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Subgingival microbiota associated with puberty: studies of pre-, circum-, and postpubertal human females

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Abstract

Children frequently exhibit gingivitis, while rarely exhibiting periodontitis. This age-associated difference in disease susceptibility may be due to host changes, microbial changes, or both. While age-related shifts in the "normal" microbiota of man and animals have been well documented, attempts to associate pubertal human development with changes in the subgingival microbiota remain unclear. This study determined whether pubertal changes in dental, skeletal, chronological, and sexual development in a population of 22 human females aged 7 to 16 years were correlated with changes in the distribution of subgingival plaque microorganisms. The predominant cultivable microbiota recovered from subgingival plaques generally comprised 48% Gram-negative rods, with 44% of the microbiota consisting of strict anaerobes. Saccharolytic black-pigmented Bacteroides *species (BPBs) were detected in 64% of the subjects;* Haemophilus actinomycetemcomitans *were detected in 27%.* Actinomyces *species, found in 68% of the subjects, were predominant microorganisms. Spirochetes, detected in 73% of the subjects, represented approximately 6% of the microscopic count.* H. actinomycetemcomitans *was unrelated to subject maturation, while the levels of* Actinomyces naeslundii *were lower in subjects with greater degrees of skeletal, dental, and chronologic maturation. The percentage of BPB species in the subgingival plaque was higher in those subjects who were soon to experience menarche, as compared to that recovered from sexually more immature or mature females in this study. No association existed between the detection of BPBs and chronologic age.*

Periodontal diseases are a group of bacterially induced inflammatory diseases which result in loss of connective tissue and bone support for the teeth. Two of these diseases, gingivitis and adult periodontitis, affect 3 out of 4 American adults.[1] Adult periodontitis is thought to begin as a chronic gingivitis, which, if left untreated, may shift to a progressing periodontitis which involves the clinical loss of periodontal attachment. The progression from gingival health to periodontitis is associated with shifts from a Gram-positive, aerobic-microaerophilic microbiota in the gingival crevice, to a predominantly Gram-negative, strictly anaerobic one.[2-7] Although these studies have demonstrated an association between specific bacterial species and the periodontal diseases (i.e., *Bacteroides gingivalis, Eikenella corrodens, Wolinella recta, Fusobacterium nucleatum, Bacteroides* species, and *H. actinomycetemcomitans*), our current understanding of the relationship of the indigenous microbiota and the development of these disease states in the physiologically maturing human female population is unclear.

Although children (i.e., ages 4–17) almost universally exhibit gingivitis,[8] periodontitis as defined by clinical and radiographic loss of periodontal support, has been found to affect less than 1% of adolescents.[9] While several investigations[10-13] have examined the basic clinical and biological parameters associated with periodontal disease prevalence in children, there still remains no clear *raison d'être* for the differences observed between children and adults in their "susceptibility" toward periodontitis. Several important microbiological and biological differences in the status of the gingival crevice between children and adults could account for the difference in periodontal disease prevalence in these 2 groups: for example, (1) adults have a different gingival crevice microbiota than children; (2) children have a much more immature plaque microbiota compared to adults, with the same components, but in different levels; (3) significant differences exist between the humoral and cellular defense mechanisms in adults and children; and (4) combinations of these factors may occur.

There are few studies[11,13-16] which have examined the gingival crevice microbiota in normal children, but these have not shown a clear association

between physiologic childhood development and changes in the subgingival microbiota. For example, DeAraujo and MacDonald[14] found low numbers of black-pigmented *Bacteroides* species in the subgingival plaque of preschool-aged children (i.e., 3–7 years), while both Bailet et al.[15] and Kelstrup[16] detected an increase in these microorganisms especially when examined in dental plaque samples as a function of age during childhood and adolescence (i.e., 5–15 years). Further, while these early studies indicated possible relationships between age and colonization of the gingival crevice by black-pigmented *Bacteroides* species, several other studies have indicated a greater degree of variability in the recovery of this genus from populations of young children and adolescents.[11,13] These differences in recovery of black-pigmented *Bacteroides* species might be attributed to a variety of factors, the most important of which could have been the method of sampling, microbiological culturing, and even methodological differences in clinical determinants.

Clearly then, whether any relationship exists between the oral microbiota and childhood physiological development—especially through puberty—remains to be determined. In addition to the confounding of microbiological results described above, these early studies were further confounded by the fact that chronologic age was the sole developmental assessment used in these microbiological studies. Numerous studies[17–19] have revealed that during puberty, there are wide individual variations in sexual, somatic, and dental development, and therefore, determination of these developmental factors, in addition to the chronologic age of the subject, are important in any studies which attempt to associate biological changes with age (i.e., puberty). This is especially important when studying microbiological changes, since it is already known that changes in hormonal levels have an effect on the composition of the subgingival microbiota.[20]

Therefore, this investigation sought to determine if there were any significant changes in the subgingival microbiota of young human females during developmentally defined stages of puberty, and whether these microbiological changes could be related to physiological developmental changes.

Methods and Materials

The subject population consisted of 22 healthy females, aged 7–16 years, who represented pre-, circum-, and postpubertal individuals. Each subject had available recent dental and skeletal (hand-wrist) radiographs taken as pretreatment orthodontic records. All subjects denied a history of recent dental prophylaxis or recent use of antibiotics.

Clinical Characterization

Chronologic age was recorded in years and months. Skeletal age (reflecting somatic development) was determined using radiographic evaluation of ossification on hand-wrist films, with the Skeletal Maturation Assessment system.[21] Sexual maturation was assessed both by report of menarche, and by physical examination of breast and pubic hair development by a pediatric endocrinologist (SKR) as described by Tanner.[22] Dental age was determined using radiographic assessment of crown and root formation of the developing mandibular canines, first and second premolars, and first, second, and third molars.[19]

Standard periodontal measurements of sampled teeth included the Plaque Index,[23] the Papillary Bleeding Index,[24] and probing depth measurements with a Michigan 'O' probe[a] in millimeters from the gingival margin.

Subgingival plaque was sampled from the mesiobuccal aspects of both mandibular first permanent molars by procedures routine in our laboratory when small quantities of plaque are anticipated. The site to be sampled was isolated with cotton rolls, dried, and supragingival plaque removed with a sterile Gracey curette;[b] subgingival plaque from each site then was removed from the sulcus with a second sterile Gracey curette. The curette tip was placed into a sterile vial containing glass beads and 0.5 ml of prereduced transport fluid (RTF)[25] without ethylenediamine-tetraacetate, and agitated so that adherent plaque was deposited into the vial. The sample vials for each subject were transported directly to a Coy anaerobic chamber,[c] and processed.

Plaque samples in RTF were dispersed by vortexing, and a portion of the RTF sample was counted directly using phase contrast and darkfield optics. The remainder of the sample was diluted and plated by Spiral Plating[d] onto selective and nonselective media, and incubated in the anaerobic chamber (5% CO_2 + 10% H_2 + 85% N_2) for 5–7 days. Two dilutions per sample were plated to Mitis-Salivarius (M-S) agar,[26] *H. actinomycetemcomitans* medium,[4] and in duplicate onto Enriched Trypticase Soy Agar (ETSA).[27] Total facultative anaerobic colony-forming units (CFU) were enumerated on ETSA after incubation at 37°C in 5% CO_2 plus air for 5–7 days; total anaerobic CFU were enumerated on ETSA after incubation in the anaerobic chamber for 5–7 days. The predominant cultivable microbiota of the sampled subgingi-

[a] Hu-Friedy: Chicago, IL.
[b] Hu-Friedy: Chicago, IL.
[c] Coy Manufacturing Co: Ann Arbor, MI.
[d] Spiral Systems: Bethesda, MD.

TABLE 1. Distribution of Subjects According to Developmental Assessment

Chronologic age (years)	7	8	9	10	11	12	13	14	15	16		
Number of subjects	1	1	0	1	7	7	0	3	1	1		
Mean = 12.4 ± 2.0 years												
Dental age (years)	5	6	7	8	9	10	11	12	13			
Number of subjects	1	1	0	1	3	4	4	7	1			
Mean = 10.9 ± 2.0 years												
Skeletal maturation	<1	1	2	3	4	5	6	7	8	9	10	11
Number of subjects	2	0	1	2	1	2	2	3	4	0	3	2
Tanner breast stage	1	2		3	4	5						
Number of subjects	4	3		7	5	3						
Tanner pubic hair stage	1	2		3	4	5						
Number of subjects	4	4		5	6	3						
Menarchal status	Premenarchal						Postmenarchal					
Number of subjects	12						10					
Menarchal stages	>1 year*			<1 year*			<1 year†			>1 year†		
Number of subjects	6			6			4			6		

* Menarche predicted by breast and pubic hair maturation.
† Postmenarchal status based on report by subject and parent.

val sites was determined from these anaerobically incubated ETSA plates. Sectors were scribed onto each ETSA plate by means of a stainless steel wedge-shaped template. Thirty to 50 colonies within specific sectors of the template were counted for the calculation of the total CFU per sample, and representative colonies within the sector were subcultured to other ETSA plates, purified, and further characterized (see below).

After ascertaining the purity of each isolate, cells were transferred to Basal Anaerobic Broth (BAB) with 1% (w/v) glucose and Basal Esculin Nitrate Broth (BEN).[28] The pure cultures also were diluted into sterile skim milk with 1% (v/v) DMSO, frozen in liquid nitrogen, and stored at −80°C for further biochemical tests as required. All black-pigmented *Bacteroides* species were picked into broth for inoculation of the Minitek biochemical series[e] and into sterile water for the An-Ident System[f] for determination of rapid carbohydrate utilization and aminopeptidase activity.

Streptococcal CFU were enumerated on M-S agar after incubation at 37°C in 5% CO_2 plus air for 48 hr; the *H. actinomycetemcomitans* selective medium was incubated in the anaerobic chamber for 4 days. Colonies which developed were enumerated under a binocular dissecting microscope. Catalase activity of the representative colony types was determined by placing a drop of 3% (v/v) H_2O_2 on the colony, and observing O_2 evolution. Presumptive *H. actinomycetemcomitans* colonies, characterized as circular, convex, translucent colonies with starlike inner structure, with positive catalase activity, were then subcultured and further characterized by biochemical methods for confirmation as described above.

All pure cultures were subjected to a computer-assisted bacterial identification system, using current schemes.[29,30] Black-pigmented *Bacteroides* species were classified according to Holdeman and Johnson[31] and Johnson and Holdeman.[32]

Clinical and developmental parameters were tested for correlation with the microbial findings, using the Spearman rank correlation coefficient. In addition, correlations between stages of sexual maturation and levels of specific bacterial species were tested using the Kruskal-Wallis one-way analysis of variance by ranks, and the Mann-Whitney *U*-test.

Results

As summarized in Table 1, subjects in this investigation represented a broad range of somatic, dental, and sexual development. Chronologically, the 22 subjects ranged in age from 7.3 to 16.9 years, with a mean age of 12.4 ± 2.0 years; dental age, based on calcification of forming teeth, revealed the subjects to range between 5.3 and 13.5 years, with a mean dental age for the group of 10.9 ± 2.0 years; and the Skeletal Maturation Assessment of hand-wrist films demonstrated the full circumpubertal range of skeletal calcification. On physical examination of breast and pubic hair development, the 22 subjects ranged from clearly prepubertal (Tanner stage 1) to fully mature (Tanner stage 5). Ten of the 22 subjects had experienced onset of menses, from 3 to 58 months prior to participating in the study. By means of a cluster analysis (Fig 1), the 3 indicators of sexual maturation (menarche, breast development, and pubic hair development) grouped subjects into four stages: (1) pre-

[e] Minitek Biochemical Differentiation Discs—BBL: Cockeysville, MD.
[f] API Diagnostic Laboratories: Plainview, NY.

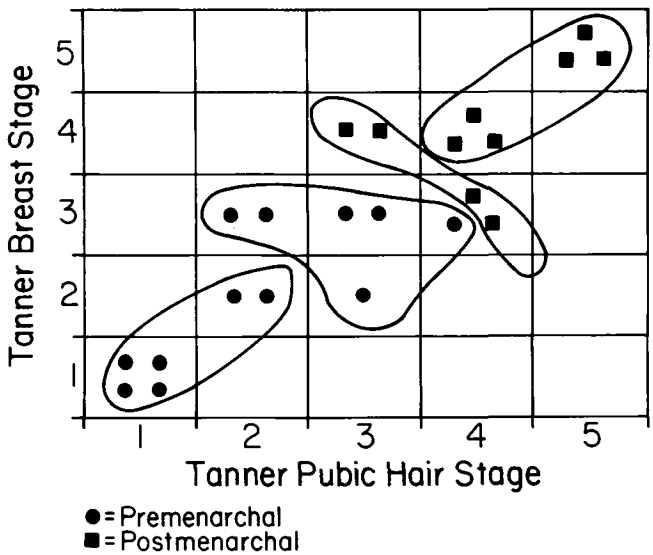

FIG 1. Cluster analysis using the 3 indicators of sexual maturation (menarche, breast development, and pubic hair development) to group subjects into the following 4 groups: (1) premenarchal subjects with absent or incipient signs of sexual development; (2) premenarchal subjects with sexual maturation and predictive of menarche within about 1 year; (3) postmenarchal subjects who had experienced onset of menses from 1 to 12 months before participation in the study; and (4) physically mature subjects with menarche more than 1 year earlier.

menarchal subjects with absent or incipient signs of sexual development; (2) premenarchal subjects with sexual maturation predictive of menarche within about 1 year; (3) postmenarchal subjects who had experienced onset of menses from 1 to 12 months before participation in the study; and (4) physically mature subjects with menarche more than 1 year earlier.

Tested for correlation with the Spearman rank correlation coefficient, all growth parameters were significantly related to each other at the 0.0005 level of significance, with correlation coefficients ranging from 0.73 to 0.88.

Clinical evaluation of gingival health of the 44 molar sites sampled subgingivally in the 22 subjects are shown in Table 2. The subjects showed a mean Plaque Index of 1.36 (3/44 sites with PI = 0; 23/44 sites = 1; 17/44 sites = 2; 1/44 sites = 3), and a mean Bleeding Index of 1.27 (2/44 sites = Bleeding Index = 0; 28/44 sites = 1; 14/44 sites = 2; 0/44 sites = 3). The mean probing depth measurement was 2.75 mm (1/44 sites = 1 mm; 12/44 sites = 2 mm; 28/44 sites = 3 mm; 3/44 sites = 4 mm). Clearly, then, all of the subjects taken as a mean possessed sample sites which exhibited gingivitis associated with demonstrable plaque, and with no loss of periodontal attachment from the cementoenamel junction at any site. Unlike a previously reported peak in frequency of gingivitis

TABLE 2. Periodontal Description of Sampled Sites

	Mean	*SD*	*Range of Scores*
Plaque Index*	1.36	0.64	0–3
Papillary Bleeding Index†	1.27	0.54	0–2
Probing depth (mm)	2.75	0.61	1–4

* Löe and Silness, 1963.
† Saxer and Mühlemann, 1975.

at about age 13 for females,[33] no increase in "puberty gingivitis" was seen in the present study.

The overall distribution of the various morphological bacterial species recovered from the predominant cultivable microbiota of the 44 subgingival sites in the 22 study subjects demonstrate significant subject-to-subject variability (Table 3). Gram-negative rods were the predominant morphological type observed, and comprised a mean of 48.2 ± 24.5% of the predominant cultivable microbiota, with a broad range of 2.5–100%. In this population of children, Gram-positive rods were the next most frequently isolated morphological type, and constituted 31.0 ± 23.3% of the cultivable microbiota. Gram-negative and Gram-positive cocci comprised 4.1 and 7.1% of the cultivable microbiota, respectively; they never exceeded 34.2 or 41.2%, respectively, of the cultivable microbiota from any site. When tested for correlation with developmental parameters, the highly variable levels of each of these bacterial morphological types in the cultured subgingival plaque were unrelated to maturation.

Bacterial strains requiring a strict anaerobic environment for growth constituted a mean of 44.0 ± 24.6% of the predominant cultivable microbiota of all sampled sites, and ranged from 1.2 to 92.6%, reflecting the highly variable nature of bacterial plaque sampled from subgingival sites in these children.

Actinomyces species, representative of Gram-positive facultatively anaerobic rods, were isolated from the subgingival bacterial plaque samples from 68.2% of the subjects, with levels in the predominant cultivable microbiota ranging from nondetectable to 64.9% of the microbiota, with a mean of 9.5 ± 14.8% (Table 4). *Actinomyces naeslundii, A. viscosus,* and *A. odontolyticus* were recovered in a ratio of 87:7:1 from the subgingival plaque of the subjects. When analyzed with the Spearman rank correlation coefficient, the total *Actinomyces* species (mean of 2 sites) in the predominant cultivable microbiota of the 22 subjects was negatively correlated to the developmental parameters of chronologic age, dental age, and skeletal maturation ($P < 0.05$). In contrast, the levels of total *Actinomyces* species were unrelated to all indicators of sexual maturation when tested with the Spearman rank correlation coefficient, as well as being unrelat-

TABLE 3. Bacterial Morphology of Predominant Cultivable Microbiota Recovered from 44 Subgingival Sites in 22 Circumpubertal Females

	Mean	SD	Range
Gram-negative rods	48.2	24.5	2.5–100
Gram-positive rods	31.0	23.3	0–86.5
Gram-negative cocci	4.1	9.8	0–37.3
Gram-positive cocci	7.1	11.0	0–34.2

ed to the menarchal stages (composite sexual maturation) when tested with the Kruskall-Wallis 1-way analysis of variance by ranks.

Bacterial isolates with the colonial morphology described as "surface translocating" on primary isolation included *Capnocytophaga, Wolinella,* and *Eikenella* species in this study population. These surface translocating bacteria were detected in 17 of the 22 subjects (77.3%), and constituted a mean of 5.7 ± 7.1% of the predominant cultivable microbiota of the gingival crevice for the study population. The levels of this colonial morphotype varied between subjects and between sites within subjects, and ranged from nondetectable to 35.7% of the predominant cultivable microbiota. Statistical analysis of the relationship of levels of these surface translocating bacteria in the subgingival bacterial plaque to development status of the subjects revealed a significant negative correlation between this colonial morphotype and the sexual maturational indices of Tanner breast stage (i.e., Spearman rank order correlation: $P < 0.02$; Kruskall-Wallis 1-way ANOVA: $P < 0.05$), Tanner pubic hair stage (Spearman: $P < 0.01$; Kruskall-Wallis: $P < 0.01$), and menarchal stages (Kruskall-Wallis: $P < 0.01$). A similar correlation could not be demonstrated for chronologic, dental, and skeletal developmental indices for these subjects.

Employing an *H. actinomycetemcomitans* selective medium, this Gram-negative facultatively anaerobic rod (which is strongly implicated in the etiology of periodontal destruction in the circumpubertal age group) was isolated in low levels from subgingival bacterial plaque samples from 6 of the 22 subjects (27.3%). Detection was unrelated to any developmental or periodontal parameter.

Spirochetes represented a mean of 5.7 ± 9.3% of the microscopic count for all sites sampled, and ranged from nondetectable to 41.2% of the microscopic count. Seen in bacterial subgingival plaque samples from 16 of the 22 subjects (72.7%) of all developmental stages, neither detection nor levels of treponemes in the microscopic count were related to subjects' maturational or periodontal indices for these children and adolescents with plaque-associated gingivitis.

TABLE 4. Isolation of Selected Species from the Gingival Crevice

	% of Predominant Cultivable Microbiota			Isolation (Subjects)
	Mean	SD	Range	
Total *Actinomyces* species	9.5	14.8	0–64.9	15/22 (68.2%)
A. naeslundii	8.7	13.9	0–64.9	13/22 (59.1%)
A. viscosus	0.7	3.2	0–20.0	4/22 (18.2%)
A. odontolyticus	0.1	0.5	0–2.9	2/22 (9.1%)
Total surface-translocating bacteria	5.7	7.1	0–35.7	17/22 (77.3%)
Total *F. nucleatum*	2.2	4.4	0–17.2	9/22 (40.9%)
Total *Bacteroides* species	5.3	10.0	0–54.0	14/22 (63.6%)
B. intermedius	4.5	9.5	0–54.0	13/22 (59.1%)
B. melaninogenicus	0.6	2.3	0–11.5	5/22 (22.7%)
B. denticola	0.2	0.9	0–5.7	3/22 (13.6%)
B. gingivalis	0.0			0/22 (0%)

The black-pigmented *Bacteroides* species, Gram-negative obligate anaerobic rods, were isolated in the predominant cultivable microbiota of the gingival crevice of 14 of the 22 subjects (Table 4). All *Bacteroides* species isolated were saccharolytic, and comprised a mean of 5.3 ± 10.0% of the predominant cultivable microbiota. *Bacteroides intermedius* was the most commonly isolated *Bacteroides* species (13/22 subjects) and was routinely recovered at higher levels than other *Bacteroides* species. *Bacteroides intermedius* comprised 4.5% of the predominant cultivable microbiota, while *B. melaninogenicus* and *B. denticola* were detected in 5 and 3 subjects, respectively. *Bacteroides gingivalis* was not isolated from any of these 22 children with gingivitis. Statistical analysis revealed that the levels of these black-pigmented *Bacteroides* species in the predominant cultivable microbiota of the subgingival plaque in the 22 subjects were related to only 1 developmental assessment, that of menarchal stage, or composite sexual maturation (Kruskall-Wallis: $P < 0.05$). Further analysis with the Mann-Whitney *U*-test demonstrated significantly higher levels of black-pigmented *Bacteroides* species in the subgingival plaque from females within about 1 year of menarche, as compared to less sexually mature subjects (predicted greater than 1 year until menarche), and those who had already experienced menarche within the past year.

Discussion

The predominant cultivable microbiota and microscopic levels of spirochetes recovered from the subgingival plaque of pre-, circum-, and postpubertal children was ascertained to determine if certain microorganisms already considered important "candidates" in the pathogenesis of adult periodontitis could be found only in postpubertal individuals, thus providing some insight into the role of physiological

endocrinologic changes in the shift of the microbial ecology from one of "health" to one of "disease." The absence of detectable shifts in these microbial populations during puberty, or the presence of an "adult" microbiota at a very young age in this population, would provide evidence to indicate possible changes in host defense factors during the aging and developmental process.

The mean percentage of Gram-negative rods observed in this study (48 ± 25%) was considerably higher than that reported by Slots[34] (13%) for adult gingival health; it was similar to that reported for adult gingivitis.[35] Similar to a study by Gusberti et al. of diabetic children, we found no statistically significant changes in the per cent recovery of Gram-negative rods as a function of the subject developmental status. In fact, the wide range of recoveries of Gram-negative rods precludes the detection of any statistically significant differences in their levels as a function of the developmental process. Regardless of the absence of statistical significance, there were higher numbers of Gram-negative rods in the subgingival microbiota of younger children than has previously been assumed.

Gram-positive rods (31%) cultivated from subgingival plaque of children was intermediate between the 45% Gram-positive rods reported for adult gingivitis[34] and the 26% found in adult gingivitis,[35] while the Gram-negative cocci (only 4%) were also very similar to those reported (2%) in adults with healthy gingiva[34] as well as in adults with gingivitis (4%[35]).

In contrast to Slots,[34] we recovered a mean of 7% Gram-positive cocci in our subjects, compared to the 40% recovery in adult gingival health.[34] This low number of Gram-positive cocci in children with gingivitis is similar to the 3% found in adult gingivitis,[35] and is comparable to those of Gusberti et al.[36] for diabetic children with gingivitis.

The total number of obligate anaerobes recovered from the subgingival plaque (i.e., 44%) is identical to that of Slots et al.[35] from the subgingival microbiota of adult gingivitis, and differs from the 24% obligate anaerobes found in gingival health in adults.[34] It is also similar to Gusberti et al.,[36] who found more than 50% obligate anaerobes in the subgingival microbiota of diabetic children. Since no significant increase in the per cent of obligate anaerobes recovered from our normal subjects as a function of developmental age was found in our study or that of Gusberti et al.,[36] the establishment of significant numbers of anaerobes in the gingival crevice appears to occur in the very young child, rather than during the circumpubertal period.

The high levels of *A. naeslundii* compared to *A. viscosus* and *A. odontolyticus* in the present study is also in agreement with Slots et al.[35] for subgingival plaque taken from human gingivitis sites. Ellen[37] found up to 24 times more catalase-positive *Actinomyces*-like isolates (presumably *A. viscosus*) than catalase-negative isolates (*A. naeslundii*) in dental plaque from children, using a selective medium for recovery of *Actinomyces* species. Gusberti et al.[36] also used a selective medium for the recovery of *Actinomyces* species, but found approximately equal proportions of *A. naeslundii* and *A. viscosus* in the subgingival plaque from diabetic children. We found a decreasing proportion of *A. naeslundii* in the subgingival plaque as a function of increasing chronological, dental, and skeletal development; however, there was no statistically significant change in the levels of *Actinomyces* species with sexual maturation, also at variance with Gusberti et al.,[36] who reported elevated levels of *Actinomyces* species in plaque samples from Tanner stage 3 subjects. Although their Tanner stage 3 subjects exhibited significantly higher levels of *A. naeslundii* than that recovered from other developmental groups, the ratio of *A. naeslundii* to *A. viscosus* remained approximately equal.

The numbers of selected surface translocating bacteria (STBs) and age of onset of the various forms of periodontal disease has been of interest (see for example, Moore et al.[38]). We observed a significant negative correlation between the levels of these STBs (i.e., *Capnocytophaga, Wolinella*) in the subgingival plaque and both breast and pubic hair development, as well as menarchal status, supporting the suggestion of Moore et al.[38] that STBs are found in higher levels in young children with gingivitis than in older subjects with experimental gingivitis. Interestingly, while a statistically significant relationship between decreasing levels of STBs and developmental status could be demonstrated for sexual development in this study, a similar relationship could not be demonstrated for somatic or dental indices of development.

The low recovery of *H. actinomycetemcomitans* (27% of the subjects) is similar to that of Slots et al.[4] While *H. actinomycetemcomitans* has been considered to be part of the normal subgingival microbiota of adolescents,[4] high levels of this species in the subgingival plaque of adolescents may be suggestive of a clinical observation of juvenile periodontitis. Similar detection rates in all developmental stages (pre-, circum-, and postpubertal subjects) suggests microbial colonization of the gingival crevice of children well before puberty.

The number of spirochetes observed represented approximately 6% of the microscopic count, somewhat higher than that reported for diabetic children of the same age range.[36] We observed spirochetes in the subgingival plaque in 73% of these subjects, in-

consistent with Yanover and Ellen's[39] observations of the absence of spirochetes in the subgingival plaque of adolescents. However, their subjects had less plaque, gingivitis, and shallower probing depths. The children and adolescents in our study have a detection rate and levels of spirochetes in the subgingival plaque consistent with that of adults with gingivitis.[40]

Finally the detection of saccharolytic BPBs in our subjects is not surprising, as this species has previously been detected in varying amounts both in adults and children displaying every state of gingival health.[11,15,41] We have not been able to demonstrate a direct relationship between increasing chronologic age and increasing incidence of BPBs in the gingival crevice similar to that reported previously.[15,16] However, our finding of a significant relationship between levels of BPBs and menarchal stage is of interest in that the near-menarche subjects, who had higher levels of BPBs than either the early premenarchal group (prepubertal) or the early postmenarchal group (menarche had occurred within 1 year), were undergoing a large number of physiologic changes which might influence the subgingival microbiota. At present there are very little data concerning pubertal host defense changes.[10,12]

Less subtle but of potentially equal or greater importance to microbiological changes are the increasing levels of circulating hormones which appear during puberty. Although studies have not specifically related changes in steroid hormone levels at puberty with periodontal disease or the subgingival microbiota, laboratory experiments and observations of the gingival and bacterial responses to altered-hormone states (i.e., pregnancy, oral contraceptive use) suggest an important relationship might exist between steroid hormone levels and changes in the subgingival microbiota, especially with BPBs.[20,42] Thus, similar changes in the subgingival microbiota may be occurring at puberty, as progesterone and estrogen levels are known to increase dramatically.[43]

Conclusions

Based on the findings of this study, the following conclusions were made.

1. The predominant cultivable microbiota of subgingival plaque in this group of children was comparable to that reportedly found in adults with healthy gingiva as well as those adults with gingivitis.
2. Significant changes in components of the microbiota of the gingival crevice were associated with the skeletal, sexual, dental, and/or chronologic age of subjects.
3. Observations suggest that it may be possible to associate some of these changes in the microbiota of the gingival crevice during puberty with alterations in hormonal levels.

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Quotable Quote: ice eases toothache pain

University experts have discovered a simple way to ease the throbbing pain of a toothache—simply apply ice on the web of skin between the thumb and index finger.

Doctors at Canada's McGill University applied ice cubes wrapped in gauze to the hand on the same side of the body as the toothache pain for 7 min or until the area felt completely numb, whichever came first. They found that ice massage relieved the intensity of pain by at least 50% in the majority of patients, according to the *Body Bulletin.*