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Bacteriology of Human Experimental Gingivitis: Effect of Plaque and Gingivitis Score

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The plaque flora isolated from discrete dentogingival sites during a human gingivitis experiment was analyzed as a function of the plaque score and of the gingivitis score. When the gingivitis score was plotted as a function of the plaque score, a nonbleeding gingivitis was associated with a proportional increase in the *Actinomyces* sp. at the expense of the *Streptococcus* sp. In particular, the percentage of *Actinomyces israelii* increased significantly, while the percent *Streptococcus sanguis* decreased significantly. *A. israelii* also increased significantly when a bleeding gingivitis developed. When the plaque score was plotted as a function of the gingivitis score, *A. israelii* increased significantly as the nonbleeding gingivitis developed, but *A. viscosus* and *Bacteroides melaninogenicus* increased significantly when the bleeding gingivitis developed. The availability of a sufficient number of plaques with a plaque score of 2.0 permitted the examination of the interrelationship of gingivitis and flora minus the effect of plaque biomass. The bacteriological profile showed that when bleeding occurred, the levels and proportions of *A. viscosus* and *B. melaninogenicus* increased significantly. These findings raise the possibility that proportional changes in the gingival plaque flora may uniquely contribute to the development of gingival inflammation in this experimental model.

The bacteriological changes occurring in plaque during human experimental gingivitis have been evaluated as a function of length of time without oral hygiene (16, 33, 34). The rate of formation of plaque and the onset and rate of gingivitis can vary from individual to individual, as well as from site to site within the same mouth (10, 16), indicating that some variable(s) in addition to time is operable. Some of the interactions between plaque score and flora and gingivitis score and flora can be obscured when the data are analyzed as a function of time. Thus, a proliferation of one or more bacterial species in association with an increase in plaque and/or gingivitis score could be masked. Such information would be important to know to determine whether specific bacterial types are etiologically associated with the various forms of periodontal disease (19, 31). In the present investigation, the plaque flora isolated from discrete dentogingival sites during a human gingivitis experiment was analyzed as a function of the plaque score and of the gingivitis score.

MATERIALS AND METHODS

Subjects. Twenty-four male dental students and one female dental student, after being brought to optimal gingival health, suspended all oral hygiene procedures for a 3-week period. The students were sorted into six groups of four or five students, and each group was consecutively brought through the no-hygiene experimental period (33).

Sample sites. Efforts were made to remove all the plaque present at the gingival margin of three interproximal test sites, i.e., the mesial gingival surface of the upper left premolar, tooth no. 13; the mesial gingival surface of the lower left central incisor, tooth no. 24; and the mesial gingival surface of the lower right first molar, tooth no. 30. The plaque sample sites were rotated among the participants so as to give an approximately similar number of 0-, 1-, 2-, and 3-week-old plaque samples from each tooth (33). At the 3-week sampling period, additional plaques were collected from maxillary premolars and anterior teeth (teeth numbered 4 to 12) that exhibited obvious plaque and gingivitis.

Bacteriological procedures. These have been described in detail in another report (33) and will be briefly summarized. The plaques were collected in 10 ml of reduced transport fluid, dispersed by 5 s of sonic disruption under a stream of oxygen-free gas (85% N_2, 5% CO_2, and 10% H_2), and were brought into an anaerobic chamber where they were serially diluted in reduced transport fluid and plated in duplicate on MM10 sucrose agar. Each colony on one MM10 sucrose plate was subcultured and partially characterized using a taxonomic scheme which permitted the recognition of 29 species or groups of organisms (33). Approximately 200 to 400 isolates were characterized

each week, and during the entire study about 8,500 isolates were characterized.

Plaque and gingivitis scores. The amount of plaque and gingivitis about each site was estimated by modification of the plaque index and gingivitis index (14, 23). Two examiners concurred on each score. The plaque score had been shown to significantly reflect wet weight (20, 22), total viable bacterial count, and, to a lesser extent, DNA content in micrograms (33). The gingivitis score was based upon the presence of bleeding and the degree of blood flow along the gingival margin (24, 33).

Statistical analysis. The clinical and bacteriological data were analyzed by an analysis of variance and by the nonparametric Kruskal-Wallis test. Whenever significance was found by analysis of variance, the comparisons between entries were performed using the Scheffe test (33).

RESULTS

The frequency distribution of the plaque scores as a function of the gingivitis score is shown in Table 1. Plaque scores of 1.5 and 2.0 were most commonly encountered, and they tended to be associated with a gingivitis score of 1.0. The plaque and gingivitis scores for all time periods were significantly related to each other (i.e., correlation coefficient $r = 0.52$, $P < 0.01$ [33]; Kruskal-Wallis test, $P < 0.001$; analysis of variance, $P < 0.001$ [Tables 2 and 5]). The gingivitis score, plaque DNA content (micrograms) and total viable count are displayed for each plaque score in Table 2. All three parameters were significantly related by an analysis of variance to the plaque score. Large and significant increases in the gingivitis score were observed as the plaque score increased from 0.5 to 1.0 and from 2.0 to 2.5. The gingivitis scores associated with a plaque score of 2.5 were significantly higher than the other gingivitis scores (Table 2). The total viable counts associated with plaque scores of 2.0 and 2.5 were each significantly different from the viable counts associated with the other plaque scores.

The viable counts of the various numerically dominant cultivable flora were stratified according to the plaque score (Table 3). All counts increased as the plaque score increased, as would be expected when starting from essentially a zero base line. However, those for *Actinomyces viscosus*, *Actinomyces israelii*, *Actinomyces naeslundii*, *Veillonella*, *Micrococcus* sp., *Streptococcus mitis* (*mitior*), and *Campylobacter sputorum* increased significantly. The levels of *A. israelii*, *A. naeslundii*, *Propionibacterium acnes*, and *C. sputorum* at a plaque score of 2.5 were significantly higher than their levels at any other plaque score.

The viable counts for each species were converted to a percentage of the total viable count and examined as a function of the plaque score (Table 4). As the plaque score increased, the proportions of gram-positive rods increased significantly, the proportions of gram-positive cocci decreased significantly, and the proportions of gram-negative organisms remained relatively constant. Among the various species, only the percentages of *A. israelii* and *C. sputorum* increased significantly. *A. israelii* exhibited a large and significant increase as the plaque score progressed from 0.5 to 1.0. This proportional increase in *A. israelii* coincided with a significant increase in the proportions of the total gram-positive rods (Table 4). At the higher plaque scores, the proportions of *A. israelii* and the total gram-positive rods tended to remain constant at about 25 and 45%, respectively, of the viable count. *C. sputorum* was undetected at plaque scores of 0.0 and 0.5, but increased significantly from 0.3 to 1.0% of the flora as the plaque score went from 1.5 to 2.0 (Table 4). The percentages of *Bacillus* sp., *Streptococcus sanguis*, and *S. mitis* (*mitior*) decreased as the plaque score increased. The *Bacillus* sp. represented 5% of the flora at a plaque score of 0.0, but declined rapidly to less than 1% of the flora at the higher plaque scores. *S. sanguis* and *S. mitis* (*mitior*) were the predominant species in the flora at the low plaque scores, accounting for about 55 to 60% of the isolates (Table 4). Despite this numerical advantage in the early plaque, the proportions of *S. sanguis* and *S. mitis* (*mitior*) declined as the plaque accumulated and aged.

Not all sites developed gingivitis, despite the accumulation of plaque (Table 1). To determine whether a different profile of organisms could be associated with gingivitis, the bacteriological data were next analyzed as a function of the gingivitis score. An average of 13 days elapsed before the development of an obvious nonbleeding gingivitis, i.e., gingivitis score of 1.0, and then an additional 7 days passed before the appearance of an obvious bleeding gingivitis, i.e., gingivitis score of 2.0 (Table 5). There were no significant changes in the physical parameters of

TABLE 1. *Frequency distribution of plaque scores as a function of the gingivitis score*

Plaque score	Gingivitis score				
	0.0	0.5	1.0	1.5	2.0
0.0	10	4	0	0	0
0.5	6	1	2	0	0
1.0	2	3	8	1	2
1.5	2	3	14	4	4
2.0	0	8	23	8	7
2.5	0	1	1	1	6

TABLE 2. *Relation of plaque score to gingivitis score and other plaque parameters about selected teeth*[a]

Determination	Parameter[b]				n
	Gingivitis score	Plaque DNA content (μg)	Total colony-forming units ($\times 10^6$)	Age of plaque (days)	
Plaque score					
0.0	0.14 (0–0.5)	7.6 (ND-21)	4.4 (0.001–53)	0.5 (0–7)	14
0.5	0.31 (0.1)	0.9 (ND-7)	7.8 (0.001–24)	0.0	9
1.0	0.94 (0–2)	13.4 (ND-66)	29.8 (0.01–80)	9.1 (0–21)	16
1.5	1.09 (0.2)	17.6 (ND-74)	49.8 (7–230)	15 (7–21)	27
2.0	1.17 (0.5–2)	32.1 (ND-120)	77.6 (5–220)	17 (7–21)	46
2.5	1.67 (0.5–2)	29.0 (ND-64)	129.7 (24–310)	21 (21)	9
Significance (*P*)					
Analysis of variance	0.000	0.001	0.000		
Kruskal-Wallis test	0.000	0.001	0.000		

[a] Teeth no. 13, 24, and 30 were measured at 0, 1, 2, and 3 weeks; additional teeth (4 to 12) were measured at week 3.

[b] Mean values; range of values given in parentheses. ND, Not detectable. All values connected by arrows are significantly different by the Scheffe test. All values within boxes are significantly different by the Scheffe test from all other values in that column.

TABLE 3. *Relation of plaque score to bacterial levels (mean values) of cultivable bacteria in marginal plaque about selected teeth*[a]

Bacterial type	Bacterial count ($\times 10^6$) at plaque score[b]:						Significance (*P*)	
	0.0	0.5	1.0	1.5	2.0	2.5	Analysis of variance	Kruskal-Wallis test
Gram-positive rods								
A. viscosus	0.32	0.16	1.77	3.82	7.6	12.22	0.034	0.002
Esculin-negative *Actinomyces*	0.48	0.04	5.15	2.29	4.5	3.11		0.001
A. israelii	0.16	0.61	8.99	9.08	17.4	41.1	0.000	0.000
A. naeslundii	0.11	0.31	1.91	4.0	4.62	9.32	0.015	0.000
Diphtheroids	<0.01	0.0	0.18	0.01	0.47	0.88		
P. acnes	0.0	<0.01	0.18	1.03	0.86	2.90		
Bacillus sp.	<0.01	0.0	0.62	0.07	0.96	0.74		
Total	1.07	1.12	18.8	20.3	36.4	70.3		
Gram-positive cocci								
S. sanguis	1.24	5.42	6.18	8.85	14.68	16.40		0.001
S. mitis (*mitior*)	1.05	0.21	5.16	8.86	10.1	17.68	0.005	0.000
Enterococci	0.1	0.06	0.0	0.31	0.38	1.25		
Micrococcus sp.	<0.01	0.01	0.64	1.13	0.37	1.75	0.038	
Total	2.39	5.7	11.98	19.15	25.53	37.1		
Gram-negative spp.								
Veillonella sp.	0.89	1.12	3.91	9.18	13.82	18.16	0.001	0.000
F. nucleatum	<0.01	<0.01	0.42	0.87	0.83	1.94		0.000
C. sputorum	0.0	0.0	0.04	0.21	0.65	2.09	0.003	
B. melaninogenicus	0.0	0.0	0.0	0.01	0.0	0.03		
Total	0.89	1.12	4.37	10.3	15.3	22.2		
n	14	9	16	46	27	9		

[a] Teeth 13, 4, and 30 were measured at 0, 1, 2, and 3 weeks; additional teeth (4 to 12) were measured at week 3.

[b] All counts $\times 10^6$ colony-forming units per site. Values within boxes are significantly different by the Scheffe test from all other values in that row. Values connected by arrows are significantly different by the Scheffe test.

TABLE 4. *Relation of plaque score to proportions (mean values) of cultivable bacteria in marginal plaque about selected teeth*[a]

Bacterial type	Bacteria (% of total)[b] at plaque score:						Significance (*P*)	
	0.0	0.5	1.0	1.5	2.0	2.5	Analysis of variance	Kruskal-Wallis test
Gram-positive rods								
A. viscosus	3.5	2.5	5.2	10.0	7.8	6.3		
Esculin-negative *Actinomyces*	3.0	3.1	9.5	5.2	6.1	4.8		
A. israelii	1.1	8.2 ⟷	20.8	21.1	22.0	26.4	0.0006	0.000
A. naeslundii	2.7	3.6	4.4	8.2	6.9	4.4		
Diphtheroids	0.1	0.0	0.7	0.1	0.4	0.8		
P. acnes	0.0	<0.1	0.3	2.4	1.6	1.5		
Bacillus sp.	[5.1]	0.6	1.2	0.2	0.8	0.4	0.06	
Total	15.5	18.0 ⟷	42.1	47.2	45.6	44.6	0.000	0.000
Gram-positive cocci								
S. sanguis	23.4	[52.1]	21.5	11.8	19.7	10.6	0.0003	0.03
S. mitis (*mitior*)	32.2 ⟷	11.8	22.6	16.4	14.3	19.3	0.04	
Enterococci	2.1	0.5	0.0	0.8	0.9	2.8		
Micrococcus sp.	3.7	2.8	2.6	1.7	0.7	1.7		
Total	61.4	67.2 ⟷	46.7 ⟷	30.7	35.6	34.4	0.0001	0.001
Gram-negative spp.								
Veillonella sp.	16.2	15.6	13.3	18.6	15.7	17.2		
F. nucleatum	0.1	<0.1	1.3	2.8	1.7	1.6		
C. sputorum	0.0	0.0	0.1	0.3 ⟷	1.0	1.9	0.01	
B. melaninogenicus	0.0	0.0	0.0	<0.1	<0.1	<0.1		
Total	16.3	15.6	14.7	21.7	18.4	20.7		
n	14	9	16	27	45	9		

[a] Teeth 13, 14, and 30 were measured at 0, 1, 2, and 3 weeks; additional teeth (4 to 12) were measured at week 3.

[b] Average value. Values within boxes are significantly different by the Scheffe test from all other values in that row. All values connected by arrows are significantly different by the Scheffe test.

TABLE 5. *Relation of gingivitis score to plaque score and other plaque parameters about selected teeth*[a]

Determination	Plaque parameter[b]			
	Plaque score	Plaque DNA content (μg)	Total colony-forming units (×10⁶)	Age of plaque (days)
Gingivitis score				
0.0	[0.43 (0–1.5)]	4.7 (ND-21)	[8.58 (<0.001–77.0)]	1.4 (0–14)
		↕		
0.5	[1.36 (0–2.5)]	29.4 (ND-120)	68.7 (0.006–220)	10.5 (0–21)
1.0	1.65 (0.5–2.5)	23.5 (ND-110)	60.8 (0.002–230)	13.4 (0–21)
1.5	1.86 (1–3)	18.4 (ND-64)	48.1 (7–110)	17.0 (7–21)
2.0	1.97 (1–3)	24.9 (ND-120)	81.8 (5.6–310)	20.6 (14–21)
Significance (*P*)				
Analysis of variance	0.000	0.03	0.001	
Kruskal-Wallis test	0.000	0.007	0.000	

[a] Teeth 13, 24, and 30 were measured at 0, 1, 2, and 3 weeks; additional teeth (4 to 12) were measured at 3 weeks.

[b] Mean values (range given in parentheses). ND, Not detectable. Values within boxes are significantly different by the Scheffe test from all other values in that column. Values connected by arrows are significantly different by the Scheffe test.

the plaque, i.e., plaque score, plaque DNA content (micrograms), or total viable count, as the gingivitis score progressed from 1.0 to 2.0. In fact, the significant changes that occurred in the plaque came at the low gingivitis scores and appeared to be related to increases stemming from the low base-line values (Table 5).

The total counts of the numerically dominant cultivable flora were stratified according to the gingivitis score (Table 6). The counts of all organisms increased as the gingivitis developed, as would be expected given the base-line nature of the 0.0 gingivitis score. Only the counts of *A. viscosus*, *A. israelii*, the enterococci, *Veillonella*, and *Bacteroides melaninogenicus* increased significantly by an analysis of variance (Table 6). The Scheffe test revealed that the levels of *A. viscosus* and *B. melaninogenicus* were significantly higher when the gingivitis score was 2.0 than at any other gingivitis score. Of these two organisms, *A. viscosus* was the more prominent, outnumbering *B. melaninogenicus* by about 260 to 1. The counts of *A. israelii* increased significantly in the intervals going from 0.0 to 0.5 and from 1.5 to 2.0. The enterococci counts peaked significantly at a gingivitis score of 0.5. The *Veillonella* counts at a gingivitis score of 0.0 were significantly lower than at the other gingivitis scores. *A. naeslundii* did not exhibit any obvious relationship to the development of gingivitis. Although there was no overall significance by an analysis of variance for *S. sanguis* and *S. mitis* (*mitior*), the Scheffe test did show that these species increased significantly going from a gingivitis score of 0.0 to 0.5. The levels of the streptococci then declined slightly at the higher gingivitis scores.

The viable counts of each organism were converted to a percentage of the total count. The proportions of the gram-positive rods increased significantly as the gingivitis score increased; the proportions of the gram-positive cocci decreased significantly; and the proportions of the gram-negative species remained relatively unchanged

TABLE 6. *Relation of gingivitis score to levels (mean values) of cultivable bacteria in plaque about selected teeth*[a]

Bacterial type	Bacterial count at gingivitis score[b]:					Significance (*P*)	
	0.0	0.5	1.0	1.5	2.0	Analysis of variance	Kruskal-Wallis test
Gram-positive rods							
A. viscosus	0.28	3.28	5.27	2.75	[13.01]	0.005	0.001
Esculin-negative *Actinomyces*	1.64	4.32	3.78	2.06	2.49		0.01
A. israelii	1.03 ⟷	13.49	16.77	7.16 ⟷	21.99	0.016	0.000
A. naeslundii	0.64	4.46	3.80	2.71	6.41		0.003
Diphtheroids	0.00	0.16	0.40	0.06	0.59		
P. acnes	0.04	0.61	1.17	1.06	0.99		
Bacillus sp.	0.46	0.92	0.34	0.19	0.87		
Total	4.09	27.24	31.53	15.99	46.35		
Gram-positive cocci							
S. sanguis	2.21 ⟷	13.58	11.99	11.03	9.54		0.003
S. mitis (mitior)	1.46 ⟷	10.77	9.13	6.85	9.65		0.001
Enterococci	0.02	[1.04]	0.33	0.14	0.00	0.077	
Micrococcus sp.	0.01	0.53	0.60	0.77	1.20		
Total	3.70	25.92	22.05	18.79	20.39		
Gram-negative spp.							
Veillonella sp.	[0.87]	13.14	9.82	10.76	12.10	0.04	0.000
F. nucleatum	0.18	0.76	0.41	1.43	1.45		0.001
C. sputorum	0.00	0.74	0.28	0.26	1.22		
B. melaninogenicus	0.00	0.01	<0.01	<0.01	[0.05]	0.03	
Total	1.05	14.65	10.51	12.45	14.82		
n	20	20	48	14	19		

[a] Teeth 13, 24, and 30 were sampled at weeks 0, 1, 2, and 3; additional teeth (4 to 12) were sampled at week 3.

[b] Average values × 10^6. Values within boxes are significantly different by the Scheffe test from all other values in that row. Values connected by arrows are significantly different by the Scheffe test.

(Table 7). An analysis of variance showed that the proportions of *A. viscosus*, *A. israelii*, *C. sputorum*, and *B. melaninogenicus* increased significantly with the severity of the gingivitis, whereas those of the *Bacillus* species decreased significantly. The proportions of *A. viscosus* and *B. melaninogenicus* at a gingivitis score of 2.0 were significantly higher than their proportions at any other gingivitis score. *A. viscosus* was now a prominent plaque organism, accounting for 14% of the flora, whereas *B. melaninogenicus* was a minor member of the flora, comprising less than 0.1% of the cultivable organisms. The significant increases in the percentage of *A. israelii* occurred primarily in the intervals going from 0.0 to 0.5 and from 0.5 to 1.0, and were coincident with the increases observed in the same intervals for the total gram-positive rods (Table 7). The percentage of *C. sputorum* also increased significantly with severity of the gingivitis score, but this significance could not be associated with any interval increment. The *Bacillus* species accounted for about 5% of the flora at a gingivitis score of 0.0 and thereafter decreased to less than 1.0% of the flora. The percentage of the total gram-positive coccal flora was significantly higher at a gingivitis score of 0.0 than at the higher gingivitis scores. The proportions of *S. sanguis* and *S. mitis* steadily declined as the gingivitis developed. The proportions of *A. naeslundii* were relatively constant, ranging from 4.3 to 7.2% at the various gingivitis scores.

Forty-six plaques had a plaque score of 2.0 (Table 1). This large number of plaques presented an opportunity to examine the interactions of plaque age, gingivitis scores, and bacterial composition when the plaque biomass was held constant, i.e., plaque score equals 2.0. The effect of plaque age on this subset of plaques

TABLE 7. *Relation of gingivitis score to proportions of the prominent cultivable plaque organisms (mean values) about selected teeth*[a]

Bacterial type	Bacterial proportion (% of total) at gingivitis score[b]:					Significance (*P*)	
	0.0	0.5	1.0	1.5	2.0	Analysis of variance	Kruskal-Wallis test
Gram-positive rods							
A. viscosus	2.2	5.6	6.5	6.5	[14.1]	0.003	0.007
Esculin-negative *Actinomyces*	4.7	8.0	5.3	5.0	4.5		
A. israelii	[3.1]	13.1 ⟷	26.0	15.9	24.8	0.000	0.001
A. naeslundii	4.3	5.0	6.5	7.2	6.7		
Diphtheroids	0.0	0.1	0.4	0.1	0.9		
P. acnes	0.1	1.2	1.4	2.9	1.7		
Bacillus sp.	[4.7]	0.6	0.4	0.3	0.7	0.019	
Total	[19.2]	33.6 ⟷	46.5	37.9	53.4	0.000	0.001
Gram-positive cocci							
S. sanguis	32.1	20.7	18.8	20.1	11.6		
S. mitis (mitior)	23.8	19.6	16.9	16.6	14.5		
Enterococci	1.5	2.1	0.9	0.3	0.0		
Micrococcus sp.	3.6	1.8	1.2	1.4	1.3		
Total	[61.0]	44.2	37.8	38.4	27.4	0.001	0.01
Gram-negative spp.							
Veillonella sp.	13.6	17.1	15.6	20.9	16.2		
F. nucleatum	2.0	1.5	1.3	2.6	1.2		
C. sputorum	0.0	0.9	0.4	0.8	1.5	0.048	
B. melaninogenicus	0.0	<0.01	<0.01	0.01	[0.08]	0.047	
Total	15.6	19.5	17.3	24.3	18.9		0.03
n	20	20	48	14	19		
Total count ($\times 10^6$)	8.6	68.7	60.8	48.1	81.8		

[a] Teeth 13, 24, and 30 were measured at 0, 1, 2, and 3 weeks; additional teeth (4 to 12) were measured at week 3.

[b] Average values. Values within boxes are significantly different from all other values in that row. Values connected by arrows are significantly different by the Scheffe test.

was determined, and the only interrelationships that were significant by an analysis of variance are shown in Table 8. The gingivitis score increased significantly as the plaque aged from 1 to 2 weeks (Table 8). This coincided with a significant increase in the proportions of *A. israelii* and a significant decrease in the proportions of *S. sanguis*. No other species, analyzing either their absolute or their relative levels, showed any signficant relationship with the development of gingivitis as a function of time. However, if the proportions of *A. viscosus* and *A. naeslundii* were combined, there was an overall significant increase in their number as the plaque aged. It was of interest that in this subset of plaques, the total viable count was highest in the 1-week-old plaques and declined with plaque age (Table 8).

The same 46 plaques were stratified according to their gingivitis scores. The absolute and relative levels of the various species at each gingivitis score were examined by an analysis of variance, and those showing significance and/or prominence are displayed in Table 9. *A. viscosus* and *B. melaninogenicus* were the only organisms that increased significantly at a gingivitis score of 2.0. When the gingivitis scores were 0.5, 1.0, and 1.5, *A. viscosus* accounted for about 5×10^6 organisms and for about 5% of the flora. At a gingivitis score of 2.0, the absolute and relative levels of *A. viscosus* increased fourfold (Table 9). *A. naeslundii, A. israelii,* and total gram-positive rods showed no similar changes with increasing gingivitis score. The percentage of gram-positive cocci decreased as the gingivitis worsened, whereas the proportions of the gram-negative species remained unchanged (Table 9). *B. melaninogenicus* was the only gram-negative species positively associated with the higher gingivitis scores, but even then its numbers comprised only about 0.2% of the flora. The *Veillonella* were proportionately prominent at all gingivitis scores, but their contribution to the viable flora remained unchanged as the gingivitis developed. The total viable count decreased as the gingivitis score increased, due mainly to a significant 45% reduction as the gingivitis score went from 0.5 to 1.0.

DISCUSSION

The increase in plaque and gingivitis scores as a function of plaque age is reproducible (15, 27) and has served as the basis for studies of the various host factors (7, 12, 27) possibly involved. The present investigation examined those plaque factors related to the numerically dominant cultivable plaque flora to determine which, if any, species or groups of organisms could be associated with the development of gingivitis. Uncultivable organisms such as the potentially pathogenic spirochetal species (18) would not be considered in this analysis.

Plaque accumulation and gingivitis were statistically related but not in a linear fashion. When the gingivitis score was plotted as a function of the plaque score (Fig. 1A), the gingivitis score increased in two large and significant increments. The first increment was associated with the development of a nonbleeding gingivitis (gingivitis score, 1.0) at a plaque score of 1.0, and the second increment with the development of a bleeding gingivitis (gingivitis score, 2.0) at a plaque score of 2.5. The nonbleeding gingivitis occurred at about day 9 of no brushing at a time when the viable counts were relatively low, i.e. about 20% of those obtained at a plaque score of 2.5 (Table 2). The most apparent flora change was a proportional increase in the *Actinomyces* species, led by *A. israelii*, at the expense of the

TABLE 8. *Relation of plaque age to gingivitis score and proportions of plaque bacteria at a plaque score of 2.0*

Determination	No. of plaques	Parameter[a]				
		Gingivitis score	Bacterial count ($\times 10^6$)	Proportion (% of total) of bacteria		
				S. sanguis	*A. israelii*	*A. viscosus* + *A. naeslundii*
Age of plaque (weeks)						
1	8	0.75	102	46.7	8.9	3.3
		↕			↕	
2	13	1.15	81	21.4	30.4	11.4
3	25	1.30	67	10.2	21	19.3
Analysis of variance (P)		0.01		<0.001	0.04	0.01

[a] Average values. All values connected by arrows are significantly different by the Scheffe test. Values within boxes are significantly different by the Scheffe test from all other values in that column.

TABLE 9. *Relation of gingivitis score to proportions of plaque bacteria and other parameters at a plaque score of 2.0*[a]

Determination	Other parameters					Proportion (avg %) of bacteria									
	No. of plaques	Age of plaque (days)	Bacterial count ($\times 10^6$)			Gram-positive rods			Gram-positive cocci			Gram-negative spp.			
			Total	*A. viscosus*	*B. melaninogenicus*	*A. israelii*	*A. viscosus*	*A. naeslundii*	Total	*S. sanguis*	*S. mitis (mitior)*	Total	*Veillonella* sp.	*C. sputorum*	*B. melaninogenicus*	Total
Gingivitis score																
0.5	8	13.2	126.5[b]	5.4	0.027	15.9	3.3	5.7	32.1	26.2	14.8	44.0	16.3	2.5	0.01	20.3
1.0	23	16.1	69.8[b]	5.6	0.002	26.4	5.7	5.2	45.9	19.9	15.7	37.1	15.4	1.5	<0.1	17.3
1.5	8	18.3	54.3	2.6	0.002	21.2	5.7	9.1	45.2	23.7	11.3	36.2	14.2	2.5	0.01	18.1
2.0	7	20	73.6	20.4	0.11	17.8	20.6	10.6	56.1	7.4	12.3	19.9	17.7	0.2	0.16	20.0
Analysis of variance (P)			0.07	0.06	0.06		0.004								0.06	

[a] Values within boxes are significantly different by the Scheffe test from all other values in that column.
[b] Values are significantly different by the Scheffe test.

Streptococcus species (Table 4). There were minimal changes in the gingivitis score as the plaque score increased to 2.0, despite the fact that the total viable count had significantly increased in number and the plaque was 17 days old (Table 2). The onset of the bleeding gingivitis occurred at a plaque score of 2.5 and was coincident with a significant upsurge in the viable count, dominated by a significant increase in *A. israelii*. Both increases in the gingivitis score as a function of the plaque score were associated with a proliferation of *A. israelii* in the plaque.

When the plaque score was plotted as a function of the gingivitis score (Fig. 1B), the plaque score increased significantly at gingivitis scores of 0.5 and 1.0 and then plateaued at the higher gingivitis scores. From this perspective, plaque accumulation was associated with the development of the nonbleeding gingivitis and coincided with a significant increase in the proportions of the gram-positive rods and *A. israelii* (Table 7). This would suggest that *A. israelii's* contribution to the nonbleeding gingivitis was mediated by an increase in plaque size or biomass. *A. israelii* also increased as the bleeding gingivitis developed. However, this gingivitis was dominated by an absolute and relative increase in the levels of *A. viscosus* and, to a lesser extent, *B. melaninogenicus* (Tables 6 and 7). The numbers of *A. viscosus* were approximately 200 times those of *B. melaninogenicus*, which suggests from a numerical argument that the *A. viscosus* changes were the more important when bleeding occurred.

The availability of a sufficient number of plaques with a plaque score of 2.0 permitted the examination of the interrelationship of gingivitis and flora minus the effect of plaque biomass. The total viable counts in this subpopulation significantly decreased as the gingivitis score increased from 0.5 to 1.0, and thereafter remained relatively constant (Table 9). Thus we have the surprising situation of the plaque score remaining constant and the viable count actually decreasing as the gingivitis develops. This set of events does not support a primary etiological role for plaque size or bacterial numbers per se in the gingivitis. Rather, the length of exposure of the gingival tissues to the plaque accumulations (Table 8) and/or an intraplaque shift to a more virulent flora (Table 9) appeared to account for the gingivitis. The bacteriological profile showed that, when bleeding occurred, the levels and proportions of *A. viscosus* and *B. melaninogenicus* increased significantly. Since the *A. israelii* levels and/or proportions did not change as the gingivitis developed, this would indicate that the *A. israelii* contribution to the

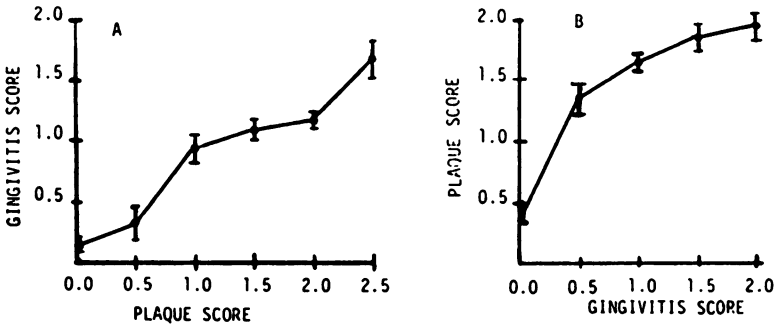

FIG. 1. *Interactions between plaque and gingivitis scores during a 3-week period of no oral hygiene in young adults. Mean values ± standard error.*

bleeding gingivitis, seen previously when the entire data set was analyzed, was mediated through an increase in plaque size or biomass. In contrast, the *A. viscosus* and *B. melaninogenicus* contributions to the bleeding gingivitis were mediated through their ascendancy in the plaque flora independent of an obvious clinical increase in plaque size.

The demonstration of a proportional increase in the levels of one or more bacterial species as the gingivitis developed is not by itself adequate evidence that these organisms caused the gingivitis. In fact, the increase of *B. melaninogenicus*, from undetectable to about 0.2% of the cultivable flora, could well be the result of the bleeding that occurred, since these organisms tend to localize where their nutritional needs for hemin and vitamin K can be satisfied (17). Nonetheless, the emergence of *B. melaninogenicus* in the predominant flora is not a good omen, as certain subspecies of this organism are pathogenic in experimental mixed anaerobic infections (32); are isolated in high numbers from mentally retarded individuals with periodontal disease (21), from periodontal pockets (28), and from periodontal abscesses (Syed, unpublished data; V. Grinenko, M. S. Weiner, H. J. Karge, I. Angel, M. G. Newman, and T. N. Sims, J. Dent. Res. **56**:B121, 1977); and produce collagenase and other end products capable of destroying tissue (4, 31). The considerably higher numbers of *A. israelii* and *A. viscosus* in the plaques associated with bleeding, however, is suggestive of some role for these organisms in the development of gingivitis. *A. israelii* has been implicated in oral actinomycotic infections (3), but not in gingivitis.

Immunological studies indicate that *A. viscosus* antigens can penetrate the gingival tissue and elicit a T-cell response in various forms of periodontal disease (1, 5, 8, 11, 25, 30). This possibility was examined for by assaying recent isolates of *A. viscosus, A. naeslundii, S. sanguis, B. melaninogenicus, Veillonella,* and *Fusibacterium nucleatum* for their ability to elicit a T-cell blastogenesis in other subjects during the period of no oral hygiene (29). Only antigens derived from *A. viscosus* significantly increased the stimulation index as the gingivitis developed. When oral hygiene was reinstituted, the T-cell response to *A. viscosus* returned to the base-line values. A similar decrease in T-cell response to *A. viscosus* antigens following treatment of naturally occurring gingivitis has been reported (26).

The present investigation separated the catalase-positive *A. viscosus* strains from the closely related catalase-negative *A. naeslundii* strains (6). *A. naeslundii* could not be statistically associated with any stages of the gingivitis. If *A. naeslundii* and *A. viscosus* were identical, then one would expect no differences between them as the gingivitis developed. Further evidence that these organisms are distinct comes from the observation that *A. naeslundii* colonization of humans precedes *A. viscosus* colonization by several years (2).

These findings raise the possibility that proportional changes in the gingival plaque flora may uniquely contribute to the development of gingival inflammation in this model. This is contrary to the prevalent opinion, which sees the gingivitis developing as a result of the nonspecific proliferation of the plaque flora (15, 31). This opinion dictates that periodontal therapy be directed towards the mechanical debridement of the dentogingival surfaces so as to keep the plaque mass within limits that can be tolerated by the approximating gingival tissues (19). However, if specific organisms are etiologically involved in gingivitis and other forms of periodontal disease, then treatment can be delivered according to the tenets of the specific plaque hypothesis (19). This treatment approach suggests that short-term intensive application of effective antimicrobial agents may have prolonged residual effects on the plaque flora.

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