The Bacteriology of Acute Necrotizing Ulcerative Gingivitis*

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Accepted for publication 25 September 1981

PLAQUE SAMPLES from 22 ulcerated sites in eight patients with ANUG were cultured using quantitative anaerobic procedures and were examined microscopically. The partial characterization of the predominant cultivable flora revealed a constant flora comprised of a limited number of bacterial types and a variable flora composed of a heterogeneous array of bacterial types. This constant flora would appear to be pathognomonic of acute necrotizing ulcerative gingivitis (ANUG) and included the various *Treponema* and *Selenomonas* sp., which comprised about 32 and 6%, respectively, of the microscopic count; *B. melaninogenicus* ssp. *intermedius* and *Fusobacterium* sp., which averaged 24 and 3%, respectively, of the viable count. One week of metronidazole treatment caused a prompt resolution of clinical symptoms, which coincided with a significant reduction in the plaque proportions of the *Treponema* sp., *B. melaninogenicus* ssp., *intermedius* and *Fusobacterium* sp. for at least 2 to 3 months following treatment. Thus, the same anaerobic species which were numerically associated with the ANUG lesion were also selectively reduced in the plaque flora following resolution of the infection. This supports a role for the above species in the ulcerative stage of the lesion but does not demonstrate that these specific anaerobes initiated the infection. Although not confirmed by the data, it was proposed that these particular anaerobic species gained ascendency in the plaque as a result of being selected through the availability of host-derived nutrients in individuals who had undergone certain physiological and psychological stresses.

Acute necrotizing ulcerative gingivitis (ANUG) is a relatively rare clinical entity, which generally has been described in young individuals.[1] The incidence of ANUG increases under conditions of physiological[2] and psychological stress,[1,3], a phenomenon which has been well documented among military personnel.[1,4,5] A fusospirochetal bacterial component has been identified on the basis of microscopic examination of plaque samples,[6,7] the demonstration of spirochetes in the tissue of the lesion[8] and the prompt resolution of the clinical lesion upon treatment with penicillin or metronidazole.[9-11] The antimicrobial studies indicate that the microbes present or dominant in the plaque contribute to the clinical symptoms, but this does not demonstrate that these organisms initiated the infection. Rather, the fusospirochetal complex could be opportunistic pathogens overgrowing in the plaque during those periods when the tissue defense mechanisms had been compromised by stress,[1] nutritional disturbances[2] or viral infections,[12] among others.[3]

The evidence for the involvement of the fusospirochetal organisms is based upon the microscopic appearance of plaque smears. Only a few attempts at cultivation of this flora have been reported and these, with the exception of the extensive investigations by Rosebury and his colleagues,[7] have been unsuccessful.[3,13] Rosebury used anaerobic methods and complex media to isolate what appeared to be representative members of the plaque flora. These studies were not quantitative in nature and resulted in the isolation and partial characterization of 58 strains comprising 11 taxonomic groups. Various large combinations of these strains were capable of causing abscess formation when injected into the groin of a guinea pig.[14] Macdonald et al.[15] simplified these combinations to a group of four organisms, which contained *Bacteroides melaninogenicus* but no spirochetes or fusiforms. Eventually, *B. melaninogenicus* was identified as the essential pathogen in this mixture.[16] Thus, the one

* This research was supported by Public Health Service Grants DE 03011, DE 02731 and F32 DE 05172 from the National Institute of Dental Research.

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cultural study of plaque taken from ANUG sites implicated *B. melaninogenicus* rather than the fusospirochetal organisms as the potential pathogen.

Anaerobic culturing methodology has improved in recent years, to the extent that the most fastidious anaerobic species can be cultured.[17] However, the quantitative isolation of the various spirochetal species remains a problem because these organisms are lysed by the procedures used to disrupt the plaque to obtain the single cells necessary for the isolation of pure colonies.[18] Accordingly, spirochetes have been identified and quantitated only by microscopic methods. In the present study plaque samples taken from discrete sites of ulceration were cultured using quantitative anaerobic procedures and were examined microscopically.

MATERIALS AND METHODS

Patients. Eight patients with one or more sites of interproximal necrosis and ulceration were seen in the research clinic. Four patients had one to three lesions and were afebrile. Another four patients had multiple sites of ulceration, were irritable and three of them had a mild fever. Three of these four patients had been unsuccessfully treated by a regimen of mechanical debridement, peroxide mouthwashes and oral hygiene. After bacteriological samples were taken from representative ulcers in these latter patients, they were given metronidazole, 250 mg, three times daily for 7 days. Bacteriological sampling was repeated immediately after the completion of the metronidazole and again 2 to 3 months later.

Cultural Procedure. Plaque was removed from discrete sites of ulceration by a curette, and the adherent plaque on the tip was transferred to a vial containing 0.5 ml of a reduced transport fluid (RTF) without EDTA.[19]

The plaque sample was immediately placed within the anaerobic chamber[20] and after dispersing for 20 seconds with a Vortex mixer, a 50 μl aliquot was removed for microscopic examination. The remaining sample was diluted to 4 ml, sonically dispersed for 20 seconds with a Kontes sonifier,* serially diluted in RTF and plated automatically with a spiral plater† on a variety of media. This dispersal procedure appears to give optimal recoveries of Gram-negative organisms from plaque samples, but lyses the spirochetes.

The total anaerobic count, the count of black-pigmented *Bacteroides* (BPB) species, *Capnocytophaga* species, *Fusobacterium* species and the red-brown-pigmented colonies of *Actinomyces odontolyticus* were obtained from growth on enriched trypticase soy agar (ETSA).[21] Either all or representative colonies of BPB were subcultured and speciated on their ability to use glucose, to hydrolyze esculin and to produce indole using the scheme shown in Table 1. *Actinomyces viscosus* and

Table 1
Scheme Used to Differentiate Black Pigmented Bacteroides Species

Species	Glucose fermentation	Indole production	Esculin hydrolysis
B. melaninogenicus			
ssp. *intermedius*	+	+	−
ssp. *melaninogenicus*	+	−	+
B. gingivalis	−	+	−

Actinomyces naeslundii were differentiated and enumerated on a cadmium sulfate-metronidazole selective medium.[22] *Streptococcus sanguis* and *Streptococcus mutans* were identified by their colony morphology on MM10 sucrose agar.[23] *Veillonella* colonies were enumerated on a medium containing the MM10 base minus the sucrose and blood, but supplemented with lactate, vancomycin[24] and a 0.004% bromocresol purple indicator. The total count of facultative organisms was obtained from growth on ETSA agar containing 20 μg/ml of metronidazole, which was incubated anaerobically.

In certain plaque samples the proportions of the cited species accounted for about 20% of the total viable count. In order to determine the identity of the other colonies, each colony on a high dilution plate that contained from 20 to 100 colonies was subcultured and partially characterized by its Gram stain and its ability to grow aerobically, ferment glucose, reduce nitrate, produce indole, hydrolyze starch and esculin and to exhibit gelatinase and catalase activity.

From the aliquot removed for microscopic examination, 10 μl was placed on a glass slide under a 22 × 30 mm cover glass, sealed and viewed by dark-field microscopy. Either 200 organisms or the number of organisms in 20 high power fields (hpf) were enumerated, depending on which event occurred first. The single cells were identified as *Treponema* species (spirochetes), large spirochetes, selenomonads, motile rods, fusiforms, nonmotile rods and cocci.[25] The proportions of each of these categories in the sample were determined. The protein in the sample was measured by a fluorometric method using fluorescamine.[26] The presence of blood and tissue debris in the plaque samples, however, reduced its usefulness in quantitation of the plaque sample.

Statistical Analyses. The total counts and the proportions of organisms in each cultural and microscopic sample were determined and statistically analyzed using the computer programs available in the Michigan terminal system. Comparisons between independent samples were analyzed by the parametric Student *t* test and the nonparametric Kruskal Wallis ranking procedure.[27] The effect of metronidazole treatment on the bacterial flora was evaluated by the paired *t* test and the Wilcoxon test.

RESULTS

Patients with ANUG were rarely encountered. In 3

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years only eight patients with ulceration were examined and from these, 22 sites were cultured. For the first three patients all isolates appearing on an ETSA plate were subcultured and partially characterized. Five hundred and twenty two isolates were obtained, which on the basis of the nine taxonomic characteristics used, could be placed into about 70 groups. In order to reduce this variability, some characteristics that were of a low frequency within a Gram stain category were omitted so as to provide the listing of the groups shown in Table 2. Even so, some 31 groupings could be identified.

The most frequently encountered groups were *B. melaninogenicus* ssp. *intermedius* (present in 11/11 sites); a Gram-positive anaerobic, asaccharolytic, nitrate negative rod (10/11 sites); *Fusobacterium* species (11/11 sites); Gram-positive facultative streptococci that were either esculin negative or positive (7/11 sites) and an anaerobic coccus that was asaccharolytic, nitrate negative and esculin variable (7/11 sites) (Table 2). No obvious pattern was discernible except that *B. melaninogenicus* ssp. *intermedius* accounted for 18% of the total isolates, mainly because it was so prominent in patients 1 and 3 (Table 2). Patient 2 has low proportions of *B. melaninogenicus* ssp. *intermedius* isolates, but had high proportions of

Table 2
Predominant Cultivable Flora Isolated From ANUG Sites

Patient		1*			2*			3*			Total	
No. of sites		4			4			3			11	
No. of isolates		156			222			144			522	
Statistical descriptors†	F	M	R	F	M	R	F	M	R	F	M	R
Species												
Gram + Rods		21.0%			23.8%			39.4%			27.3%	
A. odontolyticus	3/4	2.5	ND–5	2/4	5	ND–18	0/3	0	ND	5/11	2.7	ND–18
A. viscosus	1/4	2	ND–9	0/4	0	ND	1/3	2	ND–5	2/11	1.3	ND–9
A. naeslundii	3/4	3	ND–8	1/4	0.3	ND–1	1/3	7	ND–21	5/11	3.1	ND–21
Fac/S^-/N^+‡	2/4	2	ND–9	2/4	2	ND–6	0/3	0	ND	4/11	1.5	ND–9
S^- N^-	1/4	1	ND–3	1/4	0.5	ND–2	1/3	0.7	ND–2	3/11	0.6	ND–3
S^+ N^-	3/4	4	ND–10	3/4	6	ND–12	1/3	0.7	ND–2	7/11	3.5	ND–12
An S^- N^+	1/4	1	ND–5	0/4	0	ND	3/3	8	ND–15	4/11	2.7	ND–15
S^- N^-	3/4	4	ND–10	4/4	7	ND–12	3/3	13	7–22	10/11	7.5	ND–22
S^+ N^+	1/4	1	ND–3	0/4	0	ND	3/3	4	2–11	4/11	1.8	ND–11
S^+ N^-	1/4	0.5	ND–2	2/4	3	ND–9	3/3	4	3–8	6/11	2.6	ND–9
Gram + Cocci		26.3%			31.0%			19.7%			25.8%	
Fac/S^- N^-/E^-	3/4	4.8	ND–8	1/4	0.5	ND–2	0/3	0	ND	4/11	1.9	ND–8
S^+ N^- E^-	2/4	3	ND–9	3/4	3	ND–6	2/3	5	ND–7	7/11	3.5	ND–9
S^+ N^- E^+	3/4	7	ND–14	2/4	5	ND–19	2/3	7	ND–14	7/11	6.1	ND–19
S^+ N^+ E^+	2/4	3.5	ND–12	2/4	3	ND–10	1/3	2	ND–5	5/11	2.8	ND–12
S^- N^+ E^-	0/4	0	ND	1/4	1.5	ND–6	0/3	0	ND	1/11	0.5	ND–6
An S^+ N^- E^v	1/4	1	ND–5	1/4	2	ND–9	1/3	0.7	ND–2	3/11	1.5	ND–9
S^+ N^+ E^-	2/4	5	ND–14	2/4	1	ND–3	2/3	2	ND–4	6/11	2.7	ND–14
S^- N^- E^v	2/4	2	ND–5	4/4	15	4–22	1/3	3	ND–8	7/11	6.8	ND–22
Gram (−) Rods		50.3%			45.5%			38.7%			44.0%	
B. melaninogenicus												
ssp *intermedius*	4/4	32	26–40	4/4	1.4	0.5–6	3/3	23	12–43	11/11	18.4	0.5–43
ssp *melaninogenicus*	1/4	3	ND–13	1/4	0.5	ND–2	1/3	0.7	ND–2	3/11	1.5	ND–13
B. gingivalis	3/4	3	ND–5	1/4	3.5	ND–14	1/3	2	ND–7	5/11	1.9	ND–14
Bacteroides sp.	1/4	1	ND–5	4/4	9.5	3–20	0/3	0	ND	5/11	3.9	ND–20
Fusobacterium sp.	4/4	3	0.1–10	4/4	8	1–22	3/3	13	2–23	11/11	7.6	0.1–23
Capnocytophaga sp	2/4	1.5	ND–3	0/4	0	ND	0/4	0	ND	2/11	0.5	ND–2
Eikenella sp	1/4	1.8	ND–7	1/4	2	ND–6	0/3	0	ND	2/11	1.2	ND–7
Selenomonas sp	1/4	1	ND–3	4/4	18	9–25	0/3	0	ND	5/11	6.7	ND.25
"vibrio"												
$S^-E^-N^+$	2/4	1	ND–3	2/4	2	ND–6	0/3	0	ND	4/11	1.0	ND–6
$S^+E^-N^+$	0/4	0	ND	2/4	2	ND–8	0/3	0	ND	2/11	0.7	ND–8
Fac $S^+E^vN^-$	3/4	3	ND–5	0/4	0	ND	0/3	0	ND	3/11	1.1	ND–5
Gram (−) Cocci		4.7%			2.0%			0%			2.5%	
Veillonella sp	3/4	4.2	ND–9	2/4	2	ND–6	0/3	0	ND	5/11	2.3	ND–9
Neisseria sp	1/4	0.5	ND–2	0/4	0	ND	0/3	0	ND	1/11	0.2	ND–2

* Mean percentage for each individual derived by averaging the percentages from each of the sites samples in each subject.
† F = frequency, M = mean, R = range; ND = not detected in dilution ranges examined.
‡ Biochemical descriptors: S = saccharolytic, N = nitrate reduction, E = esculin hydrolysis, + = positive, − = negative, v = variable, An = anaerobic, Fac = facultative.

Selenomonas sp. and nonpigmented *Bacteroides* sp., both of which were rarely encountered in the other patients.

This partial characterization of each isolate indicated great variability between sites, even within the same mouth, and was most time consuming. Accordingly, the plaques from the subsequent patients, as well as those from the first three patients, were analyzed by enumerating those species which could be identified reliably by their colony morphology on selective or nonselective media. Only the black-pigmented bacteroides species were subcultured and speciated according to the scheme given in Table 1.

Three of the patients had a temperature of about 100°F, had multiple sites of ulceration and had been refractory to conventional treatment. The other five patients had no fever at the time of sampling, had limited sites of ulceration and had not been treated previously. The plaque flora removed from the sites of ulceration in these two groups of patients was compared in terms of levels (numbers) of the bacteria in the plaque samples (Table 3), or the proportions of these bacteria in the samples (Table 4).

More bacteria were cultured from plaques removed from the febrile patients (Table 3). However, no significant differences, with one exception, were found when the levels of the various bacteria in the plaques taken from the febrile patients were compared by either the Student *t* test or the Kruskal-Wallis test with their levels in the plaques taken from the nonfebrile patients. The exception was the significantly higher levels of *A. odontolyticus* in the febrile plaques (Table 3).

The proportions of the identified species in the plaque samples were calculated by dividing the level of each species in the plaque by the total number of cultivable colony forming units (CFU) in the plaque. Also the proportion of each morphological entity seen in the microscopic count was determined by dividing the levels of the morphological entity by the total number of organisms counted microscopically. There were no differences in the proportions of the various identified organisms in plaques taken from the febrile or the nonfebrile patients when analyzed by either the Student *t* test or the Kruskal-Wallis test (Table 4).

In both groups *B. melaninogenicus* ssp. *intermedius* was the most prominent species demonstrated by the cultural method and *Treponema* sp. were the dominant morphologic types identifiable in the microscopic flora (Table 4). The difference in the levels, but not the proportions of *A. odontolyticus* did not warrant the separation of the febrile group from the nonfebrile group. Accordingly, the values from all the patients were combined (Tables 3 and 4). *B. melaninogenicus* ssp. *intermedius,* the *Treponema* sp. and the *Fusobacterium* sp. were found in all plaque samples. *B. melaninogenicus* ssp. *intermedius* accounted for 24% of the cultural count, and the *Treponema* sp. for 30% of the microscopic count.

The *Fusobacterium* sp. represented about 2.6% and the *Capnocytophaga* sp. about 0.8% of the cultural count. Fusiforms, which would include both *Fusobacterium* and *Capnocytophaga* sp., comprised 4.9% of the microscopic count. The *Selenomonas* sp. were present in most microscopic samples,[17-20] but were not readily identifiable on

Table 3
Levels of Suspected Odontopathic Organisms in the Predominant Cultivable Flora of Plaque Taken From ANUG Sites in Febrile and Nonfebrile Patients

	Patients				Frequency
	Febrile (12)*		Nonfebrile (10)*	Total (22)*	
	C.F.U. × 10⁶ organisms per plaque sample				
Total count	171.3 ± 250.0†		65.6 ± 66.0	123.0 ± 194.0	
Facultative count	22.5 ± 38.2		13.5 ± 19.6	18.0 ± 30.0	
B. melaninogenicus ssp. *intermedius*	20.2 ± 19.8		19.7 ± 42.0	19.9 ± 31.0	22/22
B. gingivalis	<0.01‡		0.4 ± 1.3	0.2 ± 0.9	5/22
Capnocytophaga sp.	0.4 ± 0.9		0.5 ± 0.6	0.5 ± 0.8	15/22
Fusobacterium sp.	3.6 ± 6.1		1.0 ± 1.2	2.4 ± 4.7	22/22
A. odontolyticus	6.8 ± 12.3	(0.05)§	1.0 ± 2.0	4.1 ± 9.5	18/22
A. viscosus	0.5 ± 0.5		0.2 ± 0.2	0.3 ± 0.4	14/18
A. naeslundii	<0.01		0.01 ± 0.03	<0.01	1/18
S. sanguis	1.1 ± 12.4		0.9 ± 1.8	1.0 ± 1.6	16/18
S. mutans	<0.01		<0.01	<0.01	3/18
Veillonella	3.7 ± 9.2		2.7 ± 6.4	3.1 ± 7.6	13/18
Microscopic count organisms/hpf	46.0 ± 45.0		41.0 ± 100.0	43.1 ± 78	
Protein	70.0 ± 6.0		63.0 ± 13.0	67.0 ± 10.0	

* Number of sites in parentheses.
† Average ± standard deviation.
‡ <0.01 means that species was not detected at lowest dilution.
§ *P* value for Kruskal-Wallis Test, significance between febrile and nonfebrile.

Table 4
Proportions of Suspected Odontopathic Organisms in the Plaque Taken From ANUG Sites in Febrile and Nonfebrile Patients

Bacterial species	Patients		Total (22)*
	Febrile (12)*	Nonfebrile (10)*	
	Cultivable Flora		%
B. melaninogenicus ssp. *intermedius*	26.4 ± 22.4†	21.0 ± 20.0	24.0 ± 21.1
B. gingivalis	<0.01	1.9 ± 6.0	0.9 ± 4.0
Capnocytophaga sp.	0.3 ± 0.4	1.4 ± 3.0	0.8 ± 1.8
Fusobacterium sp.	2.4 ± 1.6	2.7 ± 3.0	2.6 ± 2.3
A. odontolyticus	3.2 ± 4.1	1.2 ± 2.0	2.3 ± 3.3
A. viscosus	2.1 ± 2.5	1.0 ± 2.0	1.5 ± 2.1
A. naeslundii	<0.01	0.2 ± 0.6	0.1 ± 0.4
S. sanguis	3.5 ± 4.1	1.5 ± 2.0	2.4 ± 3.0
S. mutans	<0.01‡	<0.01	<0.01
Veillonella sp.	1.6 ± 1.6	5.1 ± 12.0	3.5 ± 9.5
Facultative organisms	28.9 ± 23.8	18.0 ± 18.0	23.4 ± 21.0
	Per Cent of Microscopic Counts		
Treponema sp.	29.0 ± 17.0	31.0 ± 25.0	30.2 ± 21.1
Large	12.0 ± 13.0	7.0 ± 9.0	9.9 ± 11.5
Selenomonads	5.0 ± 2.0	9.0 ± 9.0	6.9 ± 7.3
Motile rods	2.0 ± 2.0	2.0 ± 4.0	2.2 ± 2.9
Fusiforms	5.0 ± 5.0	5.0 ± 6.0	4.9 ± 5.3
Rods & Cocci	61.0 ± 16.0	54.0 ± 19.0	57.4 ± 18.0

* Number of sites given in parentheses.
† Average ± standard deviation.
‡ No significant differences found between plaques taken from febrile or nonfebrile patients. Student *t* or Kruskal-Wallis tests.

the nonselective ETSA medium. *A. odontolyticus* was present in the majority of the plaque samples. No other sought-after organism was found either in all the samples or in high proportions in the samples.

Metronidazole has been effective in the treatment of ANUG patients.[9–11] As this agent is active only against anaerobic species,[28, 29] it was of interest to determine what changes metronidazole treatment would cause among the monitored plaque anaerobic species. Three patients who had been refractory to conventional treatment were given metronidazole for 1 week. Prior to treatment, the plaque from 10 sites in these patients harbored primarily an anaerobic flora with high proportions of *B. melaninogenicus* ssp. *intermedius* and *Treponema* sp. (Table 5). Immediately after the metronidazole treatment the same sites were no longer ulcerated and had decreased plaque, as evident by the reduced viable and microscopic counts. The plaque was now dominated by facultative organisms, which accounted for 79% of the flora.

Anaerobic species such as *B. melaninogenicus* ssp. *intermedius, Fusobacterium* sp., *Selenomonas* sp. and *Treponema* sp. were significantly reduced (Table 5). Among the monitored facultative and microaerophilic species, *S. sanguis* and the *Capnocytophaga* sp. increased significantly (Table 5), as would be expected if the patients took the metronidazole as directed.

This ascendency of the facultative flora did not persist, inasmuch as 2 to 3 months later the proportions of facultative organisms and of *S. sanguis* and *Capnocytophaga* sp. had returned to or had fallen below pretreatment values. This reemergence of anaerobic species in the plaque was not accompanied by a rebound in the proportions of *B. melaninogenicus* ssp. *intermedius, Fusobacterium* and *Treponema* sp., as these organisms were still significantly reduced compared to pretreatment values (Table 5). However, the proportions of these species had increased relative to the immediately-after-treatment values, and in the case of the spirochetes and selenomonads this increase was significant (Table 5). There was no return of the gingivitis to these cultured sites.

DISCUSSION

The cultural methods used in this investigation permit the isolation of about 60 to 70% of the organisms that can be counted microscopically. Thirty per cent of the microscopic count of the ANUG plaque samples consisted of the various spirochetal species whereas the remaining 70% probably represented the organisms that were also enumerated in the viable count. In order to determine the identity of these cultivable organisms, over 500 isolates obtained from 12 diseased sites were subcultured and partially characterized. This combined microscopic-cultural approach revealed both a constant flora comprised of a limited number of bacterial species and a variable flora composed of a heterogeneous array of bacterial types, most of which could not be identified to the genus or species level (Table 2). The constant flora would include the various *Treponema* sp., *B. melaninogenicus* ssp. *intermedius* and the *Fusobacterium* sp. There was the suggestion that the *Selenomonas* sp. by virtue of their presence microscopically in most plaque samples and their prominence in the viable count of one patient (patient 2 in Table 2) might also be a member of this constant flora.

This constant flora would appear to be pathognomonic of ANUG. As members of this flora can be identified by either microscopic appearance or by colony morphology on nonselective media, most of these organisms had been previously associated with ANUG. Thus the present bacteriological findings confirm the long-documented association of the fuso-spirochetal organisms in ANUG[6, 7] and introduce *B. melaninogenicus* ssp. *intermedius*, and possibly *A. odontolyticus* and the various *Selenomonas* sp., as contributors to the pathosis. Macdonald et al.[15] described an experimental fusospirochetal infection in guinea pigs, in which *B. melaninogenicus* was demonstrated to be the essential pathogen. The taxonomic status of oral isolates of *B. melaninogenicus* has been clarified recently,[30] and it is likely that the proteolytic black-pigmented bacteroides strains which Macdonald studied would now be classified as *Bacteroides gingivalis*. Thus, the demonstration of the involvement of *B.*

Table 5
Effect of One Week Metronidazole Treatment on Proportions of Suspected Odontopathic Organisms in Plaque Taken From ANUG Sites

		After treatment	
No. of Sites	Prior to treatment	Immediately	2 to 3 Mos.
	10 (3)	10 (3)	8 (2)
	Percent of Cultivable Flora		
B. melaninogenicus ssp. *intermedius*	26.9 ± 22.0*	0.02 ± .04	2.7 ± 7.2
B. gingivalis	<.1	<.1	<.1
Capnocytophaga sp.	1.4 ± 2.6	5.0 ± 6.9	0.3 ± 0.3
Fusobacterium sp.	3.1 ± 1.4	0.8 ± 1.4	0.6 ± 0.6
A. odontolyticus	1.7 ± 1.9 →	(.02)† ← 0.2 ± 0.4	5.3 ± 13.5
A. viscosus	2.3 ± 2.5	0.9 ± 1.5	0.2 ± 0.5
A. naeslundii	<.1 ± <.1	0.1 ± 0.4	4.4 ± 11.0
S. sanguis	3.2 ± 3.6	25.9 ± 20.7	3.0 ± 7.0
S. mutans	<.1 ± <.1	0.7 ± 2.0	0.6 ± 1.1
Veillonella sp.	1.3 ± 1.5	1.2 ± 3.3	1.2 ± 1.1
Facultative organisms	28.9 ± 23.7	79.1 ± 26.7	15.3 ± 12.1
	Percent of Microscopic Count		
Treponema sp.	32.6 ± 20.1	2.4 ± 3.4 → (.04) ←	12.4 ± 12.0
Selenomonas	7.3 ± 10.0	0.3 ± 0.9	3.2 ± 3.1
Motile rods	0.6 ± 0.6	1.0 ± 2.1	4.5 ± 4.0
Fusiforms	1.7 ± 1.4	4.9 ± 13.9	10.5 ± 13.3
Rods & Cocci	69.0 ± 15.0	93.0 ± 6.0	70.2 ± 18.3
Total Count × 10^6 CFU	150 ± 278	33 ± 28	90 ± 103
Microscopic count per high power field	48 ± 111	6 ± 4	7 ± 6

* Number in box significantly different from other values in row by paired *t* test or Wilcoxon test; $P \leq 0.05$.
† → ← Values connected by arrows are significantly different by paired *t* test, $P \leq 0.05$.

melaninogenicus ssp. *intermedius* in the ANUG lesion may indeed be new. (J. Slots, at SUNY in Buffalo reports a similar finding, personal communication.) Rosebury et. al.[7] found spirilla organisms in 56% of their ANUG cases. The present cultural study identified these spirilla-like organisms as *Selenomonas* species, because they were Gram-negative, motile, anaerobic organisms that were saccharolytic.[31] Several biotypes or species were present, as the isolates were variable in nitrate reduction and in esculin hydrolysis.

The prompt resolution of clinical symptoms following metronidazole treatment is presumptive evidence (given the spectrum of antimicrobial activity of this agent) of the anaerobic members of the plaque flora being responsible for the symptoms. Previous studies have shown, by microscopic means, the diminution of the fusospirochetal organisms in the plaque after metronidazole treatment,[11, 32] In the present investigation the proportions of *B. melaninogenicus* ssp. *intermedius*, the *Fusobacterium* sp. and *Treponema* sp. were significantly reduced for at least 2 to 3 months after treatment (Table 5). Proportions of the *Selenomonas* sp., were significantly reduced only immediately after treatment. Thus the same anaerobic species that were numerically associated with the ANUG lesion, were also selectively reduced in the plaque flora after resolution of the infection. This is convincing evidence for their specific involvement in the ulcerative stage of the lesion, but because the proportions of these organisms during the formative stage of the lesion were not known these data do not demonstrate that these specific anaerobes initiated the infection.

A considerable amount of epidemiological data indicates that ANUG occurs primarily in young individuals who have been subjected to physiologic or psychologic stresses.[1-3] Such stress can cause multiple changes in tissue metabolism, which in turn could compromise the defense mechanisms of the host. For example, elevated levels of corticosteroids could so weaken the gingival inflammatory response to plaque antigens and products that bacterial penetration could not be prevented; or elevated levels of norepinephrine could reduce the blood flow to the interdental papilla to the extent that a relative ischemia would ensue,[33] thus making the tissue vulnerable to bacterial invasion by motile anaerobes such as the spirochetes.[34]

If such scenarios are illustrative of the pathogenesis of ANUG, then many members of the plaque flora should be able to exploit this opportunity to invade the host.

Yet all the bacteriological studies indicate that only a finite number of species, namely the fusospirochetal organisms and *B. melaninogenicus* ssp. *intermedius* seem to overgrow. How does such bacterial specificity evolve from host-mediated events? Some understanding in this regard comes from the nutritional requirements of the prominent ANUG organisms such as the *Treponema* sp. and *B. melaninogenicus* ssp. *intermedius.*

The few small *Treponema* sp. that have been cultivated have growth factors, such as long chain fatty acids[35] and α-2-globulin in *T. denticola*[36] and polyamines in *T. macrodentium*,[37] that can be provided by the host. All the black-pigmented bacteroides species have a requirement for hemin, and *B. gingivalis* and *B. melaninogenicus* ssp. *intermedius* have a need for a factor that can be fulfilled by vitamin K or menadione.[16]

Recently it has been found that steroid hormones such as progesterone and estradiol can substitute for the vitamin K requirement in certain strains of *B. melaninogenicus* ssp. *intermedius.*[38] Thus this species, like the various *Treponema* sp., is dependent upon host-derived products for specific growth factors. If the levels of these host products were to become increased in the gingival crevice area, as a result of a stress-induced tissue alteration, then the proportions of these organisms might increase, as they would have a selective nutrient advantage over the other plaque species.[39] Their ascendency in the plaque could enhance still more the inflammatory exudate, thereby perpetuating a cycle that would assure their continued access to their nutrient needs. In this manner host changes could lead to the selection of a specific configuration of organisms from the diverse microbial populations that exist in the plaque. In fact such a host-flora interaction appears to explain the etiology of pregnancy gingivitis.[40]

The increased proportions of *B. melaninogenicus* in ANUG raises the possibility that steroid hormones may be involved as microbial nutrients. In this context, the corticosteroids are being evaluated for their ability to support *in vitro* the growth of black pigmented bacteroides species.

ANUG, by analogy with the pregnancy gingivitis model, occurs when stress-induced changes in the gingiva make available host products, which can serve as nutrients for certain periodontopathic members of the plaque flora. If a particular constellation of these organisms already exists in the plaque (such as the spirochetes and *B. melaninogenicus* ssp. *intermedius*), then this nutrient availability assures their ascendency in the plaque at the time that ulceration is present. The cycle is interrupted and health restored when the periodontopathic organisms are removed by either mechanical debridement or antibacterial agents, or the nutrients diminish because of restored host equilibrium. The specificity of the *Treponema* sp. and *B. melaninogenicus* ssp. *intermedius* in ANUG would appear in this context to be host mediated. ANUG could therefore be categorized as an example of the specific plaque hypothesis[41] as it relates to periodontal disease.

ACKNOWLEDGMENT

The assistance of Natalie S. Grossman is acknowledged.

REFERENCES

1. Goldhaber, P., and Giddon, D. B.: Present concepts concerning the etiology and treatment of acute necrotizing ulcerative gingivitis. *Int Dent J* **14:** 468, 1964.

2. Enwonwu, C. O.: Epidemiological and biochemical studies of necrotizing ulcerative gingivitis and noma (cancrum oris) in Nigerian children. *Arch Oral Biol* **17:** 1357, 1972.

3. Wirthlin, M. R., and Devine, L.: Venery and Vincent's. Fifteen case reports and discussion. *J Periodontol* **49:** 449, 1978.

4. Pindborg, J. J.: Influence of service in armed forces on incidence of gingivitis. *J Am Dent Assoc* **42:** 517,1951.

5. Smitt, P. A.: Some clinical and epidemiological aspects of Vincent's gingivitis. *Dent Pract* (Bristol) **15:** 281, 1965.

6. Smith, D. T.: Oral spirochetes and related organisms in fusospirochetal disease. Williams and Wilkins Co. Baltimore, 1932.

7. Rosebury, T., MacDonald, J. B., and Clark, A. R.: A bacteriologic survey of gingival scrapings from periodontal infections by direct examination, guinea pig inoculation, and anaerobic cultivation. *J Dent Res* **29:** 718, 1950.

8. Listgarten, M. A.: Electron microscopic observations on the bacterial flora of acute necrotizing ulcerative gingivitis. *J Periodontol* **36:** 328, 1965.

9. Shinn, D. L.: Vincent's disease and its treatment. Metronidazole. S. M. Finegold. (ed), pp. 334, *Excerpta Medica*, 1977.

10. Emslie, R. D.: Treatment of acute necrotizing ulcerative gingivitis. A clinical trial using chewing gums containing metronidazole or penicillin. *Br Dent J* **122:** 307, 1967.

11. Duckworth, R., Waterhouse, J. P., Britton, D. E. R., Nuki, K., Sheiham, A., Winter, R., and Blake, G. C.: Acute ulcerative gingivitis: A double blind controlled clinical trial of metronidazole. *Br Dent J* **120:** 599, 1966.

12. Rosebury, T., and Sonnerwirth, A. C.: Bacterial indigenous to man. Ch. in *Bacterial and Mycotic Infection of Man.* R. J. Dubos (ed), J. B. Lippincott Co., Philadelphia, 1958.

13. King, J. D.: Nutritional and other factors in "Trench Mouth" with special reference to the nicotinic acid component of the Vitamin B_2 complex. *Br Dent J* **74:** 113, 1943.

14. Rosebury, T., Clark, P. R., Macdonald, J. B., and O'Connell, D. C.: Studies of fusospirochetal infection. III. Further studies of a guinea pig passage strain of fusospirochetal infection, including the infectivity of sterile exudate filtrates of mixed cultures through ten transfers and of recombined pure cultures. *J Infect Dis* **87:** 234, 1950.

15. Macdonald, J. B., Sutton, P. M., Knoll, M. L., Madlener, E. M., and Grainger, R. M.: The pathogenic components of an experimental fusospirochetal infection. *J Infect Dis* **98:** 15, 1956.

16. Macdonald, J. B., Gibbons, R. J., and Socransky, S. S.: Bacterial mechanisms in periodontal disease. *Ann NY Acad Sci* **85:** 467, 1960.

17. Loesche, W. J.: Oxygen sensitivity of various anaerobic bacteria. *Appl Microbiol* **18:** 723, 1969.

18. Loesche, W. J.: Periodontal disease and the Treponemes. Ch. in the *Biology of the Parasitic Spirochetes.* R. C. Johnson (ed), Academic Press, New York, pp. 261, 1976.

19. Syed, S. A., and Loesche, W. J.: Survival of human dental plaque flora in various transport media. *Appl Microbiol* **24:** 638, 1972.

20. Aranki, A., Syed, S. A., Kenney, E. B., and Freter, R.: Isolation of anaerobic bacteria from human gingiva and mouse cecum by means of a simplified glove box procedure. *Appl Microbiol* **17:** 568, 1969.

21. Syed, S. A., Svanberg, M., and Svanberg, G.: Predominant cultivable dental plaque flora of beagle dogs with gingivitis. *J Periodont Res* **15:** 123, 1980.

22. Kornman, K. S., and Loesche, W. J.: New medium for the isolation of *Actinomyces viscosus* and *Actinomyces naeslundii* from dental plaque. *J Clin Microbiol* **7:** 514, 1978.

23. Loesche, W. J., and Syed, S. A.: The predominant cultivable flora of carious plaque and carious dentine. *Caries Res* **7:** 201, 1973.

24. Rogosa, M.: The genus *veillonella. J Bacteriol* **87:** 162, 1964.

25. Listgarten, M. A., and Helldin, L.: Relative distribution of bacteria at clinically healthy and periodontally diseased sites in humans. *J Clin Periodontol* **5:** 115, 1978.

26. Minah, G. E., and Loesche, W. J.: Development of methods to analyze sucrose metabolism by small dental plaque suspensions in microbial aspects of dental caries. Stiles, H. J., Loesche, W. J. and O'Brien, T. C. (eds), *Microbiol Abst Spec Suppl* **2:** 491, 1976.

27. Siegal, S.: *Nonparametric Statistics for the Behavior Sciences.* McGraw-Hill, New York, 1956.

28. Muller, M., Lindmark, D. G., and McLaughlin, J.: Action mechanism of metronidazole in anaerobic microorganisms. Metronidazole, S. Finegold, (ed), *Excerpta Medica* 12, 1977.

29. Sutter, V. L., and Finegold, S. M.: In vitro studies with metronidazole against anaerobic bacteria. *Metronidazole,* S. M. Finegold (ed), Excerpta Medica, 279, 1977.

30. Coykendall, A. L., Kaczmacek, F. S., and Slots, J.: 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, 1980.

31. Loesche, W. J., and Gibbons, R. J.: A practical scheme for the taxonomy of oral gram-negative anaerobic rods. *Arch Oral Biol* **10:** 723, 1965.

32. Blake, G. C.: The microbiology of acute ulcerative gingivitis with reference to the culture of oral trichomonads and spirochetes. *Proc R Soc Med* **61:** 131, 1968.

33. Kardachi, B., and Clarke, N.: Aetiology of acute necrotizing ulcerative gingivitis: A hypothetical explanation. *J Periodontol* **45:** 830, 1974.

34. Listgarten, M. A., and Lewis, D. W.: The distribution of spirochetes in the lesion of acute necrotizing ulcerative gingivitis: An electron microscopic and statistical survey. *J Periodontol* **38:** 379, 1967.

35. Livermore, B. P., and Johnson, R. C.: Lipids of the Spirochaetales: Comparison of the lipids of serveral members of the genera *Spirochaeta, Treponema,* and *Leptospira. J Bacteriol* **120:** 1268, 1974.

36. Socransky, S. S., and Hubersak, C.: Replacement of ascitic fluid or rabbit serum requirement of *Treponema dentium* by alpha-globulin. *J Bacteriol* **94:** 1795, 1967.

37. Socransky, S. S., Loesche, W. J., Hubersak, C., and Macdonald, J. B.: Dependency of *Treponema microdentium* on other oral organisms for isobutyrate, polyamines and a controlled oxidation reduction potential. *J Bacteriol* **88:** 200, 1964.

38. Kornman, K. S., and Loesche, W. J.: Effect of estradiol and progesterone on *Bacteroides melaninogenicus* and *Bacteroides gingivalis.* In press. *Infect Immun* 1982.

39. Loesche, W. J.: Importance of nutrition in gingival crevice microbial ecology. *Periodontics* **6:** 245, 1968.

40. Kornman, K. S., and Loesche, W. J.: The subgingival microbial flora during pregnancy. *J Periodont Res* **15:** 111, 1980.

41. Loesche, W. J.: Chemotherapy of dental plaque infections. *Oral Sci Rev* **9:** 63, 1976.

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