Some Serological Studies on *Bacteroides corrodens*

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Bacteria currently known as *Bacteroides corrodens* and *Eikenella corrodens* have been described by Henriksen (1937), Henriksen (1948), Holm (1950), Eiken (1958), Reinhold (1966), Khairat (1967), Dowell & Hawkins (1968), Henriksen (1969), Hill, Snell & Lapage (1970), Marsden & Hyde (1971), Jackson, Goodman, Bel, Wong & Whitehouse (1971), Wong & Jackson (1971) and Jackson & Goodman, (1972). Their most striking feature is the tiny pits in the surface of blood agar which they produce initially (Reinhold, 1966; Khairat, 1967). Failure to grow in the presence of oxygen led Eiken (1958) to name them *Bacteroides corrodens*. However, Henriksen (1969) isolated oxygen-tolerant strains which showed a preference for microaerophilic conditions. The two types were shown to be biochemically unresponsive though not totally inert, morphologically similar, and manifested colonial forms which were indistinguishable one from the other.

The dilemma which then arose was whether to allow the *Bacteroides* genus to claim the strictly anaerobic variety and consider its aerobic counterpart as more appropriate to the *Haemophilus*, or, if the pitting phenomenon were to be considered of paramount importance, to include them both in such a genus as would allow for the representation of both anaerobic and aerobic species as in the case of *Streptococcus*. Jackson & Goodman (1972) proposed that a new genus, *Eikenella*, be created to accommodate the facultatively anaerobic strains, and that the strictly anaerobic strains be excluded from the *Bacteroides*. These recommendations were based mainly on the considerable differences in their GC ratios (28 to 30 % for strictly anaerobic strains as against 57 to 58 % for facultative strains), and lack of any demonstrable serological relationship between strictly anaerobic and facultatively anaerobic strains (Jackson *et al.* 1971; Wong & Jackson, 1971; Jackson & Goodman, 1972). Failure to show such a relationship was possibly due to the limited number of anaerobic strains they tested. In this study a number of strictly anaerobic strains and facultatively anaerobic strains of *B. corrodens* are compared serologically with several species of *Bacteroides* and *Haemophilus*.

METHODS

The bacteria used were either freshly isolated or obtained from public or private culture collections.

Strictly anaerobic strains (27). These organisms had failed to grow in the presence of a demonstrable amount of oxygen during at least 50 subcultures and had on at least one occasion produced pits in agar surface. Their laboratory reference numbers are prefixed 'NC-'.

Facultatively anaerobic strains (29). These organisms were facultatively anaerobic and had at least once produced agar pits. Their laboratory numbers are prefixed 'AC-'.

Preparation of antiserum. This was programmed in two phases, NC-1, AC-5, *Bacteroides*

fragilis (NCTC 8560) and *B. melaninogenicum* (NCTC 9336) being selected for the first series, and NCL-20, AC-7, *Sphaerophorus necrophorus* (NCTC 10575) and *Fusobacterium necrogenes* (NCTC 10723) for the second. Luxuriant growth of NC-1 and NCL-20 was obtained by the addition of potassium nitrate (0.1 %, w/v) to Brain Heart Infusion Blood Agar and by the addition of cystine (0.005 %, w/v) for strains AC-5 and AC-7. The *Bacteroides* species grew well on the blood agar without further enrichment. Colonial material was harvested into pyrogen-free normal saline, heated at 60 °C for 15 min, and then centrifuged at 2400 g for 20 min. The deposit was resuspended in saline, washed twice more and then emulsified in saline to give a density equivalent to 50 International Opacity Units. To 1.75 ml of this concentrate was added 0.25 ml Freund's Complete Adjuvant and this constituted the immunogenic material used to raise antibodies in 4-months-old New Zealand White rabbits. Injections of 0.25 ml were given into both shoulders and hindquarters, and repeated 2 weeks later after 20 ml blood had been taken from the ear. Similar blood samples were taken at fortnightly intervals up to 12 weeks. Working samples of antisera, preserved with merthiolate (0.015 %, w/v) were kept at 4 °C, and the remainder pooled and stored at -20 °C. (Antiserum was not inactivated at this stage because heating at about 50 °C was found to reduce the sensitivity of precipitation tests.)

Antigenic material used in tests. With the exception of that stored at -20 °C and thawed for use, all extracts were prepared fresh. Two methods were used: fresh boiled - bacterial growth was emulsified in 0.15 M-NaCl to a density of thin cream and boiled for 15 min; alkali extracted - similar emulsions were made in 0.05 N-NaOH, boiled for 15 min, neutralized with 0.2 N-HCl in phenol red (0.02 %, w/v), and separated by centrifugation at 1780 g for 10 min.

In addition to the extractions, a freshly prepared, untreated emulsion of organisms in saline was used. The most sensitive preparations for the various tests were found to be as follows: agglutination - fresh untreated suspensions for all strains except *Sphaerophorus necrophorus* and *Fusobacterium necrogenes* which gave better reactions to freshly boiled extracts; complement fixation - frozen and thawed extracts for all strains except *Bacteroides fragilis* (freshly boiled), and *S. necrophorus* (fresh untreated); capillary ring precipitation - alkali extracts for all strains except AC-5, AC-7 (frozen and thawed), and *B. fragilis* (fresh boiled); gel diffusion precipitation - frozen and thawed for all strains except NC-1 (alkali extract) and *F. necrogenes* (fresh untreated).

Slide-agglutination tests. Bacterial growth was emulsified in the normal fashion and reactions were rapid. Auto-agglutinability was overcome by using 36-h cultures in the case of facultatively anaerobic strains which tend to become rough after 48 h.

Tube-agglutination tests. Dreyer's conical tubes (60 mm × 7 mm), containing 0.3 ml unit vol. of antiserum dilution and antigen, were incubated in water baths for 2 h at 37 °C and then at 48 °C overnight.

Complement-fixation tests. Dreyer's tubes containing 0.1 ml unit volumes of antiserum dilution (inactivated at 56 °C for 30 min), antigen (suspended in 0.15 M-NaCl buffer) and complement (2MHD), were incubated for 1.5 h at 37 °C and then for 3 h at 4 °C. Sensitized sheep cells (5MHD), of 2.5 % were added and the whole incubated at 37 °C and read at 15 min intervals up till 1 h.

Capillary interfacial ring precipitation tests (Jones & Shattock, 1960).

Table 1. Serological results of strains used in study

Strains/species reacting with		Antisera prepared against				
Source		Slide agglutination	Tube agglutination	Complement fixation	Capillary ring precipitation	Gel diffusion precipitation
<i>Bacteroides fragilis</i>	NCTC 8560	++	1/80* NCTC 8560	1/320* NCTC 8560	++	5† NCTC 8560
<i>Sphaerophorus necrophorus</i>	NCTC 10575	++	1/1280 NCTC 10575	1/160 NCTC 10575	++	3 NCTC 10575
<i>Fusobacterium necrogenes</i>	NCTC 10723	++	1/1280 NCTC 10723	1/160 NCTC 10723	++	3 NCTC 10723
<i>Bacteroides melaninogenicum</i>	NCTC 9336	++	1/5120 NCTC 9336	1/160 NCTC 9336	++	4 NCTC 9336
<i>B. corrodens</i>	Eiken: 397/53-54	++	1/2560 NC-1 1/40 NCL-20	1/80 NC-1 1/40 NCL-20	++	3 NC-1 1 NCL-20
(anaerobic) two strains	249/54					
(anaerobic) 24 strains	Own isolates					
(anaerobic Link)	NC-1, 2, 11 to 19, 101 to 114	++	1/320 NC-1 1/640 NCL-20	1/80 NCL-20	++	1 NC-1 3 NCL-20
	NCL-20					
(facultatively anaerobic)	Henriksen: 4094/68	++	1/320 AC-5 1/320 AC-7 1/80 NCL-20	1/160 AC-5 1/80 AC-7 1/40 NCL-20	++	4 AC-5 4 AC-7
2 strains	5917/67					
27 strains	Own isolates					
	AC-3, 5, 6, 7, 9, 10, 12, 201 to 220	-	-	1/20 AC-5 1/20 AC-7 1/10 NCL-20	++	2 AC-5 2 AC-7
<i>Haemophilus influenzae</i>	Own isolates					
33 strains	H-501 to 533	-	-	1/20 AC-5 1/20 AC-7 1/20 NCL-20	++	2 AC-5 2 AC-7
	H-571-588					
<i>H. haemolyticus</i>	Own isolates	-	-	1/20 AC-5 1/20 AC-7 1/20 NCL-20	++	2 AC-5 2 AC-7
17 strains	H-571-588					

* Minimum titre obtained with any strain.

† Number of precipitation bands.

++ , Strong (intense) reaction; + , weak but definite reaction; - , no reaction.

RESULTS AND DISCUSSION

Antibodies giving good reactions in agglutination, precipitation and complement-fixation tests were produced from all eight vaccines.

Bacteroides. No serological relationship was shown between the four species used to produce antisera and any other species of *Bacteroides* tested (*Bacteroides hypermegas* NCTC 10570, *B. thetaiotaomicron* NCTC 10582, *B. vulgatus* NCTC 10583, *Sphaerophorus varians* NCTC 10560), nor with any of the pitting strains.

Anaerobic pitting organisms. With antiserum prepared from the typical NC-1 strain, no affinity with facultatively anaerobic strains was found but there was a serological relationship with all anaerobic strains examined. In both liquid and gel precipitating systems reactions were given with *Haemophilus influenzae* and *H. haemolyticus*. These latter reactions, although slow to develop, were unequivocal being clearly reproduced on separate occasions. NCL-20, selected for antiserum production because of its atypical reactions towards NC-1 antiserum, revealed distinct affinities with both anaerobic and facultatively anaerobic pitting strains, and was thus named NCL (link)-20. Link antiserum titres were higher with anaerobic strains than with aerobic strains whereas in the case of capillary ring precipitation, reactions were considerably stronger with the latter. In gel diffusion studies, however, reactions were stronger with anaerobic strains. In common with NC-1, AC-5 and AC-7, NCL-20 precipitation reactions were given with certain strains of *Haemophilus*.

Facultatively anaerobic strains. Although no anaerobic strains except NCL-20 reacted with AC-5 or AC-7 antisera, all facultatively anaerobic strains did so to the same degree as that shown among anaerobic strains (Table 1). Reactions with *Haemophilus* were stronger than with NC-1 and NCL-20 antisera, particularly in capillary precipitation studies. Wherever cross-reactions occurred, tests were repeated using antisera absorbed with homologous bacteria and this effectively prevented any reactions with all strains tested, giving an inference of some shared antigenicity.

Since the findings of this work seem to differ from those of Jackson *et al.* (1971) and Jackson & Goodman (1972), it is proposed to extend the scope of this work considerably before reaching any conclusions as to its taxonomic relevance.

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