THE ISOLATION AND IDENTIFICATION OF *BACTEROIDES* SPP. FROM THE NORMAL HUMAN GINGIVAL FLORA

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GRAM-NEGATIVE anaerobic bacilli are important members of the normal flora of the mouth (Hardie and Bowden, 1974) and are implicated in a variety of oral infections (Hardie, 1974). The mouth consists of several micro-environments with different microbial flora; the gingival crevice and dental plaque are the principal sites of colonisation by gram-negative anaerobic bacilli (Socransky and Manganiello, 1971; Hardie and Bowden, 1974). The surface of the tongue is almost devoid of *Bacteroides*, and saliva contains a variable number of fusobacteria derived from the gingival crevice (Hadi and Russell, 1968; 1969). Gibbons *et al.* (1963, 1964) and Loesche, Hockett and Syed (1972) found that 16·1% of the cultivable flora of the human gingival crevice, 4% of the flora from dental plaque in normal subjects, and 17% of the plaque flora in institutionalised subjects, were gram-negative anaerobic rods; Loesche and Gibbons (1965) devised a scheme for their identification.

Bacteroides melaninogenicus was first described in studies of the microflora of mucous membranes (Oliver and Wherry, 1921); pigmented strains constitute 4·8% of the cultivable flora of the normal gingival crevice (Gibbons, 1974) and 5·6% of the plaque flora from institutionalised subjects (Loesche *et al.*, 1972). Loesche, Socransky and Gibbons (1964) isolated non-pigmented strains that they called *B. oralis*; this species shares many properties with some strains of *B. melaninogenicus* and is the subject of an unresolved taxonomic debate (Holbrook and Duerden, 1974; Sundqvist, 1976; Holbrook, Duerden and Deacon, 1977; International Committee, 1977, 1979; Duerden *et al.*, 1980). The *B. melaninogenicus/oralis* group also includes human isolates of *B. ruminicola* and two recent non-pigmented additions, *B. bivius* and *B. disiens* (Holde-
man and Johnson, 1977).

In the present investigation, conventional bacteriological methods for the isolation (Holbrook, Ogston and Ross, 1978) and identification (Duerden *et al.*, 1976, 1980) of gram-negative anaerobic bacilli were used to assess the occurrence of the different species in the normal flora of the gingival crevice of healthy adults.

MATERIALS AND METHODS

Two hundred strains of gram-negative anaerobic bacilli isolated from 20 specimens of subgingival plaque from normal young adults were studied.

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Anaerobic incubation. The anaerobic procedure of Collee *et al.* (1972) was followed in all essential aspects; incubation was at 37°C in an atmosphere of H₂ 90% and CO₂ 10%. A slope of Simmons citrate medium seeded with *Pseudomonas aeruginosa* was included in each jar as a control.

Specimens. Sterile toothpicks were used to collect samples of subgingival plaque from 20 normal healthy adults. The subjects were medical students aged 19–20 years; none had any gross oral or dental pathology and none was receiving antibiotic therapy.

Isolation of *Bacteroides*. The methods and media were derived from those of Baird-Parker (1957), Loesche, Hockett and Syed (1971), Syed and Loesche (1973) and Williams *et al.* (1975); they were evaluated by Holbrook (1976) and Holbrook *et al.* (1978). The samples of plaque were seeded directly on to a sector of a plate of pre-reduced BM agar (see Williams *et al.*, 1975) with kanamycin 75 µg/ml and vancomycin 2.5 µg/ml and the inoculum was streaked over the remainder of the plate. The plates were examined after anaerobic incubation for 48 h; all colony types were noted and representative colonies were subcultured from each specimen on to plain BM agar. After incubation for a further 48 h, any additional colony types were noted and further representative colonies were subcultured to a total of 10 from each specimen. The colony types were selected in approximate proportions to their comparative numbers on the primary isolation plate. The isolates were suspended in a freezing medium of Nutrient Broth No. 2 (Oxoid) with inactivated horse serum (Wellcome) 10% and glucose 1% and held at –70°C in a liquid-nitrogen container.

Identification of isolates. The isolates were identified by the methods of Duerden *et al.* (1976, 1980). The tests were: colony morphology after 48-h incubation on blood agar; cell morphology in gram-stained smears from 48-h cultures on blood agar and in BM broth with cooked-meat particles (see Deacon, Duerden and Holbrook, 1978); pigment production on BM agar; haemolysis on human blood agar; motility in BM broth; antibiotic-disk resistance tests with neomycin 1000 µg, kanamycin 1000 µg, penicillin 2 units and rifampicin 15 µg disks; tolerance tests with taurocholate, deoxycholate, Victoria blue 4R and ethyl violet; biochemical tests for the production of indole, digestion of gelatin and hydrolysis of aesculin; fermentation tests with glucose, lactose, sucrose, rhamnose, trehalose, mannitol and xylose.

RESULTS

A heavy growth of gram-negative, non-sporing, non-motile, anaerobic bacilli was obtained on the selective medium from the 20 specimens of subgingival plaque. Ten representative colonies were selected from each subject for identification and results were obtained with 188 isolates; 13 isolates failed to grow on subculture from the preserved cultures. One hundred and seventy-six isolates representing 12 recognised species or subspecies were identified and the numbers of isolates allocated to each species are shown in table I. Nine isolates were allocated to the *B. fragilis* group, 128 to the *B. melaninogenicus/oralis* group, 10 were non-saccharolytic *Bacteroides* spp., 29 were fusobacteria, 10 were probably *Leptotrichia buccalis*, and two isolates could not be allocated to a recognised group.

B. fragilis group

The seven strains of *B. fragilis* and two strains of *B. thetaiotaomicron* were isolated from eight subjects and were present in very small numbers in comparison with other species. All were typical of the designated species except that one isolate of *B. fragilis* was inhibited by Victoria blue 4R.

TABLE I
Identification of 188 gingival isolates from 20 subjects

Species	Number of isolates (and group total)
<i>B. fragilis</i> group	(9)
<i>B. fragilis</i>	7
<i>B. thetaiotaomicron</i>	2
<i>B. melaninogenicus/oralis</i> group	(128)
<i>B. melaninogenicus</i> ss. <i>melaninogenicus</i>	33
ss. <i>intermedius</i>	22
<i>B. biviaus</i>	3
<i>B. disiens</i>	7
<i>B. oralis</i>	53
<i>B. oralis</i> group (unidentified)	4
<i>B. ruminicola</i>	6
Asaccharolytic group	(10)
<i>B. asaccharolyticus</i>	1
non-pigmented non-saccharolytic spp.	9
<i>Fusobacterium</i> group	(39)
<i>F. polymorphum</i>	12
<i>Fusobacterium</i> spp.	17
<i>L. buccalis</i>	10
<i>Bacteroides</i> spp. (unidentified)	(2)
TOTAL	188

B. melaninogenicus/oralis group

The most common species of *Bacteroides* isolated from the normal gingival flora were members of the *B. melaninogenicus/oralis* group. The 128 isolates allocated to this group accounted for 68% of the gram-negative anaerobic bacilli isolated and identified in this study. All were inhibited by taurocholate, deoxycholate and ethyl violet, resistant to kanamycin and sensitive to rifampicin, and most strains were sensitive to neomycin and penicillin.

The 33 isolates of *B. melaninogenicus* ss. *melaninogenicus* produced pigmented colonies on BM agar; some produced black colonies, but many produced pale colonies with a dark brown centre after incubation for 5–7 days and a few strains produced pigmented colonies only in the presence of a contaminant organism such as a staphylococcus. Most isolates were typical of the subspecies, but four isolates were resistant to penicillin and only one was resistant to neomycin. Eight isolates were tolerant of Victoria blue 4R; three of these isolates and five others hydrolysed aesculin. All 22 isolates of *B. melaninogenicus* ss. *intermedius* produced black or dark brown colonies on BM agar and were typical of the subspecies except that two isolates from one subject were sensitive to the kanamycin 1000- μ g disk; the same two isolates plus two others hydrolysed aesculin. The 10 isolates identified as *B. biviaus* and *B. disiens* were similar to the reference strains of those species except that two isolates identified as *B. biviaus* hydrolysed aesculin.

B. oralis was the commonest species isolated from subgingival plaque. All strains gave patterns of results typical of the published reports of the species.

Three isolates were resistant to penicillin and 20 isolates, including pairs of identical isolates from six subjects, were tolerant of Victoria blue 4R. Eighteen isolates, including pairs of identical isolates from four subjects, did not hydrolyse aesculin. Three additional isolates were identified as non-pigmented members of the *B. melaninogenicus/oralis* group but could not be allocated to a recognised species. Six isolates were identified as *B. ruminicola*; all fermented xylose and hydrolysed aesculin and five also fermented rhamnose.

Asaccharolytic group

One isolate that produced black pigmented colonies but did not ferment glucose was identified as *B. asaccharolyticus* and nine isolates were non-pigmented and non-saccharolytic but could not be further identified.

Fusobacterium group

Thirty-nine isolates had a typical fusiform morphology in gram-stained smears; 17 patterns of results were obtained with 35 isolates, and four strains with typical morphology failed to grow on the second subculture. The patterns of results obtained with the fusiform organisms are shown in table II. Twelve isolates were provisionally identified as *F. polymorphum*. They were inhibited by bile salts but tolerant of both dyes and most were sensitive to the four antibiotic disks. All produced indole and digested gelatin and one also hydrolysed aesculin, but only six isolates fermented glucose (weakly).

Ten isolates were provisionally identified as *L. buccalis*; the cells were larger than the fusobacteria in Gram-stained smears. They were sensitive to the four antibiotic disks and inhibited by bile salts. Four isolates were typical of *L. buccalis*, but the remaining isolates were less tolerant of the dyes and two isolates did not ferment glucose. The remaining 12 isolates could not be allocated to a species within the genus *Fusobacterium* on the basis of the set of tests used.

Identification of isolates from individual subjects

When identical isolates from one subject were treated as a single strain, 131 strains were isolated from the 20 subjects and the identity of these is shown in table III. The distribution of species amongst the subjects is shown in table IV. The mean number of strains isolated from each subject was 6.5 (range 4–9). A variety of species was isolated from each subject and the overall prevalence of the different species was not distorted by the isolation of series of identical isolates from individual subjects. At least one strain of *B. oralis* was isolated from each subject, and *B. melaninogenicus* was isolated from all except two subjects. Fusobacteria were isolated from all except four subjects.

TABLE II
Patterns of results obtained with 35 gingival isolates of fusobacteria and *L. buccalis*

Test	Patterns of results obtained with 35 isolates																																				
Antibiotic-disk resistance tests																																					
neomycin 1000 µg	S	S	S	S	S	R	S	S	S	S	S	S/R	S	S	R	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S					
kanamycin 1000 µg	S	S	S	R	R	S	S	S	S	S	S	S/R	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S				
penicillin 2 units	S	S	S	R	R	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S				
rifampicin 15 µg	S	S	S	S	S	S	S	S	S	S	S	R	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S				
Tolerance tests																																					
taurocholate	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I					
deoxycholate	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I					
Victoria blue 4R	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I				
ethyl violet	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I				
Pigment production	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-				
Indole production	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-				
Gelatin digestion	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+			
Aesculin hydrolysis	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-				
Fermentation of glucose	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+			
Fusiform morphology	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+			
Number of isolates conforming to recognised pattern	1	2	1	3	1	1	3	1	1	3	1	1	4	5	1	1	4	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1			
																												<i>F. polymorphum</i>					<i>L. buccalis</i>				

S = Sensitive in antibiotic resistance tests; R = resistant; S/R = marginally resistant.
 + = Positive result (growth in tolerance tests); - = negative result; I = inhibited in tolerance tests

TABLE III
Strains of bacteroides and fusobacteria isolated from 20 subjects

Species	Number of strains isolated (and group total)
<i>B. fragilis</i> group	(8)
<i>B. fragilis</i>	6
<i>B. thetaiotaomicron</i>	2
<i>B. melaninogenicus/oralis</i> group	(89)
<i>B. melaninogenicus</i> ss. <i>melaninogenicus</i>	24
ss. <i>intermedius</i>	15
<i>B. biviaus</i>	3
<i>B. disiens</i>	6
<i>B. oralis</i>	32
<i>B. oralis</i> group (unidentified)	3
<i>B. ruminicola</i>	6
Asaccharolytic group	(7)
<i>B. asaccharolyticus</i>	1
non-pigmented non-saccharolytic spp.	6
<i>Fusobacterium</i> group	(25)
<i>F. polymorphum</i>	7
<i>Fusobacterium</i> spp.	15
<i>L. buccalis</i>	3
<i>Bacteroides</i> spp. (unidentified)	(2)
TOTAL	131

Nutritional interdependence of oral strains

The gram-negative anaerobic flora of the gingival crevice is nutritionally interdependent and this caused difficulties in attempts to study pure isolates. Several attempts to isolate particular strains by subculture of a single colony on to fresh solid medium failed because the strain would grow only in mixed culture with other strains that provided essential nutrients. Eleven isolates of eight strains from seven subjects in the present study would not grow in pure culture on solid media but grew well in pure culture in BM broth or on solid media in mixed cultures where they showed marked satellitism around other gram-negative anaerobes or around contaminant colonies of organisms such as staphylococci. Most of these strains were members of the *B. melaninogenicus/oralis* group—*B. melaninogenicus* ss. *melaninogenicus*, *B. oralis* (2), *B. disiens* (2) and *B. biviaus*—but there was also a non-pigmented non-saccharolytic strain and an unidentified *Bacteroides* sp.

Colony and cell morphology

Colony morphology was helpful in the initial recognition of only a few species. In general, pigmentation developed earlier in *B. melaninogenicus* ss. *intermedius* strains than in ss. *melaninogenicus* strains, and most strains of ss. *intermedius* produced shiny black colonies. Most strains of ss. *melaninogenicus* produced brown colonies that were often larger than those of ss. *intermedius*; pigmentation usually began in the centre of the colony and the

TABLE IV
Species of bacteroides and fusobacteria isolated from gingival plaque from 20 subjects

Species	Isolation of the given species from subject number																			
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
<i>B. fragilis</i>	+		+			+	+	+	+	+								+		
<i>B. thetaiotaomicron</i>																				
<i>B. melaninogenicus</i> ss. <i>melaninogenicus</i>	+	+	+	+	+	+	+	+	+	+	+	+		+	+	+	+	+	+	+
ss. <i>intermedius</i>																				
<i>B. bivius</i>																				
<i>B. distans</i>																				
<i>B. oralis</i>																				
<i>B. oralis</i> group (unidentified)	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
<i>B. ruminicola</i>																				
<i>B. asaccharolyticus</i>																				
non-pigmented non-saccharolytic spp.	+			+	+						+	+	+		+		+			+
<i>F. polymorphum</i>			+				+	+	+	+	+	+	+	+					+	
<i>Fusobacterium</i> spp.	+				+	+	+	+	+	+	+	+	+	+		+				
<i>L. buccalis</i>						+	+	+												
<i>Bacteroides</i> spp. (unidentified)	+																	+		

+ Denotes isolation.

margin was pale, although some strains produced uniformly dark colonies. *B. oralis* strains produced a variety of colony-sizes on primary isolation. Most strains grew better and produced larger colonies on BM agar than on blood agar and some strains produced large colonies (c. 2 mm diameter) that developed a characteristic spreading margin which was low, rough and irregular in outline. The colonies of *B. bivius* and *B. disiens* were indistinguishable from the smaller colonies of *B. oralis* strains, but they did not produce large, spreading colonies.

The non-pigmented non-saccharolytic strains produced very small colonies even after prolonged incubation. Some *Fusobacterium* spp. produced characteristic colonies but others were indistinguishable from non-pigmented *Bacteroides* spp. Most isolates identified as *F. polymorphum* produced pale yellow colonies of 1–2 mm diameter that were raised, rough, irregular in outline and opaque. The colonies of other fusobacteria were not distinctive and most were small, smooth, low convex, grey and translucent. Strains identified as *L. buccalis* produced colonies similar to the small colonies of *B. oralis*.

Bacterial-cell morphology was of limited discriminatory value. *B. melaninogenicus* strains consisted of small pleomorphic bacilli with many coccobacilli and a few short filaments. *B. oralis* and other non-pigmented species in the group were similar but generally more pleomorphic. A few strains of *B. oralis* that produced large spreading colonies were predominantly long bacilli. The non-pigmented non-saccharolytic strains were small bacilli and coccobacilli.

The appearance of the fusobacteria was characteristic but varied from strain to strain. The isolates identified as *F. polymorphum* were typical fusiform bacilli with pointed ends and the remaining fusobacteria were filamentous organisms of various sizes with pointed or blunt ends. The isolates identified as *L. buccalis* were filamentous but not invariably pointed.

DISCUSSION

A heavy growth of gram-negative anaerobic bacilli was obtained from each specimen of subgingival plaque from healthy young adults without periodontal disease. This confirms the findings of Gibbons *et al.* (1963, 1964) and Loesche *et al.* (1972) that these organisms form an important part of the normal flora of the gingival crevice. The methods and medium used for the isolation of *Bacteroides* spp. from subgingival plaque were evaluated by Holbrook (1976; Holbrook *et al.*, 1978); he obtained a good isolation of *B. melaninogenicus* and other *Bacteroides* spp. from small inocula in a laboratory trial and from samples of subgingival plaque in a clinical trial with a medium derived from the BM medium of Nash (see Williams *et al.*, 1975) containing lysed blood and made selective for *Bacteroides* spp. by the addition of kanamycin and vancomycin.

The gingival strains were found to be nutritionally demanding and difficulty was experienced in isolating and maintaining some strains in pure culture.

Several strains would not grow on solid media in pure culture. They required a growth factor(s) provided by a second organism that might be another *Bacteroides* strain or a facultative organism such as a staphylococcus around which they showed marked satellitism. The dependent strains that could be grown in liquid culture were identified as members of the *B. melaninogenicus/oralis* group, and others that could not be isolated in pure culture for identification appeared to belong to the same group on the basis of their colony and microscopic morphology. It had been noted previously that some strains of *B. melaninogenicus* would grow only in mixed culture on solid media (Duerden *et al.*, 1976; Holbrook, 1976). In the present study, some dependent strains were pigmented and some were not, but a few strains of *B. melaninogenicus* ss. *melaninogenicus* that grew in pure culture produced pigment only when growing around a colony of another organism such as a staphylococcus. One of the problems encountered by earlier workers was the difficulty of obtaining pure cultures of *Bacteroides* spp. (Senos and Mattman, 1955). The recovery of many *Bacteroides* spp. is improved by the addition of 5–10% CO₂ to the anaerobic atmosphere (Watt, 1973; Stalons, Thornsberry and Dowell, 1974) and several other growth factors have been identified. Metal ions are growth factors for certain bacteroides (Caldwell and Arcand, 1974) and some species require haemin (Gibbons and MacDonald, 1960; Gilmour and Poole, 1970). Some *B. melaninogenicus* strains also require menadione (Vitamin K; Lev, 1959) or its precursors (Robins, Yee and Bentley, 1973) and succinate (Lev, Keudall and Milford, 1971). A medium containing a variety of these growth factors is generally necessary for optimal growth of a wide range of *Bacteroides* spp. The modified BM medium used in the present studies took account of the known problems and included haemin, menadione and succinate but clearly some other factor(s) as yet unidentified is needed for the growth of the most demanding strains.

Most of the *Bacteroides* spp. isolated from the gingival crevice were members of the *B. melaninogenicus/oralis* group and accounted for 68% of the isolates studied. Loesche *et al.* (1972), Gibbons (1974) and Holbrook (1976) showed that *B. melaninogenicus* is a common commensal in the gingival crevice of normal, healthy adults and *B. melaninogenicus* strains of ss. *intermedius* or ss. *melaninogenicus* or both were isolated from all except two subjects in the present study. The group of strains designated *B. oralis* was the commonest species identified and accounted for 41% of the *B. melaninogenicus/oralis*-group isolates. These non-pigmented strains gave a pattern of results in the identification tests that was similar to the published description of the species *B. oralis* (Loesche *et al.*, 1964) and similar to the results obtained with *B. melaninogenicus* ss. *melaninogenicus* strains. *B. oralis* is the subject of an unresolved taxonomic debate because the original reference strain was found to produce pigment and was re-identified as *B. melaninogenicus* ss. *melaninogenicus* (Holbrook and Duerden, 1974; International Committee, 1977). A neotype strain of *B. oralis* has not been designated (International Committee, 1979) but the isolates identified as *B. oralis* in the present investigation are similar to several reference strains that have been provisionally described as *B.*

oralis. A few non-pigmented isolates that belonged to the *B. melaninogenicus/oralis*-group were identified as *B. bivius*, *B. disiens* and *B. ruminicola*.

Hardie (1974) stated that *B. fragilis* had not been shown to be a commensal in the mouth. In the present study, only nine isolates of species in the *B. fragilis* groups were detected; they were present in very small numbers and probably represented transient carriage rather than permanent colonisation. Hadi and Russell (1968, 1969) found that *Fusobacterium* spp. were a regular part of the normal flora of the gingival crevice. In the present study *Fusobacterium* spp. and *L. buccalis* were isolated from 70% of subjects and accounted for 21% of the isolates studied. They were present in smaller numbers than the *Bacteroides* spp. on most plates and only a minority could be assigned to a recognised species on the basis of the tests used. This comparison of the numbers of *Bacteroides* and fusobacteria may underestimate the proportion of fusobacteria present in subgingival plaque. Some fusobacteria were difficult to isolate in pure culture and some were difficult to maintain in repeated subcultures and could not be identified. Moreover, the isolation of fusobacteria may have been reduced by the method adopted; studies have shown that fusobacteria are more sensitive to aminoglycoside antibiotics than most *Bacteroides* spp. (Sutter and Finegold, 1971; Duerden *et al.*, 1976) and the use of kanamycin 75 µg/ml in the primary isolation plates may have been inhibitory to some species.

Gram-negative anaerobic bacilli are important members of the normal flora of the mouth, lower gastro-intestinal tract and vagina. In parallel studies (Duerden, 1980*a* and *b*), faecal and vaginal isolates were identified by the methods used in the present study. The *Bacteroides* spp. found in the three sites form distinct populations. Most of the faecal isolates were members of the *B. fragilis* group and few strains from the *B. melaninogenicus/oralis* group were isolated. The *B. fragilis* group was rarely encountered in the mouth and vagina and is probably not part of the commensal flora of these sites which typically yielded members of the *B. melaninogenicus/oralis* group. The similarity in the species isolated from the mouth and vagina may reflect shared properties of saliva and cervical secretions and similarities in the physiological environments of the two sites (Jordan and Singer, 1976; Mason and Chisolm, 1975). The difference between the *Bacteroides* spp. isolated from the mouth and the vagina was that *B. oralis* was the commonest species isolated from the gingival crevice and *B. bivius* was the commonest species isolated from the vagina.

The present study has shown that the *Bacteroides* spp. that form part of the normal flora of the gingival crevice can be isolated and identified by conventional bacteriological methods and that most isolates are members of the *B. melaninogenicus/oralis* group. *Fusobacterium* spp. also form an important part of the normal gingival flora, but their isolation is less reliable and their identification by conventional bacteriological methods needs further development.

SUMMARY

Gram-negative anaerobic bacilli were isolated on an enriched selective

medium from specimens of subgingival plaque from 20 normal, healthy adults without periodontal disease. A heavy growth of *Bacteroides* and *Fusobacterium* spp. was obtained from all specimens and 10 representative colonies from each subject were selected for identification by conventional bacteriological methods. Results were obtained in these tests with 188 isolates; some strains of the *B. melaninogenicus/oralis* group would not grow in pure culture on solid media and showed satellitism in mixed culture, and some strains of fusobacteria did not survive repeated subculture. Most isolates (68%) belonged to the *B. melaninogenicus/oralis* group. The commonest species identified in this group were *B. oralis* (41%), *B. melaninogenicus* ss. *melaninogenicus* (26%) and ss. *intermedius* (17%). *B. oralis* was isolated from all subjects and at least one strain of *B. melaninogenicus* was isolated from 18 out of 20 subjects. Only eight strains of the *B. fragilis* group were detected and they probably represented transient carriage. *Fusobacterium* spp. and *L. buccalis* were isolated from 15 out of 20 subjects and accounted for 21% of the isolates studied, but most could not be identified specifically by the set of tests used. Asaccharolytic species of *Bacteroides* were isolated in small numbers from seven subjects.

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