Slots *et al.*, 1978). Our results concur with those of Slots *et al.* (1978) and Savitt and Socransky (1984), who also reported increased numbers of *Bacteroides intermedius*, although the probable homology group (Johnson and Holdeman, 1983) to which their isolates belong was not reported. In adult gingivitis *vs.* health, we found an increase in numbers of *B. intermedius* 8944, which is not the homology group that includes the type strain of the species.

Van Palenstein Helderman (1975) reported that the numbers of "*Vibrio (Campylobacter) sputorum*" also increased markedly with GI score and that *Bacteroides ochraceus* (which probably included all of the species now recognized in *Capnocytophaga*) and *Selenomonas sputigena*, both present in low numbers, increased slightly. It is possible that the organisms van Palenstein Helderman identified as "*V. sputorum*" (small, straight, or slightly curved rods, non-fermentative, reducing nitrate) were *Wolinella*, which we found increased in gingivitis over health, or the organisms we recognize as *Selenomonas* D04, which we did not find to increase. Neither we nor Slots *et al.* (1978) nor Savitt and Socransky (1984) found an increase

TABLE 4
PREDOMINANT PERIODONTAL FLORAS ASSOCIATED WITH GINGIVITIS AND THEIR INCIDENCE IN HEALTH AND PERIODONTITIS[a]

	Mean Percent of Flora ± S.E.M. in Samples (or Incidence[b]) from:						
	Health		Naturally-occurring Gingivitis		Periodontitis		
Taxon or Group	Child	Adult	Child	Adult	Moderate	Juvenile	Severe
Absent in Health, > in Gingivitis and Periodontitis:							
Eubacterium brachy	0.0	0.0	1.5 ± 0.63	0.8 ± 0.39	3.1 ± 0.95	1.0 ± 0.43	1.7 ± 0.76
Eubacterium nodatum	0.0	0.0	0.0	0.2 ± 0.19	5.4 ± 1.11	5.9 ± 1.78	7.9 ± 1.86
Eubacterium D11	0.0	0.0	0.1 ± 0.08	0.4 ± 0.30	2.3 ± 0.75	1.3 ± 0.64	0.6 ± 0.27
Fusobacterium alocis	0.0	0.0	0.0	0.2 ± 0.19	0.9 ± 0.36	2.1 ± 0.63	1.4 ± 0.70
Haemophilus actinomycetemcomitans	0.0	0.0	0.0	0.1 ± 0.09	0.2 ± 0.14	0.8 ± 0.67	0.0
Peptostreptococcus anaerobius	0.0	0.0	0.1 ± 0.09	0.5 ± 0.31	1.4 ± 0.43	1.5 ± 0.63	0.8 ± 0.24
Treponema denticola[b]	0.0	0.0	0.0	(2.9)	(24.6)	(19.4)	(20.9)
Treponema pectinovorum[b]	0.0	0.0	(2.9)	(8.6)	(5.8)	(19.4)	(11.6)
Treponema D[b]	0.0	0.0	(2.9)	(11.4)	(8.7)	(16.1)	(14.0)
Treponema F[b]	0.0	0.0	(2.9)	(2.9)	(4.4)	0.0	(2.3)
"Large treponeme"[bc]	0.0	0.0	0.0	(5.7)	(11.6)	(48.3)	(62.8)
> In Gingivitis and Periodontitis							
Bacteroides intermedius 4197 Homology group	0.0	0.1 ± 0.10	0.0	0.4 ± 0.18	1.5 ± 0.56	1.2 ± 0.50	4.3 ± 1.22
Eubacterium timidum	0.2 ± 0.15	0.6 ± 0.43	0.3 ± 0.34	0.8 ± 0.36	2.7 ± 0.78	2.7 ± 0.54	6.2 ± 1.53
Fusobacterium nucleatum	2.8 ± 0.79	4.4 ± 1.67	7.1 ± 1.47	6.4 ± 1.33	12.1 ± 2.15	6.6 ± 1.21	8.1 ± 1.27
Wolinella recta	1.0 ± 0.56	0.7 ± 0.33	1.7 ± 0.63	1.2 ± 0.43	2.1 ± 0.42	4.4 ± 1.47	0.7 ± 0.24
Treponema socranskii[b,d]	(13.8)	(26.0)	(14.3)	(28.5)	(58.0)	(58.1)	(58.2)
subspecies *buccalis*[b,e]			(2.9)	(11.4)			
subspecies *socranskii*[b,e]			(0.0)	(5.7)			
Did Not Survive[f]	2.4 ± 0.68	3.6 ± 1.20	3.9 ± 1.12	2.7 ± 0.56	4.6 ± 0.86	5.3 ± 1.32	5.2 ± 0.72
Treponemas observed Microscopically[b]	(6.9)	0.0	(11.4)	(40.0)	(65.2)	(83.9)	(88.4)
> In Gingivitis, no. > in Periodontitis							
Actinomyces naeslundii III	1.0 ± 0.74	2.9 ± 1.54	2.4 ± 0.94	4.4 ± 1.70	1.3 ± 0.54	2.0 ± 0.77	1.6 ± 0.56
Campylobacter concisus	0.0	0.2 ± 0.13	1.3 ± 1.36	0.7 ± 0.32	0.2 ± 0.10	0.4 ± 0.24	0.1 ± 0.10
Streptococcus anginosus	6.5 ± 1.95	1.6 ± 0.76	8.8 ± 1.70	3.3 ± 1.38	3.1 ± 0.70	1.0 ± 0.33	4.2 ± 2.13
Streptococcus sanguis I	1.1 ± 0.46	1.8 ± 0.53	1.8 ± 0.66	3.0 ± 0.83	1.7 ± 0.47	1.0 ± 0.43	0.2 ± 0.12
Treponema socranskii subspecies *paredis*[b]	(3.4)	0.0	(28.6)	(2.9)	(1.4)	0.0	(2.3)
> By 1% in Child Gingivitis and by Less Than 0.5% in Periodontitis							
Actinomyces israelii II	2.8 ± 1.54	0.5 ± 0.48	4.1 ± 1.14	0.3 ± 0.16	0.2 ± 0.11	0.0	0.6 ± 0.59
Actinomyces naeslundii (−)	1.3 ± 0.76	5.0 ± 1.17	2.8 ± 1.13	4.9 ± 1.33	1.4 ± 0.44	1.5 ± 0.72	1.7 ± 0.86
Bacteroides gracilis	0.3 ± 0.18	1.4 ± 0.48	2.1 ± 0.61	1.3 ± 0.56	0.3 ± 0.17	1.1 ± 0.82	1.4 ± 0.51
Capnocytophaga ochracea	1.9 ± 0.53	4.0 ± 1.36	3.8 ± 0.96	3.5 ± 0.94	0.4 ± 0.16	0.9 ± 0.65	1.3 ± 0.39
Coccus SM1	0.0	1.4 ± 0.71	1.0 ± 0.43	0.9 ± 0.34	0.3 ± 0.15	0.8 ± 0.40	0.2 ± 0.16
Haemophilus aphrophilus	0.1 ± 0.12	0.9 ± 0.50	1.7 ± 0.85	0.1 ± 0.09	0.5 ± 0.23	0.0	0.1 ± 0.07
Propionibacterium acnes	0.5 ± 0.28	1.6 ± 0.57	1.6 ± 0.54	1.1 ± 0.39	1.6 ± 0.69	0.8 ± 0.32	1.2 ± 0.28
Treponema V[b]	0.0	0.0	(5.7)	0.0	0.0	0.0	0.0
> By 1% in Adult Gingivitis and by Less Than 0.5% in Periodontitis							
Streptococcus morbillorum	0.7 ± 0.29	0.2 ± 0.13	0.2 ± 0.14	1.3 ± 0.72	0.0	0.0	0.0
Treponema L[b]	0.0	0.0	0.0	(2.9)	0.0	0.0	(2.3)
Veillonella parvula	17.4 ± 2.49	3.3 ± 0.70	9.1 ± 1.37	5.8 ± 1.16	2.3 ± 0.62	3.6 ± 1.32	1.4 ± 0.47
% Of Non-treponeme Flora Represented Here	40.0	34.2	55.4	44.3	49.6	45.9	50.9
Total Non-treponemal Isolates	915	1053	1129	1178	1685	1064	1711
Total Non-treponemal Taxa	93	107	105	133	150	116	145
Total samples[g]							
Non-treponeme	29	33	35	38	53	32	46
Treponeme	29	27	35	35	69	31	43
Total subjects	4	20	10	11	21	21	21

[a]Species that comprise 0.7% or more of the flora in any category and that are associated with disease are listed. Data for moderate, juvenile, and severe periodontitis are, in part, from Moore *et al.* (1983, 1985, and 1982a, respectively).

[b] ( ) Treponeme data are mean % of persons from whom the taxon was isolated. Other data are mean % of strains isolated from the population.

[c]Observed microscopically; not cultured.

[d]Figures exclude incidence of *T. socranskii* subsp. *paredis*.

[e]Subspecies data are incomplete.

[f]Colonies that did not survive through identification.

[g]The number of samples equals "N" for the S.E.M.

TABLE 5
COMPARISON OF PREDOMINANT PERIODONTAL FLORAS NOT ASSOCIATED WITH GINGIVITIS[a]

	Mean Percent of Flora ± S.E.M. in Samples (or Incidence[b]) from:						
	Health		Naturally-occurring Gingivitis		Periodontitis		
Taxon or Group	Child	Adult	Child	Adult	Moderate	Juvenile	Severe
Associated with Health:							
Actinomyces israelii (−)[c]	3.3 ± 1.00	1.2 ± 0.49	1.7 ± 0.64	1.4 ± 0.62	0.6 ± 0.20	0.4 ± 0.24	0.6 ± 0.25
Actinomyces naeslundii I	8.8 ± 1.86	7.6 ± 2.96	5.8 ± 1.81	6.6 ± 1.58	2.0 ± 0.87	0.9 ± 0.52	0.6 ± 0.28
Actinomyces NV[d]	5.4 ± 1.74	5.4 ± 2.16	3.8 ± 1.36	2.3 ± 1.01	2.6 ± 1.40	1.1 ± 0.59	0.5 ± 0.21
Actinomyces odontolyticus (−)	0.3 ± 0.17	1.5 ± 0.55	0.2 ± 0.13	0.7 ± 0.34	1.0 ± 0.29	0.8 ± 0.70	0.1 ± 0.11
Actinomyces odontolyticus I	3.4 ± 0.80	1.9 ± 1.09	0.7 ± 0.30	0.2 ± 0.11	0.7 ± 0.23	0.7 ± 0.49	0.1 ± 0.10
Actinomyces WVa963[e]	5.2 ± 1.79	3.1 ± 1.37	3.3 ± 0.98	2.2 ± 0.71	0.4 ± 0.18	1.2 ± 0.53	0.1 ± 0.08
Rothia dentocariosa	0.1 ± 0.11	1.4 ± 0.85	0.1 ± 0.09	0.2 ± 0.18	0.0	0.0	0.0
Streptococcus D07	5.0 ± 1.29	5.6 ± 1.61	1.4 ± 0.55	1.3 ± 0.50	0.1 ± 0.12	0.2 ± 0.20	0.1 ± 0.14
Streptococcus mitis	1.4 ± 0.51	2.3 ± 0.77	0.5 ± 0.28	0.7 ± 0.33	0.8 ± 0.45	0.0	0.6 ± 0.60
Less Than 1% Increase in Gingivitis or Periodontitis							
Actinomyces israelii I	0.2 ± 0.16	0.9 ± 0.61	0.2 ± 0.19	0.4 ± 0.22	1.1 ± 0.57	0.3 ± 0.23	0.9 ± 0.44
Actinomyces meyeri (−)	0.0	0.6 ± 0.34	0.8 ± 0.68	0.4 ± 0.23	0.8 ± 0.33	1.4 ± 1.28	0.6 ± 0.49
Actinomyces viscosus II	1.1 ± 0.47	0.6 ± 0.22	1.8 ± 0.94	1.2 ± 0.69	0.2 ± 0.10	1.9 ± 1.14	1.0 ± 0.57
Bacteroides intermedius 8944 Homology group	1.3 ± 0.73	0.7 ± 0.42	1.3 ± 0.57	1.4 ± 0.70	0.7 ± 0.56	1.0 ± 0.65	0.9 ± 0.34
Bacteroides oralis	0.1 ± 0.12	0.2 ± 0.13	0.0	0.5 ± 0.31	0.1 ± 0.06	1.1 ± 0.63	0.4 ± 0.18
Bacteroides pneumosintes	0.0	0.3 ± 0.16	0.2 ± 0.17	0.5 ± 0.29	0.9 ± 0.65	0.8 ± 0.39	0.8 ± 0.31
Capnocytophaga gingivalis	1.7 ± 0.45	1.3 ± 0.57	1.4 ± 0.62	2.0 ± 0.53	0.3 ± 0.13	0.3 ± 0.22	0.0
Capnocytophaga sputigena	0.2 ± 0.16	0.4 ± 0.22	0.7 ± 0.47	1.2 ± 0.46	0.2 ± 0.20	0.0	0.1 ± 0.15
Eubacterium alactolyticum	0.0	0.0	0.0	0.0	0.7 ± 0.27	0.6 ± 0.52	0.8 ± 0.23
Haemophilus segnis	0.0	0.5 ± 0.33	0.2 ± 0.13	0.8 ± 0.38	0.0	0.0	0.0
Propionibacterium avidum	0.0	0.0	0.0	0.0	0.1 ± 0.06	0.0	0.6 ± 0.57
Selenomonas sputigena	1.2 ± 0.60	0.5 ± 0.24	0.0	0.4 ± 0.29	1.5 ± 0.71	0.4 ± 0.19	0.8 ± 0.26
Selenomonas D04	0.8 ± 0.44	0.9 ± 0.66	1.2 ± 0.56	0.6 ± 0.31	0.7 ± 0.25	0.9 ± 0.40	1.0 ± 0.44
Streptococcus sanguis II	1.4 ± 0.74	5.3 ± 1.02	2.2 ± 0.83	2.8 ± 0.90	1.8 ± 0.44	3.3 ± 1.64	0.3 ± 0.17
Streptococcus D16	2.7 ± 0.81	2.3 ± 1.30	0.7 ± 0.34	2.8 ± 0.97	0.4 ± 0.19	0.0	0.0
Streptococcus SM	0.8 ± 0.32	1.0 ± 0.42	0.2 ± 0.12	0.2 ± 0.11	1.0 ± 0.77	0.5 ± 0.26	0.1 ± 0.10
Veillonella atypica	0.6 ± 0.36	0.7 ± 0.31	0.3 ± 0.20	0.8 ± 0.39	0.0	0.0	0.1 ± 0.07
Wolinella curva	0.0	0.1 ± 0.10	0.2 ± 0.14	0.5 ± 0.25	0.8 ± 0.33	0.5 ± 0.43	0.6 ± 0.40
Wolinella X	0.3 ± 0.20	0.0	1.0 ± 0.42	0.7 ± 0.27	0.3 ± 0.15	0.7 ± 0.44	0.7 ± 0.32
Less Than 1% Increase in Gingivitis, > in Periodontitis							
Bacteroides denticola	0.0	0.3 ± 0.16	0.1 ± 0.07	0.3 ± 0.19	0.7 ± 0.41	1.5 ± 0.53	1.3 ± 0.49
Bacteroides gingivalis	0.0	0.0	0.0	0.0	3.6 ± 1.20	1.1 ± 0.97	0.3 ± 0.21
Bacteroides M1	0.0	0.0	0.0	0.0	0.0	1.7 ± 1.50	0.0
Bacteroides oris	0.1 ± 0.11	0.2 ± 0.14	0.0	0.2 ± 0.12	0.5 ± 0.22	2.5 ± 1.17	1.2 ± 0.68
Eubacterium D06	0.1 ± 0.12	0.1 ± 0.10	0.0	0.1 ± 0.09	1.0 ± 0.32	2.1 ± 0.77	1.8 ± 0.53
Eubacterium D08	0.0	0.0	0.0	0.1 ± 0.08	1.6 ± 0.66	6.3 ± 2.21	2.6 ± 0.79
Lactobacillus minutus	0.2 ± 0.21	0.3 ± 0.16	0.0	0.2 ± 0.18	1.1 ± 0.33	2.7 ± 1.25	4.2 ± 1.58
Lactobacillus D02	1.2 ± 0.53	0.6 ± 0.41	0.0	0.4 ± 0.21	1.6 ± 0.75	1.7 ± 0.69	1.7 ± 0.49
Peptostreptococcus micros	1.3 ± 0.58	2.4 ± 1.14	1.7 ± 0.66	1.5 ± 0.63	5.3 ± 0.97	2.6 ± 0.76	4.8 ± 1.31
Streptococcus D06	0.1 ± 0.11	0.2 ± 0.13	0.2 ± 0.11	0.5 ± 0.33	0.2 ± 0.09	0.3 ± 0.22	0.1 ± 0.13
Streptococcus D39	0.0	0.0	0.0	0.0	0.0	0.0	2.2 ± 2.20
Mycoplasma[b]	0.0	0.0	0.0	0.0	(21.7)	(48.3)	(53.5)
Treponema vincentii[b]	0.0	0.0	0.0	0.0	(4.4)	(6.4)	(2.3)
Treponema E[b]	0.0	0.0	0.0	0.0	(1.4)	(3.2)	0.0
Treponema G[b]	0.0	0.0	0.0	0.0	(4.4)	0.0	0.0
Treponema N[b]	0.0	0.0	0.0	0.0	0.0	0.0	(2.3)
Treponema U[b]	0.0	0.0	0.0	0.0	(1.4)	0.0	0.0
% Of Non-treponeme-mycoplasma Flora Represented Here	48.3	50.4	31.9	36.1	35.4	41.5	32.6

[a]Species that comprise 0.7% or more of the flora in any category and that are not associated with gingivitis or periodontitis are listed. Data for moderate, juvenile, and severe periodontitis are, in part, from Moore *et al.* (1983, 1985, and 1982a, respectively). Totals for numbers of isolates, taxa, samples, and subjects are given at the end of Table 4.

[b] ( ) Treponeme and mycoplasma data are mean % of persons from whom the taxon was isolated. Other data are mean % of strains isolated from the population.

[c](−) Serologically negative, but strains have the phenotypic characteristics of the species.

[d]Uniform serogroup that reacts with both *A. naeslundii* and *A. viscosus* antisera.

[e]Undescribed species are identified by letters and numbers.

in *Selenomonas sputigena*. Our results confirmed neither the two-fold increase in numbers of catalase-positive actinomyces (usually *A. viscosus* II and *Actinomyces* NV) reported by Slots *et al.* (1978), nor the marked increase in numbers of *Capnocytophaga gingivalis* and *Eikenella corrodens* reported by Savitt and Socransky, although in our study both showed slight increases in naturally-occurring adult gingivitis as compared with health (*C. gingivalis*, 1.3 to 1.8%; *E. corrodens*, 0.19 to 0.34%).

H. actinomycetemcomitans was included in our list of taxa especially associated with gingivitis because no strains were detected from our healthy subjects, but low levels were detected in naturally-occurring gingivitis in both adults and children, and the levels increased further in juvenile periodontitis but not in either the moderate or severe periodontitis populations studied.

Eubacterium brachy, *Eubacterium* D-11 (a non-fermentative species), *Peptostreptococcus anaerobius*, *Streptococcus anginosus*, *Wolinella recta*, *Treponema pectinovorum*, *Treponema* D, *Treponema* F, *Treponema socranskii* subsp. *paredis* (children), *Treponema denticola* (adults), *Eubacterium nodatum* (adults), *Fusobacterium alocis* (adults), *Bacteroides gracilis*, *Campylobacter concisus*, and other species that appeared to be especially associated with naturally-occurring gingivitis, were not considered by the other investigators, who did not attempt culture of treponemes and were concerned principally with Gram-negative components of the flora cultured. We found that *S. anginosus*, *C. concisus*, *T. socranskii* subsp. *paredis*, *A. naeslundii* III, and *S. sanguis* I were more numerous in samples from gingivitis in both children and adults than in samples from healthy persons or persons with periodontitis. *B. gracilis*, *C. ochracea*, *P. acnes*, and *Treponema* V were more common in gingivitis in children than in healthy children, and *S. morbillorum*, *Treponema* L, and *V. parvula* were associated with adult gingivitis (Table 4). *E. brachy*, *Eubacterium* D11, and *P. anaerobius* were not detected in healthy samples, were isolated from gingivitis samples, and were even more numerous in samples from persons with periodontitis.

The incidence of *T. socranskii* subsp. *paredis* in particular is considerably higher in children with gingivitis than in samples from any of the other populations studied (Table 4). *T. socranskii* subsp. *buccalis* and *T. socranskii* subsp. *socranskii* can be distinguished only by serological tests, which have not yet been completed on all of the isolates. The incidence of these two subspecies combined increased in gingivitis as compared with health and increased considerably in periodontitis as compared with gingivitis. Savitt and Socransky (1984) reported a significant increase in the incidence of small spirochetes, as determined by dark-field microscopy, in samples from gingivitis as compared with samples from healthy persons. Their observed increase in the incidence of medium-sized spirochetes was not statistically significant. *T. socranskii*, *Treponema* D, and *Treponema* F are small spirochetes. *T. pectinovorum*, along with *T. denticola* and *T. vincentii*, are medium-sized spirochetes.

The absence of major differences in composition between the floras of naturally-occurring and experimental gingivitis suggests that the experimental model represents the natural condition reasonably well bacteriologically. However, the fact that the floras differed among different GI scores in the experimental model and in the healthy adults, but not in subjects with naturally-occurring gingivitis, suggests that the (probably) longer duration of higher GI scores in the latter population influences the distribution of species among sites. Much of the distinctive flora associated with gingivitis is distributed among both inflamed and uninflamed sites in subjects with naturally-occurring gingivitis. This further suggests that sites within people are not independent bacteriologically, especially in people with naturally-occurring gingivitis and greater than 0.5 full-mouth GI scores, as compared with the experimental gingivitis model or periodontal health.

The lack of a demonstrable difference between the floras of children and adults with naturally-occurring gingivitis was surprising in light of the results obtained in the experimental gingivitis studies (Moore *et al.*, 1982b; 1984b). The differences between naturally-occurring gingivitis and previous studies of experimental gingivitis might be attributed to the variation among floras of different people (Moore *et al.*, 1984a). Comparisons of the subjects' floras in the present study suggest that the four people in each experimental gingivitis study may have been insufficient to represent their population groups. With the present subjects, 64 of 121 possible comparisons of individual children's flora *vs.* adults' flora showed the minimum possible probability of similarity based on only two to four samples for each person.

On the other hand, gingivitis induced experimentally with complete absence of tooth-brushing may stimulate specific portions (*e.g.*, *Capnocytophaga* and *Selenomonas* species) of the flora uniquely in children, as seen in the previous experiments (Moore *et al.*, 1982b; 1984b). Species of *Capnocytophaga* and *Selenomonas* did not increase uniquely in children with naturally-occurring gingivitis (Table 4).

The lower plaque and gingival indices in the healthy adult control group than in the adults with naturally-occurring gingivitis match the expected positive relationship between the amount of plaque and severity of gingival inflammation. Thus, the gingivitis appears to be correlated with relative oral hygiene rather than with an especially high sensitivity to plaque bacteria. The higher plaque levels relative to gingival scores in children are consistent with previous reports of greater resistance to gingivitis prior to puberty (Mackler and Crawford, 1973; Mattson, 1978; Moore *et al.*, 1984b).

In summary, the composition of the subgingival flora of gingivitis was significantly different from the corresponding floras of health and of periodontitis. The composition was consistent with a flora in transition between that associated with health and that associated with periodontitis. In contrast to previous results from the experimental gingivitis model, the flora composition of sites with different GI scores was not significantly different, which indicates considerable interdependence among the floras of various sites within the mouth of persons with relatively high GI scores of indeterminant duration. The significant difference between the composition of the floras of adults and children found previously in the experimental gingivitis model was not found in the present study of naturally-occurring gingivitis. In other major respects, however, the bacteriology of naturally-occurring gingivitis and that of the experimental gingivitis model are similar.

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REFERENCES

GOOD, I.J. (1982): An Index of Separateness of Clusters and a Permutation Test for its Significance, *J Statist Comput Simul* 15:81–84.

HOLDEMAN, L.V.; CATO, E.P.; and MOORE, W.E.C. (Ed.) (1977): **Anaerobe Laboratory Manual,** 4th ed. Blacksburg, VA: Virginia Polytechnic Institute and State University.

JOHNSON, J.L. and HOLDEMAN, L.V. (1983): *Bacteroides intermedius* comb. nov. and Descriptions of *Bacteroides corporis* sp. nov. and *Bacteroides levii* sp. nov., *Int J Syst Bacteriol* 33:15–25.

LÖE, H. and SILNESS, J. (1963): Periodontal Disease in Pregnancy. I. Prevalence and Severity, *Acta Odontol Scand* 21:533–551.

LÖE, H.; THEILADE, E.; and JENSEN, S.B. (1965): Experimental Gingivitis in Man, *J Periodontol* 36:177–187.

LOESCHE, W.J. and SYED, S.A. (1978): Bacteriology of Human Experimental Gingivitis: Effect of Plaque and Gingivitis Score, *Infect Immun* 21:830–839.

MACKLER, S.B. and CRAWFORD, J.J. (1973): Plaque Development and Gingivitis in the Primary Dentition, *J Periodontol* 44:18–24.

MATTSON, L. (1978): Development of Gingivitis in Pre-school Children and Young Adults, *J Clin Periodontol* 5:24–38.

MOORE, W.E.C.; HASH, D.E.; HOLDEMAN, L.V.; and CATO, E.P. (1980): Polyacrylamide Slab Gel Electrophoresis of Soluble Proteins for Study of Bacterial Floras, *Appl Environ Microbiol* 39:900–907.

MOORE, W.E.C.; HOLDEMAN, L.V.; CATO, E.P.; GOOD, I.J.; SMITH, E.P.; PALCANIS, K.G.; and RANNEY, R.R. (1985): Comparative Bacteriology of Juvenile Periodontitis, *Infect Immun* 48:507–519.

MOORE, W.E.C.; HOLDEMAN, L.V.; CATO, E.P.; GOOD, I.J.; SMITH, E.P.; RANNEY, R.R.; and PALCANIS, K.G. (1984a): Variation in Periodontal Floras, *Infect Immun* 46:720–726.

MOORE, W.E.C.; HOLDEMAN, L.V.; CATO, E.P.; SMIBERT, R.M.; BURMEISTER, J.A.; and RANNEY, R.R. (1983): Bacteriology of Moderate (Chronic) Periodontitis in Mature Adult Humans, *Infect Immun* 42:510–515.

MOORE, W.E.C.; HOLDEMAN, L.V.; SMIBERT, R.M.; CATO, E.P.; BURMEISTER, J.A.; PALCANIS, K.G.; and RANNEY, R.R. (1984b): Bacteriology of Experimental Gingivitis in Children, *Infect Immun* 46:1–6.

MOORE, W.E.C.; HOLDEMAN, L.V.; SMIBERT, R.M.; HASH, D.E.; BURMEISTER, J.A.; and RANNEY, R.R. (1982a): Bacteriology of Severe Periodontitis in Young Adult Humans, *Infect Immun* 38:1137–1148.

MOORE, W.E.C.; HOLDEMAN, L.V.; SMIBERT, R.M.; GOOD, I.J.; BURMEISTER, J.A.; and RANNEY, R.R. (1982b): Bacteriology of Experimental Gingivitis in Young Adult Humans, *Infect Immun* 38:651–667.

SAVITT, E.D. and SOCRANSKY, S.S. (1984): Distribution of Certain Subgingival Microbial Species in Selected Periodontal Conditions, *J Periodont Res* 19:111–123.

SILNESS, J. and LÖE, H. (1964): Periodontal Disease in Pregnancy. II. Correlation Between Oral Hygiene and Periodontal Condition, *Acta Odontol Scand* 22:112–135.

SLOTS, J.; MÖENBO, D.; LANGEBAEK, J.; and FRANDSEN, A. (1978): Microbiota of Gingivitis in Man, *Scand J Dent Res* 86:174–181.

SYED, S.A. and LOESCHE, W.J. (1978): Bacteriology of Human Experimental Gingivitis: Effect of Plaque Age, *Infect Immun* 21:821–829.

VAN PALENSTEIN HELDERMAN, W.H. (1975): Total Viable Count and Differential Count of *Vibrio (Campylobacter) sputorum, Fusobacterium nucleatum, Selenomonas sputigena, Bacteroides ochraceus* and *Veillonella* in the Inflamed and Non-inflamed Human Gingival Crevice, *J Periodont Res* 10:294–305.