

## Capacity of Anaerobic Bacteria from Necrotic Dental Pulp to Induce Purulent Infections

GÖRAN K. SUNDQVIST,<sup>1, 2\*</sup> MATS I. ECKERBOM,<sup>1</sup> ÅKE P. LARSSON,<sup>3</sup> AND ULF T. SJÖGREN<sup>1</sup>

Departments of Endodontics<sup>1</sup> and Oral Microbiology,<sup>2</sup> University of Umeå, S-901 87 Umeå, and Department of Oral Pathology, University of Lund, S-221 01 Lund,<sup>3</sup> Sweden

Received for publication 30 April 1979

Combinations of bacteria isolated from the root canals of teeth with necrotic pulps and periapical bone destruction were tested for their capacity to induce abscess formation and transmissible infections when inoculated subcutaneously into guinea pigs. Transmissible infections could be induced with combinations obtained from teeth with purulent apical inflammation, but not with combinations from symptomless teeth with chronic apical inflammation. All combinations which gave transmissible infections contained strains of *Bacteroides melaninogenicus* or *B. asaccharolyticus* (formerly *B. melaninogenicus* subsp. *asaccharolyticus*). The results suggest that purulent inflammation in the apical region in certain cases may be induced by specific combinations of bacteria in the root canal and that the presence of *B. melaninogenicus* or *B. asaccharolyticus* in such combinations is essential. However, with one exception, the strains needed the support of additional microorganisms to achieve pathogenicity. The results indicate that *Peptostreptococcus micros* was also essential. Histological sections of the lesions in the guinea pigs showed that all bacterial combinations induced acute inflammation with an accumulation of polymorphonuclear leukocytes and the formation of an abscess. However, the presence of *B. melaninogenicus* or *B. asaccharolyticus* in the combinations resulted in a failure of abscess resolution, with a gradually increasing accumulation of polymorphonuclear leukocytes.

A bacterial infection of necrotic dental pulp tissue induces inflammation in the tissues at the apex of the tooth (42). This inflammation is usually chronic and asymptomatic, but can also be acute (32). Instrumentation of an infected root canal may produce an acute exacerbation of a chronic apical inflammation (20, 36). Since exacerbation may also occur spontaneously, it has been suggested that this may be a result of an increased virulence of microorganisms in the root canal or may be due to a decreased host defence (3). No specific microorganisms have, however, been identified as the causative agents (1).

In a recent study it was shown that the root canals of teeth with necrotic pulps and periapical bone destruction and symptoms of swelling and tenderness harbored a larger number of bacteria and a more complex anaerobic bacterial flora than did the root canals of teeth with necrotic pulps and periapical bone destruction but without clinical symptoms (42). The clinical symptoms, swelling and tenderness, were invariably associated with purulent inflammation, and certain bacteria were found more frequently in the root canals of teeth with evidence of pus for-

mation than in teeth without such evidence. The purulent apical inflammation of pulpal origin may thus be caused by specific microorganisms or combinations of microorganisms.

The aim of the present study was to investigate the capacity of specific combinations of bacteria isolated from necrotic pulp tissue to induce dermal purulent inflammations and transmissible infections in guinea pigs (28).

### MATERIALS AND METHODS

**Microorganisms.** A total of 88 bacterial strains, 85 of which were anaerobic, were tested. The bacteria had been isolated from the root canals of teeth with periapical bone destruction (42). Many of the strains did not fit into recognized species, but numerical taxonomic analysis (42) showed that such strains could be put into distinct groups. The strains were used in their originally isolated combinations but also in other combinations. Seven of the combinations were from teeth with acute symptoms (combinations D, G, H, P, AB, UJB, BN), and the remaining 11 combinations were from symptomless teeth. The number of strains in the various bacterial combinations ranged between 1 and 12 (Tables 1 and 2).

**Bacteriological media and culture conditions.** PY-glucose broth and PRAS-dilution blanks were pre-

TABLE 1. Composition of the bacterial flora in combinations not causing transmissible infections in guinea pigs

Combination	Organism(s)	Combination	Organism(s)
B	<i>Bacteroides ochraceus</i>	AC	<i>Eubacterium alactolyticum</i> <i>Eubacterium lentum</i>
C	<i>P. anaerobius</i> <i>Eubacterium lentum</i> <i>Eubacterium alactolyticum</i> <i>Fusobacterium</i> sp. group 2 <sup>a</sup> <i>Lactobacillus</i> sp. group 3 <sup>a</sup> <i>Peptococcus</i> sp. group 1 <sup>a</sup>	BA	<i>Selenomonas sputigena</i> <i>F. nucleatum</i> <i>Lactobacillus</i> sp. group 1 <sup>a</sup> <i>Fusobacterium</i> sp. group 1 <sup>a</sup> <i>P. micros</i>
E	<i>S. mitis</i>		<i>Eubacterium</i> sp. group 4 <sup>a</sup> <i>Anaerobic Vibrio</i>
H <sup>b</sup>	<i>F. nucleatum</i> <i>Eubacterium</i> sp. group 1 <sup>a</sup> <i>Lactobacillus</i> sp. group 3 <sup>a</sup> <i>Fusobacterium</i> sp. group 2 <sup>a</sup> <i>B. asaccharolyticus</i> <i>Anaerobic Vibrio</i>	IN	<i>Actinomyces naeslundii</i>
M	<i>S. mitis</i>	UJA	<i>Bacteroides ochraceus</i>
U	<i>Arachnia propionica</i>	EL	<i>F. nucleatum</i> <i>Bacteroides oralis</i>
X	<i>Actinomyces naeslundii</i> <i>Propionibacterium acnes</i>		<i>F. nucleatum</i>

<sup>a</sup> These strains do not fit into recognized species and were classified by numerical taxonomy (42).

<sup>b</sup> Combination H was derived from a tooth with purulent apical inflammation.

TABLE 2. Bacteria recovered after the infection was transferred four times in guinea pigs

Bacterial species recovered	% of count with bacterial combinations					
	D	G	P	AB <sup>a</sup>	UJB	BN <sup>b</sup>
<i>F. nucleatum</i>			17 (9-35) <sup>c</sup>	6 (2-13)	5 (0-24)	6 (3-10)
<i>Fusobacterium</i> sp. group 1 or 2 <sup>d</sup>				NR <sup>c</sup>	NR	NR
<i>B. melaninogenicus</i>	65 (62-78)	42 (20-52)	20 (1-25)	32 (26-37)	76 (38-84)	
<i>B. asaccharolyticus</i>						51 (42-75)
<i>Bacteroides oralis</i>		6 (0-21)				
<i>P. anaerobius</i>	NR	6 (3-23)		8 (5-29)	5 (0-24)	
<i>P. micros</i>	28 (18-33)		49 (37-67)	32 (14-50)	14 (2-23)	20 (10-47)
<i>Peptococcus</i> sp. group 1 <sup>d</sup>			0 (0-2)	NR	NR	NR
<i>Anaerobic Vibrio</i>	3 (2-10)		0 (0-3)	0 (0-3)		NR
<i>Eubacterium</i> sp. group 1 <sup>d</sup>			4 (3-11)			0 (0-5)
<i>Eubacterium</i> sp. group 2 or 4 <sup>d</sup>			NR	0 (0-5)	NR	10 (5-13)
<i>Eubacterium alactolyticum</i>				0 (0-7)		
<i>Lactobacillus</i> sp. group 1 or 3 <sup>d</sup>	7 (2-15)	15 (5-21)		5 (0-19)	8 (6-20)	12 (0-21)
<i>L. catenaforme</i>				4 (3-9)		
<i>Veillonella</i> sp.		5 (0-12)	10 (0-19)			
<i>Selenomonas sputigena</i>					0 (0-5)	
<i>Actinomyces</i> sp.	0 (0-3)	7 (0-11)	NR			
<i>Arachnia propionica</i>			NR			
<i>S. mitis</i>		29 (18-44)				

<sup>a</sup> Two strains included in *Lactobacillus* sp. group.

<sup>b</sup> Two strains included in *Fusobacterium* sp. group.

<sup>c</sup> Median value and range expressed as percentage of total viable count in the purulent exudate.

<sup>d</sup> These strains do not fit into recognized species and were classified by numerical taxonomy (42).

<sup>e</sup> NR, Inoculated but not recovered.

pared as described by Holdeman and Moore (18). Horse blood (Statens Bakteriologiska Laboratorium, Stockholm, Sweden) was hemolysed by freeze-thawing before being used in brain heart infusion agar medium (18). Agar media were prepared in room atmosphere and were stored for at least 24 h in an anaerobic box before use. The anaerobic box had an atmosphere of 10% hydrogen and 5% carbon dioxide in nitrogen (42). Each of the different strains was cultivated on blood agar slants for 3 to 4 days at 37°C. Colonies of each strain in a combination were suspended in PY-glucose broth, and all suspensions were transferred to one tube with a final amount of 5 ml of PY-glucose broth. The cell suspension was homogenized in a glass mortar and transferred to a syringe in the anaerobic box. The concentration of the different bacterial strains was determined by viable count on blood agar. The average concentration of bacteria in the suspension was  $10^8$  cells per ml.

**Testing of pathogenicity.** According to a standardized procedure, 1 ml of the suspension was inoculated into the subcutaneous tissue of the groin of 200- to 250-g guinea pigs (Axells Djurfarm, Sollentuna, Sweden). As a control, PY-glucose broth without bacteria was inoculated. The animals were examined daily for the presence of developing lesions. Hard, nodular, caseous swellings or rapid healing was interpreted as poor pathogenicity of the combination, whereas the death of the animal, the spreading of a necrotic lesion, or localized abscess formation was seen as evidence of high virulence. In the case of abscess formation, the contents of the abscess were aspirated, and 0.5 ml was inoculated into another guinea pig; this procedure was repeated each time a purulent infection was established. A bacterial combination was considered to induce a transmissible infection when the procedure could be repeated four times. After four transmissions the contents of the abscess were analyzed; 0.1 ml of the abscess contents from the fifth animal was added to 5 ml of PY-glucose broth. To avoid oxidation of the broth, the sampling tube was flushed with oxygen-free gas (97% carbon dioxide, 3% hydrogen). From the broth 10-fold serial dilutions were made in the anaerobic box, and samples were cultivated on blood agar to estimate the concentration of viable bacteria and the relative numbers of the various bacterial strains. Some strains could be recognized by their colonial morphology. Other strains had to be identified after Gram staining and determination of their fermentation products (42). Each combination of bacteria was inoculated into four guinea pigs on three different occasions.

**Histological procedures.** Infected tissues were excised from various animals at different times, thus permitting the development of the lesions to be studied either at daily intervals for a total of 5 days or at longer intervals for a total period of 3 weeks. The specimens were fixed for 48 h in neutral buffered Formalin at room temperature, dehydrated, and embedded in paraffin. Sections (5  $\mu$ m) were taken through the midportion of the lesions and stained with hematoxylin and eosin. The Brown-Brenn staining procedure was used for the demonstration of the presence of bacteria.

**Antibody determination.** Blood was obtained from the guinea pigs by cardiac puncture before inoculation and 2 weeks thereafter. Sera were prepared, inactivated at 56°C for 30 min, frozen, and stored at -80°C until required. The presence of antibodies was demonstrated by: (i) the indirect fluorescent antibody technique, (ii) the double diffusion in gel method of Ouchterlony, and (iii), for hemagglutinating strains (*Fusobacterium nucleatum*), the hemagglutination inhibition test. The strains were grown in PY-glucose broth, washed three times in phosphate-buffered saline, pH 7.2, suspended in phosphate-buffered saline, and kept frozen at -80°C until required. The indirect fluorescent antibody (FA) technique was performed by the method of Williams et al. (48). Fluorescein isothiocyanate-labeled rabbit anti-guinea pig immunoglobulin G (IgG) serum and the same serum unlabeled were obtained from Behringswerke, Marburg, West Germany. When fluorescence occurred, its specificity was tested by blocking the fluorescence (i) by absorption of the tested serum with the strain under investigation, (ii) by adding unlabeled anti-IgG serum, and (iii) by omission of the test serum before adding the labeled anti-IgG serum (41).

A nonimmune reaction with the Fc part of the IgG molecules which gives fluorescence with the FA technique has been reported (9, 25). Species of staphylococci (10) and streptococci (22) have this nonimmune reactivity with IgG, but the presence of similar structures in other microbial species has been investigated only to a limited extent, and the possibility could not be ruled out that the fluorescence with the strains in this study was due to a non-immunological reaction. The Fc binding capacity of the strains with positive FA reactions was therefore tested with a radioimmunological assay (6) and by their ability to agglutinate sensitized sheep erythrocytes (5). These tests were made by P. Christensen, Lund, Sweden. The double diffusion in gel test was performed with the modifications described by Wadsworth (46). Bacterial intracellular material was prepared as described by Holm (19). The hemagglutination inhibition test was carried out by the method of Kwapinski (23).

## RESULTS

**Pathogenicity.** Inoculation of PY-glucose broth without microorganisms produced no visible macroscopic reactions. When the combinations of bacteria isolated from 11 symptomless teeth with chronic apical inflammation were inoculated, no transmissible infection developed. In contrast, six of the seven combinations derived from teeth with purulent apical inflammation induced transmissible infections in guinea-pigs (Table 3). Thus, there was a correlation between the ability of these bacterial combinations to induce purulent apical inflammations in humans and their ability to produce transmissible infections when inoculated subcutaneously into guinea pigs.

**Bacteriological findings.** All of the combi-

TABLE 3. Induction of transmissible infection in guinea pigs by bacterial combinations isolated from necrotic dental pulps<sup>a</sup>

Bacterial combination	Proportion of infected animals at the first inoculation in a series			No. of series in which transmissible infection was established <sup>b</sup>	
	Complete combination	<i>Bacteroides</i> excluded <sup>c</sup>	Only <i>Bacteroides</i> <sup>c</sup>	Complete combination	Only <i>Bacteroides</i> <sup>c</sup>
D	8/12	0/4	0/4	4	
G	10/12	0/4	1/4	10	0
H	0/12	0/4	0/4		
P	12/12	0/4	0/4	9	
AB	12/12	0/4	1/4	12	0
UJB	10/12	0/4	4/4	10	4
BN	8/12	0/4	0/4	5	

<sup>a</sup> Twelve animal series were tested for each complete combination and four were tested for each reduced combination.

<sup>b</sup> A bacterial combination was considered to induce a transmissible infection when the infection in the first animal in a series could be serially transferred to four other animals.

<sup>c</sup> *B. melaninogenicus* or *B. asaccharolyticus*.

nations of bacteria which produced transmissible infections contained strains of *Bacteriodes melaninogenicus* or *B. asaccharolyticus*. The combinations D, G, P, AB, and UJB contained *B. melaninogenicus* subsp. *intermedius*, and the combination BN contained *B. asaccharolyticus* (formerly *B. melaninogenicus* subsp. *asaccharolyticus*) (8). Combination H also contained the latter species but did not induce transmissible infections. When the *B. melaninogenicus* or *B. asaccharolyticus* strains were excluded from the combinations, no transmissible infections developed in the animals. One strain of *B. melaninogenicus* subsp. *intermedius* (UJB13-c) was able to produce transmissible infection in pure culture (Table 3). These results suggested that purulent inflammation in the apical region may in some cases be induced by specific combinations of bacteria in the root canal and that the presence of *B. melaninogenicus* or *B. asaccharolyticus* in these combinations was essential.

Most of the inoculated strains could be isolated after the infection had been transferred four times (Table 2). The distribution of the bacterial strains in the exudates (Table 2) suggests, however, that in addition to *B. melaninogenicus* or *B. asaccharolyticus*, *Peptostreptococcus micros* may be essential for pathogenicity. *Streptococcus mitis* may play a similar role in combination G. The inoculum had to contain 10<sup>8</sup> bacteria per ml to induce transmissible infec-

tion. The density of the bacteria in the abscess fluid was 10<sup>9</sup> to 10<sup>10</sup> cells per ml.

**Antibody determination.** The levels of antibodies in the guinea pig sera are shown in Table 4. Sera collected before the inoculation reacted not only with the *B. melaninogenicus* or *B. asaccharolyticus* strains, but also with strains of *F. nucleatum*, *Actinomyces* species, *Peptostreptococcus anaerobius*, and *Lactobacillus catenaforme*. The strains giving positive FA reactions had no Fc binding capacity with IgG, and the results indicate therefore that guinea pigs have natural antibodies against these strains. Generally, no significant increase in the titers could be detected after the abscesses had developed. However, in combinations D and BN one animal showed a significant increase in the level of antibodies.

**Histopathological observations.** At 1 day after inoculation all bacterial combinations caused a lesion that exhibited the classic features of acute inflammation, with accumulation of polymorphonuclear leukocytes (PMNs) and fluid and with loss of collagen fibers. The degree of tissue reaction did, however, vary. When *B. melaninogenicus* or *B. asaccharolyticus* was present, a large abscess formed, with a heavy central accumulation of degenerating PMNs and microorganisms. These portions were walled off by a prominent layer of PMNs, followed by edematous connective tissue diffusely sprinkled with PMNs and showing considerable loss of collagen architecture (Fig. 1).

In contrast, in the absence of *B. melaninogenicus* or *B. asaccharolyticus*, a smaller and more circumscribed abscess formed, in which densely packed microorganisms were walled off by a dense layer of PMNs, with moderate signs of degeneration and connective tissue edema. Thus, the number of PMNs in association with the microorganisms, as well as the degree of PMN degeneration and connective tissue edema, seemed to increase in the presence of *B. melaninogenicus* or *B. asaccharolyticus*.

With bacterial combinations containing *B. melaninogenicus* or *B. asaccharolyticus*, the initial abscess increased in size during the first 5 days. Large numbers of necrotic and degenerating PMNs filled the abscess cavity, which was lined by a thick layer of PMNs showing signs of gradually increased degeneration toward the cavity (Fig. 2). Peripheral to this layer, fibroblastic proliferation was evident, with a moderate production of collagen. Diffusely spread PMNs were observed among the fibroblasts, which also proliferated into the superficial muscular layer.

In the sections stained by the Brown-Brenn

TABLE 4. Presence of antibodies in guinea pigs in which transmissible infection was induced

Bacterial combination	Strain	Presence of antibodies by:				
		Ouchterlony method (test serum) <sup>a</sup>	FA technique		Hemagglutination inhibition test	
			Normal serum <sup>b</sup>	Test serum	Normal serum	Test serum
D	<i>Actinomyces</i> sp.	—	+ (4-8) <sup>c</sup>	+ (4-128)		
	<i>P. anaerobius</i>	—	+ (4-32)	+ (4-128)		
	<i>B. melaninogenicus</i>	—	+ (4-16)	+ (4-512)		
G	<i>Bacteroides oralis</i>	—	—	+ (4-16)		
	<i>B. melaninogenicus</i>	—	+ (4-32)	+ (4-32)		
P	<i>F. nucleatum</i>	+	—	+ (4-16)	—	+ (4)
	<i>B. melaninogenicus</i>	—	+ (4)	+ (4-16)		
AB	<i>F. nucleatum</i>	—	+ (4)	+ (4-16)	+ (8)	+ (8)
	<i>L. catenaforme</i>	—	+ (4)	+ (4-16)		
	<i>B. melaninogenicus</i>	—	+ (4-16)	+ (4-16)		
BN	<i>F. nucleatum</i> (BN11a-d)	+	+ (4-16)	+ (4-16)		
	<i>B. asaccharolyticus</i>	—	+ (4)	+ (4-32)		
	<i>F. nucleatum</i> (BN9a-l)	+	+ (4-16)	+ (4-256)	+ (16)	+ (16)
	<i>F. nucleatum</i> (BN9a-m)	+	—	+ (4-64)	—	+ (8)
UJB	<i>B. melaninogenicus</i>	—	+ (4-32)	+ (4-32)		
	<i>Selenomonas sputigena</i>	—	—	+ (4)		
	<i>F. nucleatum</i>	—	—	+ (4-16)	—	+ (8)

<sup>a</sup> Test serum was collected 7 to 10 days after an abscess had developed.

<sup>b</sup> Normal serum was collected before the inoculation.

<sup>c</sup> Numbers in parentheses represent the reciprocal of the highest dilution of serum giving fluorescence or hemagglutination inhibition.

procedure, a number of PMNs at the junction between the dense PMN layer and the surrounding fibroblastic connective tissue showed positive cytoplasmic staining, which also corresponded well with a periodic acid-Schiff staining of the cells. Apparently, these cells were actively engaged in phagocytosis of microorganisms (Fig. 3).

After a 5-day exposure to the combinations without *B. melaninogenicus* or *B. asaccharolyticus*, a small and localized abscess was seen in the animals. The abscess showed some central necrosis, which was walled off by a layer of PMNs. Reactive fibroblastic connective tissue surrounded the abscess, and adjacent to this tissue a number of PMNs showed the same stain reaction as described above, i.e. signs of phagocytosed microorganisms.

Among animals exposed to the bacterial combination for longer periods of time, extensive spread of the infection, including perforation of the skin and the peritoneal cavity, was observed in animals infected with combinations containing *B. melaninogenicus* or *B. asaccharolyticus*. In animals infected with combinations without these strains, however, a gradual resolution took place, and after 3 weeks only a few inflammatory cells could be recognized in the connective tissue at the site of infection.

## DISCUSSION

Most periapical inflammations caused by infected dental root canals are chronic and develop insidiously and without symptoms (36). Acute exacerbations, which may occur spontaneously or as a sequel to endodontic treatment, have not been associated with the occurrence of certain microorganisms (1, 13).

In the present study, combinations of bacteria isolated from the root canals of pulpless teeth with or without symptoms but with periapical bone destruction were tested for their capacity to induce transmissible infections in guinea pigs. It was repeatedly found that abscesses could be produced. However, persistent abscesses only developed as a result of infection with bacterial combinations from teeth with clinical symptoms and evidence of pus formation. The presence of *B. melaninogenicus* or *B. asaccharolyticus* was found to be essential for inducing this transmissible infection. This is in accordance with earlier observations (27, 39, 43), but the association of *B. melaninogenicus* and *B. asaccharolyticus* with the purulent inflammation of pulpless teeth has not been reported before.

Recently, *B. asaccharolyticus*, formerly considered a subspecies of *B. melaninogenicus* (18), has been elevated to species rank (8). In the

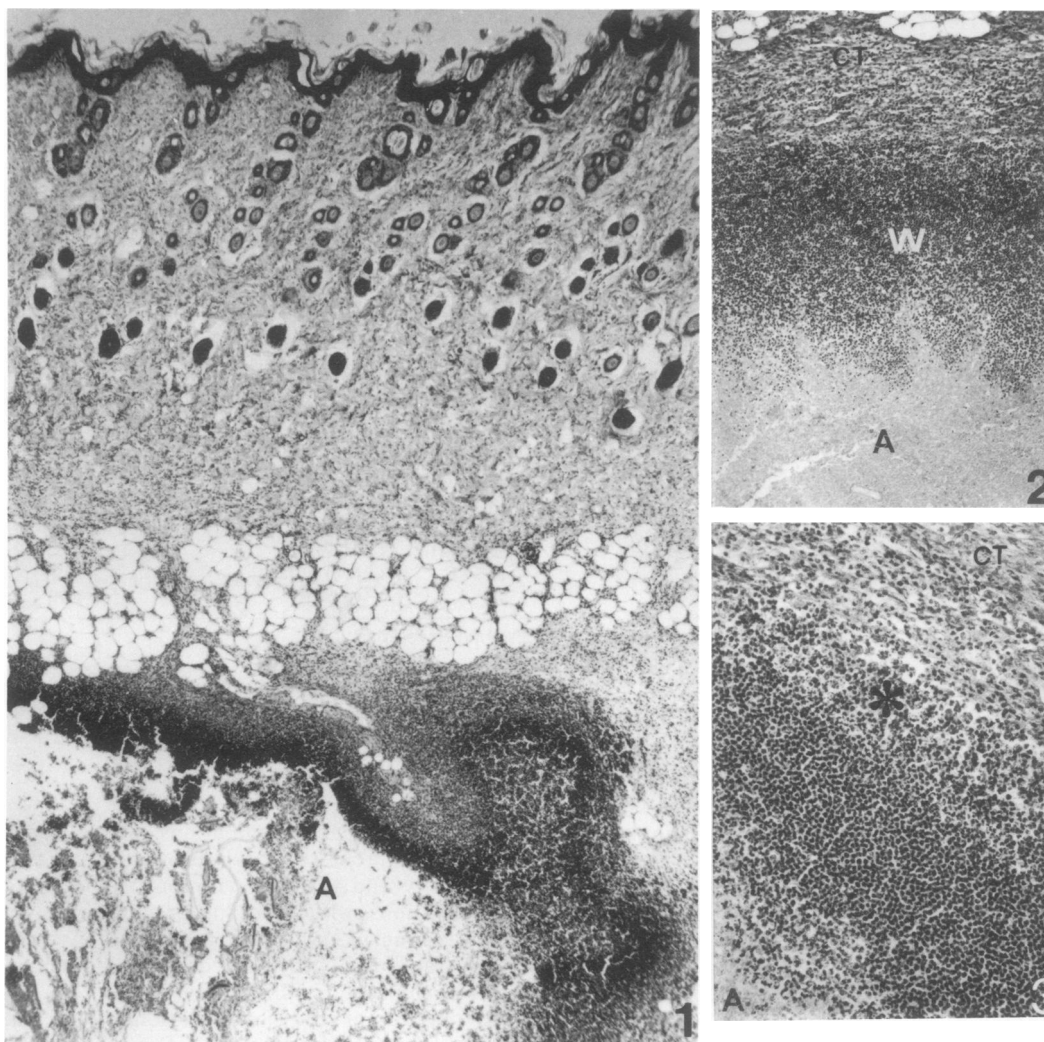


FIG. 1. Portion of skin excised 1 day after inoculation with the bacterial combination UJB, *B. melaninogenicus* included, showing edge of abscess cavity (A) in dermis walled off by a dense layer of PMNs. The preparation was stained with hematoxylin and eosin.  $\times 40$ .

FIG. 2. Wall of dermal abscess at 5 days after inoculation with the same bacterial combination described in the legend to Fig. 1. Abscess cavity (A) is filled with necrotic material and walled off by PMNs (W) and fibroblastic connective tissue (CT). The preparation was stained with hematoxylin and eosin.  $\times 75$ . See Fig. 1 for further details.

FIG. 3. Higher magnification of abscess wall corresponding to that shown in Fig. 2. In Brown-Brenn- as well as periodic acid-Schiff-stained sections, the PMNs within a well-defined area of the wall (\*) exhibited a positive stain reaction, interpreted as evidence of phagocytosed microorganisms. CT, Fibroblastic connective tissue; A, abscess cavity. The preparation was stained with periodic acid-Schiff stain.  $\times 130$ .

majority of previous reports concerning *B. melaninogenicus*, no identification to subspecies level was made and *B. asaccharolyticus* was reported as *B. melaninogenicus*. *B. melaninogenicus*, including *B. asaccharolyticus*, has been isolated from various human infections (24) and is a common inhabitant of the oral cavity in

humans (4). It is among the predominant species in the gingival crevice, making up approximately 5% of the viable counts (12, 40). Slots (37) has reported recently that *B. melaninogenicus* constitutes more than 50% of the cultivable flora in the gingival pocket in patients with advanced marginal periodontitis. Only recently, however,

has the organism been isolated regularly from root canal samples of pulpless teeth (2, 21, 42, 49).

*B. melaninogenicus*, including *B. asaccharolyticus*, contains endotoxin (16, 30) and is able to hydrolyze collagen (11, 14, 15), fibrin (47), and other proteins (27). It also produces metabolites that may be of importance in the infective process (26). The collagenolytic activity of *B. melaninogenicus* is of special interest, since tissue destruction may be the principal pathogenic factor of the organism (38). Both *B. melaninogenicus* and *B. asaccharolyticus* exhibit collagenolytic activity and also the capacity to induce transmissible infection. The similarity between *B. asaccharolyticus* and the subspecies of *B. melaninogenicus* has been shown, by numerical taxonomic analysis, to be only 63% (42). This suggests that one or more specific properties may be of particular importance in abscess formation, for example the collagenolytic capacity.

In the present study, transmissibility of the *B. melaninogenicus* or *B. asaccharolyticus* infection could be demonstrated as occurring despite a massive immigration of PMNs to the site of infection. This indicates that the virulence may be partly related to an ability to resist ingestion or intracellular killing once ingested. It is of interest that Okuda and Takazoe (33) found that a highly infective strain of *B. melaninogenicus*, which had a capsule, was not readily phagocytosed and killed. It has also been found that oral strains of the species may have a capsule and that encapsulated strains produce experimental abscesses, in contrast to non-encapsulated strains (44). Recently, strains of *B. asaccharolyticus* have also been shown to be encapsulated (29).

Moreover, we found that almost all additional strains in each combination containing *B. melaninogenicus* or *B. asaccharolyticus* survived in the tissues, which is suggestive of impaired function among the accumulated PMNs. This may be due to a leukocidal action of *B. melaninogenicus* and *B. asaccharolyticus*. In ovine foot abscesses, which are also caused by a synergistic mixed infection, one of the organisms involved (*Fusobacterium necrophorum*) produces a leukocidal toxin which protects both this and other organisms from being phagocytosed (34). The role of *B. melaninogenicus* and *B. asaccharolyticus* may be the same in other polymicrobial pyogenic infections of the oral cavity. In fact, Okuda and Takazoe (33) have demonstrated that capsular material from *B. melaninogenicus* inhibits phagocytosis and the phagocytic killing of another microorganism in an in vitro system.

In a polymicrobial infection, the pathogenicity

expressed by the bacteria is the result of synergistic actions in the tissue (35). *B. melaninogenicus* and *B. asaccharolyticus* are usually unable to induce abscesses when inoculated subcutaneously in pure culture into guinea pigs (27). Additional organisms are required in the inoculum to achieve pathogenicity. However, different strains of *B. melaninogenicus* vary as to the degree of pathogenicity. Macdonald et al. (27) were able to produce abscesses with one strain of *B. melaninogenicus* (CR2A) without the support of additional organisms. In the present study, the strain UJB13-c exhibited the same capacity. It is also clear that not all organisms in the pathogenic combinations of the present study are essential for transmissible infection to occur. The bacteria recovered from the abscesses after four subsequent transfers in the animals (Table 2) suggest that, in addition to *B. melaninogenicus* and *B. asaccharolyticus*, the anaerobic *P. micros* and the facultatively anaerobic *S. mitis* could be essential organisms. Our failure to produce transmissible infection with combination H (Table 3), which was derived from a tooth with signs of purulent inflammation, may be due to a failure to isolate an essential organism in the original sample.

We could detect an increased level of antibodies to *B. melaninogenicus*, *B. asaccharolyticus*, and *F. nucleatum* in sera collected after an abscess had formed, but we also found that the guinea pigs had naturally occurring antibodies to some of the bacterial species. In humans low levels of antibodies to *B. melaninogenicus* (7, 17), *F. nucleatum* (17), and most other gingival crevice microorganisms (31, 48) can be demonstrated. This is consistent with what is usually observed in the case of indigenous bacteria. Thus, it is possible that the reactions observed in the apical tissues in patients and in the skin of the guinea pigs may have been aggravated by immunological reactions. The lack of a consistent increase in the antibody titer after abscess formation in the guinea pigs suggests, however, that the immunological contribution to the reaction could only have been small.

The mechanical instrumentation of the infected root canal is an important part of endodontic treatment. Usually irrigating solutions are employed to facilitate this procedure. It has been shown that the contents of root canals are extruded at the apical foramen when irrigating solutions are used (45). If the extruded material is infected, it may induce an acute exacerbation (20, 36, 49). As irrigation is regularly used, it is likely that microorganisms are often forced out periapically, but the experience is that acute exacerbation occurs in relatively few cases. The

finding that certain acute exacerbations may be caused by polymicrobial infections in which special microorganisms attain pathogenicity by synergism may explain why it does not occur more often.

## LITERATURE CITED

1. **Bartels, H. A., I. J. Naidorf, and H. Blechman.** 1968. A study of some factors associated with endodontic "flare-ups." *Oral Surg. Oral Med. Oral Pathol.* **25**:255-261.
2. **Bergenholtz, G.** 1974. Micro-organisms from necrotic pulp of traumatized teeth. *Odontol. Revy* **25**:347-358.
3. **Blechman, H.** 1973. Infection of the pulp and periapical tissues, p. 237-239. *In* W. A. Nolte (ed.), *Oral microbiology*, 2nd ed. C. V. Mosby Co., St. Louis.
4. **Burdon, K. L.** 1928. *Bacterium melaninogenicus* from normal and pathogenic tissues. *J. Infect. Dis.* **42**:161-171.
5. **Christensen, P., and G. Kronwall.** 1974. Capacity of group A, B, C, D and G streptococci to agglutinate sensitized sheep red cells. *Acta Pathol. Microbiol. Scand.* **82**:19-24.
6. **Christensen, P., and V.-A. Oxelius.** 1974. Quantitation of the uptake of human IgG by some streptococci groups A, B, C and G. *Acta Pathol. Microbiol. Scand.* **82**:475-483.
7. **Courant, P. R., and R. J. Gibbons.** 1967. Biochemical and immunological heterogeneity of *Bacteroides melaninogenicus*. *Arch. Oral Biol.* **12**:1605-1613.
8. **Finogold, S. M., and E. M. Barnes.** 1977. Report of the ICSB taxonomic subcommittee on gram-negative anaerobic rods. Proposal that the saccharolytic and asaccharolytic strains at present classified in the species *Bacteroides melaninogenicus* (Oliver and Wherry) be reclassified in two species as *Bacteroides melaninogenicus* and *Bacteroides asaccharolyticus*. *Int. J. Syst. Bacteriol.* **27**:388-391.
9. **Forsgren, A., and U. Forsum.** 1970. Role of protein A in nonspecific immunofluorescence of *Staphylococcus aureus*. *Infect. Immun.* **2**:387-391.
10. **Forsgren, A., and J. Sjöquist.** 1966. Protein A from *S. aureus*. Pseudoimmune reaction with human  $\gamma$ -globulin. *J. Immunol.* **97**:822-827.
11. **Gibbons, R. J., and J. B. Macdonald.** 1961. Degradation of collagenous substrates by *Bacteroides melaninogenicus*. *J. Bacteriol.* **81**:614-621.
12. **Gibbons, R. J., S. S. Socransky, S. Sawyer, B. Kapsimalis, and J. B. Macdonald.** 1963. The microbiota of the gingival crevice area of man. II. The predominant cultivable organisms. *Arch. Oral Biol.* **8**:281-289.
13. **Grossman, L. I., E. Lee, and S. Demp.** 1962. Isolation of gas-producing organisms from root canals. *J. Dent. Res.* **41**:495.
14. **Hausmann, E., P. R. Courant, and D. S. Arnold.** 1967. Conditions for the demonstration of collagenolytic activity in *Bacteroides melaninogenicus*. *Arch. Oral Biol.* **12**:317-319.
15. **Hausmann, E., and E. Kaufman.** 1969. Collagenase activity in a particulate fraction from *Bacteroides melaninogenicus*. *Biochim. Biophys. Acta* **194**:612-615.
16. **Hofstad, T.** 1969. Serological properties of lipopolysaccharide from oral strains of *Bacteroides melaninogenicus*. *J. Bacteriol.* **97**:1078-1082.
17. **Hofstad, T.** 1974. Antibodies reacting with lipopolysaccharide from *Bacteroides melaninogenicus*, *Bacteroides fragilis* and *Fusobacterium nucleatum* from normal human subjects. *J. Infect. Dis.* **129**:349-352.
18. **Holdeman, L. V., and W. E. C. Moore (ed.).** 1975. *Anaerobe laboratory manual*. Virginia Polytechnic Institute and State University, Blacksburg.
19. **Holm, S. E.** 1967. Precipitogens in beta-hemolytic streptococci and some related human kidney antigens. *Acta Pathol. Microbiol. Scand.* **70**:79-94.
20. **Ingle, J. I., D. H. Glick, and L. D. Schaeffer.** 1976. Differential diagnosis and treatment of oral and perioral pain, p. 488-563. *In* J. I. Ingle and E. E. Beveridge (ed.), *Endodontics*, 2nd ed. Lea & Febiger, Philadelphia.
21. **Kantz, W. E., and C. A. Henry.** 1974. Isolation and classification of anaerobic bacteria from intact chambers of non-vital teeth in man. *Arch. Oral Biol.* **19**:91-96.
22. **Kronwall, G.** 1973. A surface component in group A, C and G streptococci with non-immune reactivity for immunoglobulin G. *J. Immunol.* **111**:1401-1406.
23. **Kwapinski, J. B.** 1965. *Methods of serological research*. John Wiley & Sons, Inc. New York.
24. **Lambe, D. W., Jr.** 1974. Determination of *Bacteroides melaninogenicus* serogroups by fluorescent antibody staining. *Appl. Microbiol.* **28**:561-567.
25. **Lind, I.** 1968. Nonspecific adsorption of FITC-labeled serum globulins to *Staphylococcus aureus*. *Acta Pathol. Microbiol. Scand.* **73**:624-636.
26. **Macdonald, J. B., and R. J. Gibbons.** 1962. The relationship of indigenous bacteria to periodontal disease. *J. Dent. Res.* **41**:320-326.
27. **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**(Suppl.):529-544.
28. **Macdonald, J. B., R. M. Sutton, and M. L. Knoll.** 1954. The production of fusospirochetal infections in guinea pigs with recombined pure culture. *J. Infect. Dis.* **95**:275-284.
29. **Mansheim, B. J., and D. L. Kasper.** 1977. Purification and immunochemical characterization of the outer membrane complex of *Bacteroides melaninogenicus* subspecies *asaccharolyticus*. *J. Infect. Dis.* **135**:787-799.
30. **Mergenhausen, S. E., E. G. Hampf, and H. W. Scherp.** 1961. Preparation and biological activities of endotoxins from oral bacteria. *J. Infect. Dis.* **108**:304-310.
31. **Nisengard, R. J., D. Myers, and M. G. Newman.** 1977. Human antibody titers to periodontosis-associated microbiota. *J. Dent. Res.* **56**:A73.
32. **Ogilvie, A. L.** 1976. Periapical pathosis, p. 394-439. *In* J. I. Ingle and E. E. Beveridge (ed.), *Endodontics*, 2nd ed. Lea & Febiger, Philadelphia.
33. **Okuda, K., and I. Takazoe.** 1973. Antiphagocytic effects of the capsular structure of a pathogenic strain of *Bacteroides melaninogenicus*. *Bull. Tokyo Dent. Coll.* **14**:99-104.
34. **Roberts, D. S.** 1967. The pathogenic synergy of *Fusiformis necrophorus* and *Corynebacterium pyogenes*. I. Influence of the leucoidal exotoxin of *F. necrophorus*. *Br. J. Exp. Pathol.* **48**:665-673.
35. **Roberts, D. S.** 1969. Synergic mechanisms in certain mixed infections. *J. Infect. Dis.* **120**:720-724.
36. **Seltzer, S.** 1971. *Endodontology: biologic considerations in endodontic procedures*. McGraw-Hill Book Co., New York.
37. **Slots, J.** 1977. The predominant cultivable microflora of advanced periodontitis. *Scand. J. Dent. Res.* **85**:114-121.
38. **Smith, L. DS.** 1975. *The pathogenic anaerobic bacteria*. Charles C Thomas, Publisher, Springfield, Ill.
39. **Socransky, S. S., and R. J. Gibbons.** 1965. Required role of *Bacteroides melaninogenicus* in mixed anaerobic infections. *J. Infect. Dis.* **115**:247-253.
40. **Socransky, S. S., R. J. Gibbons, A. C. Dale, L. Bortnick, E. Rosenthal, and J. B. Macdonald.** 1963. The microbiota of the gingival crevice area of man. I. Total microscopic and viable counts and counts of specific organisms. *Arch. Oral Biol.* **8**:275-280.

Downloaded from jai.asm.org at UNIVERSITY OF PENNSYLVANIA LIBRARY on May 2, 2008

41. **Sternberger, L. A.** 1974. Immunocytochemistry. Prentice-Hall, Inc., Englewood Cliffs, N.J.
42. **Sundqvist, G.** 1976. Bacteriological studies of necrotic dental pulps. Umeå University Odontological Dissertation No. 7. University of Umeå, Umeå, Sweden.
43. **Takazoe, I., and T. Nakamura.** 1971. Experimental mixed infection by human gingival crevice material. Bull. Tokyo Dent. Coll. 12:85-93.
44. **Takazoe, I., K. Okuda, and A. Yamamoto.** 1975. Distribution of a K-antigen among oral strains of *Bacteroides melaninogenicus*. Bull. Tokyo Dent. Coll. 16:1-5.
45. **Van de Visse, J. E., and J. D. Brilliant.** 1975. Effect of irrigation on the production of extruded material at the root apex during instrumentation. J. Endodont. 1:243-246.
46. **Wadsworth, C.** 1957. A slide microtechnique for the analysis of immune precipitates in gel. Int. Arch. Allergy 10:355-360.
47. **Weiss, C.** 1943. The pathogenicity of *Bacteriodes melaninogenicus* and its importance in surgical infections. Surgery 13:683-691.
48. **Williams, B. L., R. M. Pantalone, and J. C. Sherris.** 1976. Subgingival microflora and periodontitis. J. Periodont. Res. 11:1-18.
49. **Wittgow, W. C., Jr., and C. B. Sabiston, Jr.** 1975. Micro-organisms from pulpal chambers of intact teeth with necrotic pulps. J. Endodont. 1:168-171.