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Black-Pigmented *Bacteroides* Spp. in the Human Oral Cavity

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Five healthy children under 6 years of age, five healthy adults, and 10 adult periodontitis patients were examined for the prevalence and distribution of black-pigmented *Bacteroides* in the oral cavity. A total of 13 samples was obtained from each individual, including four supragingival and four subgingival dental plaques, dental occlusal surface, buccal mucosa, dorsal tongue, tonsil, and whole saliva. Black-pigmented *Bacteroides* were recovered from nine adult periodontitis patients. Healthy adults harbored the organisms in low incidence and proportions, whereas the children exhibited no cultivable black-pigmented *Bacteroides*. The organisms were isolated in highest proportions from dental plaque, especially subgingival plaque, and from the tonsil area, indicating that these sites constitute the organisms' primary ecological niche in the oral cavity. The predominant isolate was *Bacteroides melaninogenicus* subsp. *intermedius* followed by *Bacteroides gingivalis* and *B. melaninogenicus* subsp. *melaninogenicus*. *B. melaninogenicus* subsp. *levii* constituted low proportions of supragingival microflora of one adult periodontitis patient. A positive correlation was demonstrated between the proportion of black-pigmented *Bacteroides* (mainly *B. melaninogenicus* subsp. *intermedius*) and both the severity of gingival inflammation and the periodontal pocket depth, suggesting that these organisms may contribute to the pathogenesis of certain forms of periodontal disease.

Black-pigmented *Bacteroides* species are commonly isolated from severe anaerobic infections of the intestinal tract, the female genital tract, and the respiratory tract (8). These organisms can also be present in high numbers in adults with rapidly progressing periodontitis lesions (24) and in *Macaca arctoides* with experimentally induced periodontal disease (20).

The pathogenic potential of *Bacteroides melaninogenicus* subspecies, *Bacteroides asaccharolyticus*, and *Bacteroides gingivalis* (previously oral *B. asaccharolyticus* [6]) is well established. Strains of these species play an essential role in transmissible subcutaneous infections produced by complex mixtures of indigenous oral bacteria (15, 22); they possess endotoxins with bone resorption potential (10), they produce hydrogen sulfide, ammonia, and other cytotoxic substances (21), and they can elaborate highly proteolytic enzymes, including collagenase and trypsin (9). These organisms are also able to inhibit phagocytosis and killing of other bacteria by polymorphonuclear leukocytes (12), and they can stimulate the host immune system to produce substances with tissue-destroying potential (16).

Little data is available on the prevalence and distribution of *B. melaninogenicus* subsp. and *B. gingivalis* in the oral cavity. Such information might be valuable in elucidating the pattern of these species' oral colonization and would also be desirable and essential in treating oral infections by these organisms.

In this study, we aimed to examine the intraoral distribution of black-pigmented *Bacteroides* species in children and adults with a healthy periodontium and in adults with moderate to severe periodontal disease.

Correlations were also sought between the microbiological findings and the clinical status of the periodontal tissues.

MATERIALS AND METHODS

Subjects. A total of three subject groups was included in this study. One group was comprised of five children between the ages of 3 and 5 years with complete deciduous dentition and a healthy periodontium. One group consisted of five adults, 25 to 36 years of age, with minimal gingival inflammation and no areas of alveolar bone loss exceeding 1 mm. One group of 10 adults, 29 to 53 years of age, exhibited moderate to severe alveolar bone breakdown (≥5 mm loss of periodontal attachment) and significant gingival inflammation on more than five teeth. None of the subjects had received dental treatment or antibiotic therapy at least 6 months before this study.

Sample collection and processing. The following 13 sites were sampled in each adult periodontitis patient: supragingival and subgingival plaque from one

proximal surface of an upper left molar or premolar associated with gingivitis and alveolar bone loss; supragingival and subgingival plaque from the facial surface of a mandibular anterior tooth associated with gingivitis and alveolar bone loss; supragingival and subgingival plaque from one proximal surface of an upper left molar or premolar associated with a healthy periodontium; supragingival and subgingival plaque from the buccal surface of three to four mandibular left molars and premolars; left lateral tongue; left tonsil; left buccal mucosa; occlusal surface of the maxillary left teeth; and unstimulated whole saliva. Five adult periodontitis patients exhibited no healthy periodontal sites. In these subjects, sites with the least inflammation and pocket depth were sampled. In the children and healthy adults studied, a similar number of oral sites was sampled. The supragingival and subgingival plaque samples in these individuals were collected from healthy or slightly inflamed sites. For each plaque sample site, the pocket depth as determined by use of a Michigan probe, the gingival index (17), and the plaque index (14) were determined. No patients showed gross evidence of tonsillitis.

Supragingival plaque was sampled by a sterile periodontal curette. After removal of supragingival plaque by means of sterile cotton balls, subgingival plaque was collected on three paper points (Johnson Fine Absorbent Points; Johnson and Johnson, East Windsor, N.J.) inserted to the depth of the periodontal pocket for 10 s each. Soft tissue samples and occlusal plaque samples were collected with a Calgiswab, type II (Inolex, Glenwood, Ill.) from an area of approximately 1 cm^2. These samples were transferred within 1 or 2 s to 3 ml of prereduced, anaerobically sterilized Ringer solution. Whole, unstimulated saliva was collected by expectoration, and $\frac{1}{10}$ ml of saliva was placed in 3 ml of prereduced, anaerobically sterilized Ringer solution. Further microbiological manipulations were performed by using continuous anaerobic technique.

Samples of supragingival plaque were dispersed by pulsed sonicaton (Sonifier Cell Disrupter, model 350; Bronson Sonic Power Co., Danbury, Conn.) for 60 s at 25 W. After dispersion, the samples were serially diluted in 10-fold steps in prereduced, anaerobically sterilized Ringer solution. Samples of appropriate dilutions were plated onto tryptic soy agar (Difco Laboratories, Detroit, Mich.) supplemented with 5% rabbit blood, 5.0 μg of hemin per ml, and 0.5 μg of menadione (enriched tryptic soy agar) per ml and onto a selective medium composed of enriched tryptic soy agar supplemented with 40 μg of kanamycin (Sigma Chemical Co., St. Louis, Mo.) per ml (as described by K. Kornman, University of Connecticut, School of Dental Medicine, Farmington, Conn.). After 7 days of incubation in an anaerobic chamber (Coy Manufacturing Co., Ann Arbor, Mich.) containing 85% N_2, 10% H_2, and 5% CO_2, the total number of colonies and the total number of black-pigmented colonies on the blood agar plates were enumerated. The proportional recovery was calculated from the medium with the higher number of black-pigmented colonies. Up to 10 black-pigmented colonies selected at random were subcultured from each sample site.

Using established procedures (5, 11), each pure culture was identified by Gram stain characteristics, morphology, anaerobiosis, hemagglutination activity for sheep erythrocytes, fermentation of glucose, starch, esculin, fructose, mannose, and lactose, production of catalase and indole, hydrolysis of starch and esculin, and production of metabolic acids in peptone-yeast extract-glucose broth cultures. The key criteria for identifying black-pigmented, gram-negative anaerobic rods as to species were as follows: *B. gingivalis*-designated oral organisms which were asaccharolytic, indole positive, catalase negative, did not hydrolyze starch or esculin, exhibited strong hemagglutinating activity and produced acetic, propionic, isobutyric, *n*-butyric, isovaleric, and phenylacetic acids as metabolic acid end products. *B. melaninogenicus* subsp. *intermedius* organisms fermented glucose and fructose, were indole positive, catalase negative, did not hydrolyze starch or esculin, and produced acetic, isobutyric, and isovaleric acids as metabolic acid end products. *B. melaninogenicus* subsp. *melaninogenicus* included those organisms which fermented glucose, fructose, and raffinose, were indole negative, catalase negative, hydrolyzed esculin but not starch, and produced acetic, isobutyric, and isovaleric acids. *B. melaninogenicus* subsp. *levii* were defined as organisms which fermented glucose, were indole negative, catalase negative, did not hydrolyze esculin or starch, and produced acetic, proprionic, isobutyric, *n*-butyric, and isovaleric acids as metabolic acid end products. In addition, the identification of black-pigmented organisms as to species was substantiated by immunofluorescence with specific antisera.

RESULTS

In Table 1 the relative recovery efficiency of the nonselective blood agar medium and the kanamycin-containing selective medium for culturing organisms of the *B. melaninogenicus* group is shown. *B. melaninogenicus* subspecies and *B. gingivalis* were more frequently recovered on the selective medium than on the nonselective medium when the organisms were present in small proportions (<1%). When the organisms composed a larger portion of the microflora, they were often recovered in greater numbers on the nonselective medium than on the selective medium.

Of the 20 individuals examined, black-pigmented *Bacteroides* species were recovered from 9 of 10 adult periodontitis patients, from 3

TABLE 1. *Relative recovery efficiency of selective and nonselective media for black-pigmented Bacteroides*[a]

Total *B. melaninogenicus/B. gingivalis*	No. of sites with higher counts on the following medium[b]:	
	Selective	Nonselective
<1%	15	9
>1%	8	18

[a] A total of 50 positive sample sites.
[b] $P < 0.025$ as determined by chi-square analysis.

of 5 healthy adults, and from none of 5 healthy children.

The distribution of *B. melaninogenicus* subspecies and *B. gingivalis* in healthy adults and adult periodontitis patients is demonstrated in Table 2. Of 65 sites examined in healthy adults, 5 sites (subgingival plaque, tongue, tonsil, and occlusal surface [two sites]) yielded growth of the organisms. The adult periodontitis group exhibited cultivable *B. melaninogenicus* subspecies and *B. gingivalis* in 35% of the supragingival plaque samples, in 47% of the subgingival plaque samples, in 20% of the saliva and buccal mucosa samples, in 60% of the tonsil area samples, and in 30% of the occlusal surface samples (Table 2). The organisms were not recovered from the tongue surface of any adult periodontitis patient.

In Table 3 the proportional recovery of *B. melaninogenicus* subspecies and *B. gingivalis* from the infected sample sites is shown. In the healthy adult group, black-pigmented *Bacteroides* species comprised 2% or less of the cultivable microflora in all sample sites studied. In contrast, these organisms constituted as much as 24% of the supragingival plaque isolates, 59% of the subgingival plaque isolates and up to 11% of salivary and tonsillary isolates in the adult periodontitis group (Table 3).

B. melaninogenicus subsp. *intermedius, B. melaninogenicus* subsp. *melaninogenicus* and *B. gingivalis* were recovered from healthy adults without predominance of any species or subspecies (Table 3). In the adult periodontitis group, *B. melaninogenicus* subsp. *intermedius* was recovered from eight adult patients, and it was the only black-pigmented *Bacteroides* organism recovered in six patients. The proportional recovery of this organism was as high as 40 to 60% in seven subgingival samples, whereas the organism made up only 10 to 15% of the total isolates in four supragingival plaque samples, in one saliva sample, and in one tonsil sample (Table 3). *B. gingivalis* was recovered from five study sites and comprised maximally 12% of the cultivable microflora (Table 3). *B. melaninogenicus* subsp. *melaninogenicus* was only isolated from three sites and formed less than 1 to 10% of the cultivable flora (Table 3). One supragingival dental plaque sample contained organisms which biochemically and serologically resembled *B. melaninogenicus* subsp. *levii.*

TABLE 2. *Oral infection of black-pigmented Bacteroides species in periodontally healthy and diseased adults*

Site	No. of sites with cultivable *B. melaninogenicus* subspecies and *B. gingivalis*/total in:	
	Healthy subjects (5)	Diseased subjects (10)
Plaque (4 sites/subject)		
Supragingival	0/20	14/40
Subgingival	1/20	18/40
Saliva	0/5	2/10
Tongue	1/5	0/10
Buccal mucosa	0/5	2/10
Tonsil	1/5	6/10
Occlusal surface	2/5	3/10
Total number of sample sites	65	130
Total number of infected sites	5	45
% Infected sites	8	35

In Table 3 the relationship of black-pigmented *Bacteroides* between subgingival and supragingival dental plaque samples in infected adult periodontitis patients is shown. The organisms were recovered from subgingival sites more frequently and in higher proportions than from supragingival sites.

A total of 10 tooth surfaces harbored these bacteria both supragingivally and in the periodontal pocket. *B. melaninogenicus* subsp. *intermedius* was an infecting organism of all these 10 tooth surfaces. One tooth surface of patient 6 was subgingivally infected with *B. gingivalis*, while other sites were infected with *B. melaninogenicus* subsp. *intermedius.* In patient 9, one subgingival site harbored *B. melaninogenicus* subsp. *intermedius* and *B. melaninogenicus* subsp. *melaninogenicus* and the corresponding supragingival site was infected with *B. melaninogenicus* subsp. *intermedius, B. melaninogenicus* subsp. *levii, and B. gingivalis.*

Spearman rank order correlation (3) was used to determine the relationship between the recovery of black-pigmented *Bacteroides* and the periodontal pocket depth and the gingival inflammation index. A highly significant, positive correlation ($P < 0.001$) was found between the proportion of cultivable subgingival *B. melaninogenicus-B. gingivalis* and the periodontal pocket depth (Table 4). There were also significant positive correlations ($P < 0.01$) between the proportions of cultivable *B. melaninogenicus-B. gingivalis* in supragingival and subgingival dental plaque samples and the gingival inflammation index. No significant correlation was demonstrated between the proportions of supragingival *B. melaninogenicus-B. gingivalis* isolates and the periodontal pocket depth.

DISCUSSION

The large number of bacterial species which inhabit the oral cavity limits the number of

TABLE 3. *Distribution of cultivable B. melaninogenicus subspecies and B. gingivalis in the oral cavity of periodontally healthy and diseased adults*[a]

Patient	Tooth no.	Pocket depth (mm)	Gingival index	% of organism at the following sites[b]: Plaque: Supragingival	Plaque: Subgingival	Saliva	Tongue	Buccal mucosa	Tonsil	Occlusal surface
Healthy (3 of 5)										
1	28, M	4	1		<1, I					1, M
2							<1, G			
3									2, G	<1, I
Periodontitis (9 of 10)										
4									10, M	
5	19, B	3	1	<1, I				<1, I		
	8, M	4	1		<1, I					
6	3, M	7	1	9, I	9, I	10, I			<1, I	<1, I
	23, D	5	2	<1, I	43, I					
	4, M	3	0		<1, I					
	19, B	2	0		<1, G					
7	10, M	7	2	<1, I	49, I				<1, I	
	19, D	5	1	<1, I						
	4, D	5	1		2, I					
8	4, M	3	1	2, I	2, I	<1, I		3, I	<1, I	<1, I
	12, M	7	1	<1, I						
	18, B	3	0	<1, I						
9	31, M	5	2	12, I<1, L 12, G	49, I <1, M				2, G	
	29, D	4	2	11, I	51, I					
	24, M	6	2	13, I	59, I					
	4, M	4	2	10, I	11, I					
10	12, M	3	1	<1, I	55, I					<1, I
	18, M	6	1		<1, I					
	24, M	8	2		8, I					
11	3, D	6	1		28, I					
	30, D	8	1		30, I					
	14, M	7	1	<1, I	41, I					
12									11, I	

[a] Symbols for organisms are: I, *B. melaninogenicus* subsp. *intermedius*; M, *B. melaninogenicus* subsp. *melaninogenicus*; L, *B. melaninogenicus* subsp. *levii*; G, *B. gingivalis*.
[b] Only sites infected with black-pigmented *Bacteroides* are listed.

TABLE 4. *Statistical correlations between the proportion of B. melaninogenicus-B. gingivalis and clinical indexes in adult periodontitis patients*

Correlation	rho[a]	*P*
B. melaninogenicus-B. gingivalis cultivable from subgingival plaque and pocket depth	+0.74	0.001
B. melaninogenicus-B. gingivalis cultivable from subgingival plaque and gingival inflammation index	+0.687	0.01
B. melaninogenicus-B. gingivalis cultivable from supragingival plaque and pocket depth	+0.286	0.1[b]
B. melaninogenicus-B. gingivalis cultivable from supragingival plaque and gingival inflammation index	+0.716	0.01

[a] Spearman rank order correlation coefficient.
[b] Not significant.

subjects and sites which practically can be subjected to comprehensive microbiological characterization. Identification and enumeration of *B. melaninogenicus* organisms are facilitated by these species' black-pigmented colonies and the availability of a medium for their selective isolation. Our data, in agreement with previous findings by Kornman (personal communication), showed that the selective medium is especially suitable for recovery of black-pigmented *Bacteroides* when they are present in small numbers.

In our examination of children from 3 to 6 years of age with a complete deciduous dentition, we were unable to demonstrate any *B. melaninogenicus* subspecies or *B. gingivalis*. Berger et al. (2) reported similar findings in preschool children. De Araujo and MacDonald (7) found 4 of 15 children from 3 to 7 years of age to be infected with the organisms, and Bailit et al. (1) demonstrated in 320 children that black-pigmented *Bacteroides* increased in prevalence during the period of mixed dentition. On the basis of the available data, it seems reasonable to assume that organisms of the *B. melaninogenicus* group commonly colonize the oral cavities of children relatively late.

B. melaninogenicus subspecies and *B. gingivalis* have been reported present in low numbers and proportions in dental plaque of adults with

healthy periodontal tissues and more abundantly present in adults with advanced periodontal disease (13, 19). Our data agree with these findings. Healthy adults exhibited black-pigmented *Bacteroides* only in 1 of 40 dental plaque samples, whereas the adult periodontitis group had these organisms in proportions up to 59% in 32 of 80 plaque samples.

Study of the distribution of *B. melaninogenicus* subspecies and *B. gingivalis* on oral mucous membranes revealed that adult periodontitis patients harbored the organisms at more oral sites than did healthy adults. However, even in the most infected individuals, the organisms generally constituted less than 1% of the cultivable microflora of tongue, buccal mucosa, occlusal surface, and saliva samples. In tonsil sites, on the other hand, 6 of 10 adult periodontitis patients examined harbored black-pigmented *Bacteroides* species in proportions up to 11% of the cultivable microflora. Burdon (4) also recovered *B. melaninogenicus* in high proportions from each of 16 excised tonsils. It appears that after bacterial dental plaque, the tonsil area serves as the next most common oral site of infection for these organisms. The possibility exists that the tonsils may be a nidus for infection of other oral sites, including the periodontal pocket area, with *B. melaninogenicus* organisms.

The positive correlation between the proportion of black-pigmented *Bacteroides* in subgingival areas and periodontal pocket depth as demonstrated in this study, points to the importance of these organisms in the pathogenesis of periodontal disease. The cross-sectional nature of our study, however, excluded the determination of the organisms as primary etiological agents or secondary invaders in the periodontal disease process. Several tooth surfaces harbored the organisms subgingivally but not supragingivally, and no correlation was found between the proportion of the organisms in supragingival dental plaque and the periodontal pocket depth. This finding may reflect a less favorable growth environment in the supragingival area, but could also be due to the patients' efforts at oral hygiene. In this study we found a positive correlation between both the subgingival and supragingival proportion of black-pigmented *Bacteroides* (mainly *B. melaninogenicus* subsp. *intermedius*) and gingival inflammation. In contrast, Tanner et al. (24) only found minimal gingival inflammation associated with *B. melaninogenicus* subsp. *intermedius*. Another unidentified organism may contribute to the gingival inflammation in our adult periodontitis group or the host response of the patients examined in the two studies may have differed.

The difference in periodontopathic potential between the various species and subspecies of black-pigmented *Bacteroides* is not known. In our study, *B. melaninogenicus* subsp. *intermedius* was the predominant organism in adult periodontitis lesions. Slots (18) and Spiegel et al. (23) found *B. gingivalis* to predominate in most adult periodontitis lesions. Tanner et al. (24) reported that in a group of eight patients with advanced destructive periodontitis, two harbored mainly *B. gingivalis* and one mainly *B. melaninogenicus* subsp. *intermedius*. The predominance of *B. gingivalis* or *B. melaninogenicus* subspecies may be associated with differences in type and activity of the periodontal disease studied which cannot be satisfactorily quantitated with available, relatively insensitive clinical measurements. An alternative explanation is that *B. gingivalis* and *B. melaninogenicus* subsp. *intermedius* may not differ significantly in periodontopathic potential.

Both *B. melaninogenicus* subsp. *intermedius* and *B. gingivalis* appear to be important periodontopathic organisms. Further study is necessary to define the role of these bacteria in the pathogenesis of human periodontal disease.

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LITERATURE CITED

1. **Bailit, H. L., D. C. Baldwin, and E. E. Hunt, Jr.** 1964. The increasing prevalence of gingival *Bacteroides melaninogenicus* with age in children. Arch. Oral Biol. **9**: 435–438.
2. **Berger, V., M. Kapovits, and G. Pfeiffer.** 1959. Besiedlung kindlicher mundhohle mit anaeroben Mikroogani-smen. Z. Hyg. Infekt. **145**:564–573.
3. **Bruning, J. L., and B. L. Kintz.** 1977. Computational handbook of statistics, 2nd ed. Scott, Foresman and Co., Glenview, Ill.
4. **Burdon, K. L.** 1928. *Bacterium melaninogenicum* from normal and pathologic tissues. J. Infect. Dis. **42**:161–171.
5. **Cowan, S. T.** 1974. Cowan and Steel's manual for the identification of medical bacteria, 2nd ed. Cambridge University Press, London.
6. **Coykendall, A. L., F. S. Kaczmarek, and J. Slots.** 1980. Genetic heterogeneity in *Bacteroides asaccharolyticus* (Holdeman and Moore 1970) Finegold and Barnes 1977 (approved lists, 1980) and proposal of *Bacteroides gingivalis* sp. nov. and *Bacteroides macacae* (Slots and Genco) comb. nov. Int. J. Syst. Bacteriol. **30**: 559–564.
7. **De Araujo, W. C., and J. B. MacDonald.** 1964. The gingival crevice microbiota in five preschool children. Arch. Oral Biol. **9**:227–228.
8. **Finegold, S. M.** 1977. Anaerobic bacteria in human disease. Academic Press, Inc., New York.

9. **Gibbons, R. J.** 1974. Aspects of the pathogenicity and ecology of the indigenous oral flora of man. *In* A. Balows, R. D. Denham, V. R. Dowell, and L. B. Guze (ed.), Anaerobic bacteria: role in disease. Charles C Thomas, Publisher, Springfield, Ill.
10. **Hausmann, E., L. B. Raisz, and W. A. Miller.** 1970. Endotoxin: stimulation of bone resorption in tissue culture. Science **168**:862–864.
11. **Holdeman, L. V., E. P. Cato, and W. E. C. Moore.** 1977. Anaerobe laboratory manual, 4th ed. Virginia Polytechnic Institute and State University, Blacksburg, Va.
12. **Ingham, H. R., P. R. Sisson, D. Tharagonnet, J. B. Selkon, and A. A. Codd.** 1977. Inhibition of phagocytosis *in vitro* by obligate anaerobes. Lancet **ii**:1252–1254.
13. **Listgarten, M. A., and L. Hellden.** 1978. Relative distribution of bacteria at clinically healthy and periodontally diseased sites in humans. J. Clin. Periodontol. **5**: 115–132.
14. **Löe, H., and J. Silness.** 1963. Periodontal disease in pregnancy. I. Prevalence and severity. Acta Odontol. Scand. **21**:533–551.
15. **MacDonald, J. B., S. S. Socransky, and R. J. Gibbons.** 1963. Aspects of the pathogenesis of mixed anaerobic infections of mucous membranes. J. Dent. Res. **42**:529–544.
16. **Nisengard, R. J.** 1977. The role of immunology in periodontal disease. J. Periodontol. **48**:505–516.
17. **Silness, J., and H. Löe.** 1964. Periodontal disease in pregnancy. II. Correlation between oral hygiene and periodontal condition. Acta Odontol. Scand. **22**:121–135.
18. **Slots, J.** 1977. The predominant cultivable microflora of advanced periodontitis. Scand. J. Dent. Res. **85**:114–121.
19. **Slots, J.** 1979. Subgingival microflora and periodontal disease. J. Clin. Periodontol. **6**:351–382.
20. **Slots, J., and E. Hausmann.** 1979. Longitudinal study of experimentally induced periodontal disease in *Macaca arctoides*: relationship between microflora and alveolar bone loss. Infect. Immun. **23**:260–269.
21. **Socransky, S. S.** 1970. Relationship of bacteria to the etiology of periodontal disease. J. Dent. Res. **49**:203–222.
22. **Socransky, S. S., and R. J. Gibbons.** 1965. Required role of *B. melaninogenicus* in mixed anaerobic infections. J. Infect. Dis. **115**:247–253.
23. **Spiegel, C. A., S. E. Hayduk, G. E. Minah, and G. N. Krywolap.** 1979. Black-pigmented *Bacteroides* from clinically characterized periodontal sites. J. Periodontal Res. **14**:376–382.
24. **Tanner, A. C. R., C. Haffer, G. T. Bratthall, R. A. Visconti, and S. S. Socransky.** 1979. A study of the bacteria associated with advancing periodontitis in man. J. Clin. Periodontol. **6**:278–307.