A COMPARISON OF THE BIOCHEMICAL ACTIVITIES OF *BACTEROIDES CORRODENS* AND *EIKENELLA CORRODENS* WITH THOSE OF CERTAIN OTHER GRAM-NEGATIVE BACTERIA

A. L. JAMES AND J. V. A. ROBINSON

*Division of Microbiology, Department of Chemistry,
Newcastle upon Tyne Polytechnic, Newcastle upon Tyne NE1 8ST*

BIOCHEMICAL classification of the strictly anaerobic bacteria that comprise *Bacteroides corrodens* (Eiken, 1958) and its facultatively anaerobic counterpart *Eikenella corrodens* (Jackson and Goodman, 1972) poses problems associated with their cultivation. They grow poorly in fluid media, slowly on solid media and well only on media containing whole blood.

Previous workers (Henriksen, 1948 and 1969; Eiken, 1958; Reinhold, 1966; Khairat, 1967; Hill, Snell and Lapage, 1970; and Jackson *et al.*, 1971) reported that the organisms showed little activity when subjected to biochemical tests commonly employed in bacteriology.

These findings are supported in the present study in which the range of tests has been extended and other organisms with some similar properties to those possessed by "corrodens" bacteria have been included for comparison.

MATERIALS AND METHODS

Organisms

"Corrodens" bacteria. Sixteen strains of *B. corrodens* (NC-1, NC-2 and NC-101 to 114) belonging to the same serological group as NC-1 (Robinson and James, 1973), four strains of *B. corrodens* (NCL-20 and NCL-141 to 143) group NCL (link)-20, and 20 strains of *E. corrodens* (AC-4 to 10 and AC-202 to 214) group AC-5, were isolated from a variety of hospital specimens from the upper respiratory, genito-urinary and intestinal tracts.

Other bacteria. The following strains were obtained from the National Collection of Type Cultures, Colindale, England: *B. fragilis* (no. NCTC8560), *B. hypermegas* (no. NCTC10570), *B. thetaiotaomicron* (no. NCTC10582), *B. vulgatus* (no. NCTC10583); *B. melaninogenicus* (no. NCTC9336), *Sphaerophorus varians* (no. NCTC10560), *Sp. necrophorus* (no. NCTC10575); *Fusobacterium necrogenes* (no. NCTC10723); *Haemophilus influenzae* (NCTC nos. 4560, 4842, 8465, 8466, 7279, 8467, 8469, 8470, 10479 and 8473), *H. parainfluenzae* (NCTC nos. 4101, 7857 and 10665), *H. aphrophilus* (NCTC nos. 5886 and 5906), *H. paraphrophilus* (NCTC nos. 10556, 10557 and 10558), *H. haemolyticus* (no. NCTC8479), *H. paraphrohaemolyticus* (NCTC nos. 10670 and 10672), *H. aegyptius* (no. NCTC8502), *H. suis* (NCTC nos. 6359 and 7441); *Bordetella pertussis* (no. NCTC10739), *Bord. parapertussis* (no. NCTC8250), *Bord. bronchiseptica* (no. NCTC8344); *Acinetobacter lwoffii* (no. NCTC5866); *Streptobacillus moniliformis* (no. NCTC10651); *Moraxella liquefaciens* (NCTC nos. 7911, 10358 and 10359), *M. nonliquefaciens* (NCTC nos. 7784 and 10464), *M. osloensis* (no. NCTC10465), *M. kingii* (no. NCTC10529) and *M. lacunata* (NCTC nos. 7985 and 7986).

Received 26 Mar. 1974; accepted 14 May 1974,

In addition, six strains of *B. melaninogenicus* and nine strains of *H. haemolyticus* that were isolated in this Department respectively from the genito-urinary and upper respiratory tracts were examined.

Cultural methods

Basal culture medium (BCM). Where the presence of whole blood was contraindicated, Difco Brain Heart Infusion Agar (BHIA) containing 10% (v/v) horse plasma (inactivated at 50°C for 30 min.) and 4% (v/v) Fildes' extract was used. When a broth medium was required, Difco Brain Heart Infusion Broth was similarly supplemented and is here referred to as basal culture broth (BCB).

Addition of substrates. BHIA was autoclaved at 121°C for 15 min., cooled to 43°C and supplemented with horse plasma and Fildes' extract; chloroform-sterilised or Seitz-filter-sterilised concentrated substrate was then added to give the desired final concentration.

Indicator. Where the reading of tests depended on a pH change, phenol red (0.005%, v/v) was included in the basal medium, and doubtful results were resolved at the time of reading by the addition of phenol red (0.2%, v/v).

Propagation culture medium (PCM). BHIA autoclaved at 115°C for 15 min. was cooled to 90°C, Difco defibrinated horse blood (8%, v/v) was added, the whole cooled to 43°C, and a further addition of horse blood (8%, v/v) was then made to provide a culture medium that supported good growth of all the organisms employed in this investigation (Robinson and James, unpublished work). In tolerance studies, the requisite filter-sterilised agent was added to PCM at 43°C before the second supplement of blood.

Seeding of media. All culture media received a standard inoculum of 0.3 ml of a freshly prepared suspension of 10⁹ organisms per ml; solid media surfaces were then seeded in the usual manner, and tubes of broth were shaken to disperse the inoculum.

Incubation. Strictly anaerobic strains were cultured in an atmosphere of 90% hydrogen and 10% carbon dioxide, in Baird and Tatlock jars fitted with three cold catalysts. All other strains were incubated in sealed candle jars to provide 2-5% carbon dioxide and to prevent dehydration of media. All cultures were incubated at 37°C for 7 days and then at 30°C for 3 days and examined after 2, 5 and 10 days.

Growth factors associated with blood or blood products

Requirements and preferences were ascertained by measuring bacterial growth produced on BHIA incorporating separately the various components at the concentrations given in table I.

Biochemical methods

Unless otherwise stated, methods employed were as described by Cowan and Steel (1966). Specific products or activities were detected as follows.

Catalase: colonies on BCM were treated with hydrogen peroxide (5.0%, w/v) and observed for sustained evolution of gas. *Peroxidase:* pyrogallol (1.0%, w/v) in aqueous hydrogen peroxide (1.0%, w/v) was added to colonies on BCM and these observed for production of yellow purpurogallin (Saunders, Holmes-Siedle and Stark, 1964). *Oxidase:* colonies from BCM and PCM were tested by the methods of Kovacs and of Gordon and McLeod (see Wilson and Miles, 1964). *Nitrate reductase* and *nitrite reductase:* BCM containing potassium nitrate (0.1%, w/v) or sodium nitrite (0.001%, w/v) respectively was employed. Enzyme activity was demonstrated by the use of 0.8% sulphanilic acid in 5M acetic acid and 0.6% (w/v) 1-naphthyl-ethylenediamine hydrochloride in place of the chromogens recommended by Cowan and Steel (1966). *Carbohydrate fermentation:* this was interpreted in terms of acid production in the presence of carbohydrate (1.0%, w/v) in BCM. *Voges-Proskauer* and *methyl-red reactions:* BCB containing glucose (0.5%, w/v) and dipotassium hydrogen phosphate (0.5%, w/v) was tested respectively with O'Meara's and Barrit's reagents, and with methyl-red solution. *Amino-acid decarboxylase, phenylalanine deaminase, arginine hydrolase, hippurate hydrolase, and urease:* the recommended methods of Cowan and Steel (1966) were applied to colonies on BCM containing the relevant substrate.

Indole production: 5-day and 10-day cultures in BCB were tested with Ehrlich's and with Kovacs' reagents. *Hydrogen sulphide production:* 5-day and 10-day cultures in BCB were examined for evolution of hydrogen sulphide with lead-acetate paper, and cultures on BCM incorporating ferrous chloride (0.05%, w/v) were observed for blackening. *Glycosidases:* β -glucosidase activity against aesculin (0.1%, w/v) was detected with ferric chloride, and β -D-galactosidase activity was demonstrated with 2-nitrophenyl β -D-galactopyranoside (ONPG, 0.01%, w/v) as substrate. To extend the range of substrates, three other glycosides were separately incorporated in BCM. Hydrolysis of salicin (0.1%, w/v) and 2-naphthyl β -D-glucoside (0.01%, w/v) was detected according to a modified histochemical method by coupling the released saligenin or 2-naphthol with alkaline tetrazotised *o*-dianisidine (Fast Blue B salt, 1.0%, w/v) and observing azo dye formation (Bergmeyer, 1963). Hydrolysis of the fluorogenic reagent 4-methyl umbelliferyl β -D-glucoside (0.01%, w/v) in BCM was demonstrated by inspection of plates under a Wood's lamp to indicate the production of fluorescent 4-methyl umbelliferone (Robinson, 1956). *Phosphatases:* four substrates were separately incorporated in BCM. Enzyme activity was demonstrated as follows: sodium 1-naphthyl phosphate (0.01%, w/v) and sodium 2-naphthyl phosphate (0.01%, w/v) by coupling released naphthol with alkaline tetrazotised *o*-dianisidine (1.0%, w/v) and observing a violet azo dye formation (Bergmeyer, 1963); phenolphthalein diphosphate (0.01%, w/v) by exposure to ammonia vapour and observing the development of a pink colour; and 4-nitrophenyl disodium orthophosphate (0.01%, w/v) by formation of a yellow colour after alkalisation with 1M NaOH. *Lipases and phospholipases:* glyceryl tributyrat (1.0%, w/v) in BCM was observed for zones of clearing, and egg yolk (5.0%, w/v) in BCM for zones of opacity; hydrolysis of the substrates 2-naphthyl stearate, 1-naphthyl acetate and 2-naphthyl acetate (all 0.01%, w/v) was demonstrated in separate test cultures by the azo coupling method described previously. *Macromolecular hydrolases:* gelatin liquefaction was tested by the incorporation of charcoal gelatin disks (Oxoid) in cultures growing in BCB; gelatin hydrolysis by Frazier's method (see Cowan and Steel, 1966) using BCM containing gelatin (0.4%, w/v); and DNA hydrolysis by observing zones of clearing produced after addition of 1M HCl to BCM containing DNA (2.0%, w/v). *Carboxylic acid utilisation:* Koser's and Simmon's citrate methods, and Difco's malonate medium, were employed.

Tolerance tests

Tolerance was measured in terms of visually observed growth in PCM in the presence of certain compounds at concentrations given in table VII.

Recording of results

Differential results are tabulated (tables I–VII); those that were uniformly positive or negative for all organisms tested are given only in the text. Replicate plates (six in each case) were used and results are expressed in the following manner:

- ++ = strong positive;
- + = weak but definite positive;
- = negative;
- +v = more than 50% strains positive;
- v = less than 50% strains positive;
- V = variable.

Appropriate blanks and test organisms, as recommended by Cowan and Steel (1966) were employed to establish the validity of the methods used.

RESULTS

Growth in the presence of factors associated with blood and blood products

The results of these studies are given in table I. All "corrodens" bacteria grew well on BHIA supplemented with untreated horse, human or sheep

TABLE I(a)
Effect of factors associated with blood on the growth of various species of bacteria

Species	Growth observed* in the presence of										
	BHIAT; no additives	horse serum (10%)	horse plasma (10%)	human serum (10%)	human plasma (10%)	lysed horse blood (10%)	heated horse blood (10%)	horse blood (10%)		human blood (10%)	
								growth	haemolysis	growth	haemolysis
<i>B. corrodens</i> NC-1 group	++	++	++	++	++	+	V	++	-	++	-
<i>B. corrodens</i> NCL-20 group	+	+	+	+	+	+	V	++	-	++	-
<i>E. corrodens</i> AC-5 group	-	-	-	-	-	-	+	++	-	++	-
<i>B. fragilis</i>	++	++	++	++	++	++	+	++	+	++	+
<i>B. hypermegas</i>	++	++	++	++	++	++	+	++	+	++	+
<i>B. thetaiotaomicron</i>	++	++	++	++	++	++	+	++	+	++	+
<i>B. vulgatus</i>	++	++	++	++	++	++	+	++	+	++	+
<i>Sp. varians</i>	++	++	++	++	++	++	+	++	+	++	+
<i>Sp. necrophorus</i>	++	++	++	++	++	++	+	++	+	++	+
<i>F. necrogenes</i>	++	++	++	++	++	++	+	++	+	++	+
<i>B. melaninogenicus</i>	++	++	++	++	++	++	+	++	+	++	+
<i>H. influenzae</i>	-	-	-	++	++	++	++	++	-	++	-
<i>H. parainfluenzae</i>	+	+	+	++	++	++	++	++	-	++	-
<i>H. aphrophilus</i>	-	-	-	+	-	+	+	++	V	+	V
<i>H. paraprofitus</i>	-	-	-	-	-	+	+	+	+	+	+
<i>H. haemolyticus</i>	-	-	-	+	-	V	+	+	+	+	+
<i>H. paraprofitus</i>	-	-	-	++	-	+	+	+	-	+	-
<i>H. aegyptius</i>	-	-	-	++	+	+	+	+	-	+	-
<i>H. suis</i>	-	-	-	++	+	+	+	+	-	+	-
<i>Bord. pertussis</i>	++	++	++	++	++	++	++	++	+	++	+
<i>Bord. parapertussis</i>	++	++	++	++	++	++	++	++	+	++	+
<i>Bord. bronchiseptica</i>	++	++	++	++	++	++	++	++	+	++	+
<i>Acinetobacter Iwoffi</i>	+	+	+	++	+	++	+	+	+	+	+
<i>Streptobacillus moniliformis</i>	-	-	-	++	+	++	+	+	-	+	-
<i>M. liquefaciens</i>	++	++	++	++	++	++	++	++	+	++	+
<i>M. nonliquefaciens</i>	++	++	++	++	++	++	++	++	+	++	+
<i>M. osloensis</i>	++	++	++	++	++	++	++	++	+	++	+
<i>M. kingii</i>	++	++	++	++	++	++	++	++	+	++	+
<i>M. lacunata</i>	++	++	++	++	++	++	++	++	+	++	+

* Key: see Methods.

† BHIA = Brain Heart Infusion Agar (Difco).

TABLE I(b)
Effect of factors associated with blood on the growth of various species of bacteria

Species	Growth observed* in the presence of									
	sheep blood (10%)		factor		Fildes' extract (7.5%)		haematin (0.005%)		menaphthone	
	growth	haemolysis	X	V					NAD (sodium salt) (0.005%)	NADP (sodium salt) (0.005%)
<i>B. corrodens</i> NC-1 group	++	-	++	++	+	+	V	V	V	++
<i>B. corrodens</i> NCL-20 group	++	-	++	++	++	++	++	V	++	++
<i>B. corrodens</i> AC-5 group	++	-	++	++	++	++	++	V	++	++
<i>B. fragilis</i>	++	-	++	++	++	++	++	++	++	++
<i>B. hypermegas</i>	++	-	++	++	++	++	++	++	++	++
<i>B. thetaiotaomicron</i>	++	-	++	++	++	++	++	++	++	++
<i>B. vulgatus</i>	++	-	++	++	++	++	++	++	++	++
<i>Sp. varians</i>	++	-	++	++	++	++	++	++	++	++
<i>Sp. necrophorus</i>	++	-	++	++	++	++	++	++	++	++
<i>Sp. necrogenes</i>	++	-	++	++	++	++	++	++	++	++
<i>B. melaninogenicus</i>	+	-	++	++	++	++	++	++	++	++
<i>H. influenzae</i>	-	-	++	++	++	++	V	V	++	++
<i>H. parainfluenzae</i>	++	V	++	++	++	++	++	V	++	++
<i>H. aphrophilus</i>	V	-	++	++	++	++	++	V	V	++
<i>H. paraprofitus</i>	-	-	++	++	++	++	++	++	++	++
<i>H. haemolyticus</i>	-	-	++	++	++	++	++	++	++	++
<i>H. paraprohaemolyticus</i>	-	-	++	++	++	++	++	++	++	++
<i>H. aegyptius</i>	-	-	++	++	++	++	++	++	++	++
<i>H. suis</i>	-	-	++	++	++	++	++	++	++	++
<i>Bord. pertussis</i>	++	++	++	++	++	++	++	++	++	++
<i>Bord. parapertussis</i>	++	++	++	++	++	++	++	++	++	++
<i>Bord. bronchiseptica</i>	++	++	++	++	++	++	++	++	++	++
<i>Acinetobacter Iwofii</i>	++	-	++	++	++	++	++	++	++	++
<i>Streptobacillus moniliformis</i>	++	-	++	++	++	++	++	++	++	++
<i>M. liquefaciens</i>	++	+	++	++	++	++	++	++	++	++
<i>M. nonliquefaciens</i>	++	-	++	++	++	++	++	++	++	++
<i>M. osloensis</i>	++	+	++	++	++	++	++	++	++	++
<i>M. kingii</i>	++	+	++	++	++	++	++	++	++	++
<i>M. lacunata</i>	++	V	++	++	++	++	++	++	++	++

* Key: see Methods.

blood without producing haemolysis, whereas with lysed blood only moderate growth was observed. With heated horse blood and BHIA, good growth of organisms of the *NC-1* and *NCL-20* groups occurred but there was only moderate growth of the *AC-5* group, whereas the *NCL-20* group grew well and the *NC-1* and *AC-5* groups grew moderately on BHIA supplemented with Fildes' extract (Cowan and Steel, 1966). Horse or human serum incorporated in BHIA without additives supported growth of the *NC-1* and *NCL-20* groups but not the *AC-5* group. BHIA medium with horse or human plasma supported good growth of the *NC-1* and *NCL-20* and moderate growth of the *AC-5* group.

With some *NC-1* and *NCL-20* strains, growth on BHIA was improved by the addition of haematin (BDH), menaphthone (BDH) and NAD (BDH);

TABLE II

Results of oxido-reduction tests with cultures of various species of bacteria*

Species	Result of test for				
	catalase	peroxidase	oxidase	nitrate reduction	nitrite reduction
<i>B. corrodens</i> NC-1 group	—	+	+	—v	+
<i>B. corrodens</i> NCL-20 group	—	+	+	++	+
<i>E. corrodens</i> AC-5 group	—	+	++	++	—
<i>B. fragilis</i>	—	—	—	—	—
<i>B. hypermegas</i>	—	—	—	++	—
<i>B. thetaiotaomicron</i>	—	—	—	+	—
<i>B. vulgatus</i>	—	—	—	—	+
<i>Sp. varians</i>	—	—	—	—	+
<i>Sp. necrophorus</i>	—	—	—	—	—
<i>F. necrogenes</i>	—	—	—	—	+
<i>B. melaninogenicus</i>	—	—	—	—	+v
<i>H. influenzae</i>	—	+	—	++	—
<i>H. parainfluenzae</i>	+	+	+v	+v	—
<i>H. aphrophilus</i>	V	+	—	+	—
<i>H. paraphrophilus</i>	—v	+	—v	+v	—
<i>H. haemolyticus</i>	+v	+	—	++	—
<i>H. paraphrohaemolyticus</i>	+	+	V	V	—
<i>H. aegyptius</i>	++	+	++	+	—
<i>H. suis</i>	V	+	V	+	—
<i>Bord. pertussis</i>	++	++	—	—	—
<i>Bord. parapertussis</i>	++	++	—	—	—
<i>Bord. bronchiseptica</i>	++	++	++	++	—
<i>Acinetobacter lwoffii</i>	++	++	—	+	—
<i>Streptobacillus moniliformis</i>	—	—	—	—	—
<i>M. liquefaciens</i>	++	++	+	++	—
<i>M. nonliquefaciens</i>	++	++	+	++	—
<i>M. osloensis</i>	++	++	++	—	—
<i>M. kingii</i>	—	+	+	—	—
<i>M. lacunata</i>	++	++	+	++	—

* Key: see *Methods*.

but there was no demonstrable improvement of growth around commercially prepared disks impregnated with factors X or V (Mast Laboratories) or NADP (BDH), or tocopherol (BDH). Growth of organisms in the *AC-5* group was improved by X and V disks, by haematin, and by NAD, and in some cases by menaphthone; but not by NADP or tocopherol.

Results of biochemical tests

Our findings are summarised in tables II-VI. All "corrodens" bacteria gave positive peroxidase and oxidase reactions and were catalase negative, phenylalanine-deaminase negative and gave negative methyl-red and Voges-Proskauer reactions. *B. corrodens* strains of the *NC-1* group were mainly nitrate-reductase negative, whereas the *B. corrodens NCL-20* and the *E. corrodens AC-5* groups gave positive reactions. The *NC-1* and *NCL-20* groups were nitrite-reductase positive, whereas the *AC-5* group gave negative reactions.

None of the "corrodens" bacteria that we tested produced indole or hydrogen sulphide, yielded acid in the presence of any carbohydrate, hydrolysed arginine, hippurate or DNA, or were capable of utilising citrate or malonate as substrates for growth.

No organism tested hydrolysed salicin, 4-methyl umbelliferyl- β -D-glucoside, glyceryl tributyrat or lecithin, and none of the "corrodens" bacteria hydrolysed aesculin, 2-naphthyl- β -D-glucoside, 2-nitrophenyl- β -D-galactoside (ONPG) or 2-naphthyl stearate. In contrast, all organisms hydrolysed 2-naphthyl acetate, while only the *NC-1* and *NCL-20* groups hydrolysed 1-naphthyl acetate.

Phosphatase activity was substrate-dependent: all "corrodens" organisms hydrolysed sodium 2-naphthyl phosphate, but failed to hydrolyse phenolphthalein diphosphate, sodium 1-naphthyl phosphate or sodium 4-nitrophenyl orthophosphate.

The *NC-1* and *NCL-20* groups hydrolysed urea, were amino-acid-decarboxylase negative and gelatinase negative by both the Oxoid charcoal-gelatin disk procedure and Frazier's method. In contrast, the *AC-5* group was urease negative, lysine- and ornithine-decarboxylase positive and most strains were weakly gelatinase positive by the Frazier method.

Results of tolerance tests

The results are given in table VII. In the presence of the bile salts, sodium deoxycholate and taurocholate (1.0%, w/v) groups *NC-1* and *NCL-20* grew, but unlike *H. influenzae* growth was not markedly enhanced; the *AC-5* group failed to grow at either concentration of bile salt used. In the presence of potassium cyanide, the *NC-1* and *NCL-20* groups grew better than the *AC-5* group at the higher concentration of 0.075% (w/v). With sodium azide (0.05%, w/v), phenol (0.1%, w/v) and Merthiolate (sodium ethylmercurithiosalicylate, 0.005%, w/v) growth of the *NC-1* and *NCL-20* but not of the *AC-5* groups was observed. Sodium chloride at a concentration of 5.0% (w/v) allowed growth of the *NC-1* but not of the *NCL-20* or *AC-5* groups.

TABLE III(a)
Results* of carbohydrate-fermentation tests

Species	Acid production in the presence of the stated carbohydrate													
	arabinose	xylose	rhamnose	glucose	fructose	mannose	galactose	sorbose	mannitol	dulcitol	sorbitol	adonitol	aesculin	salicin
<i>B. corrodens</i> NC-1 group	—	—	—	—	—	—	—	—	—	—	—	—	—	—
<i>B. corrodens</i> NCL-20 group	—	—	—	—	—	—	—	—	—	—	—	—	—	—
<i>E. corrodens</i> AC-5 group	—	—	—	—	—	—	—	—	—	—	—	—	—	—
<i>B. fragilis</i>	+	+	—	+	+	+	+	—	+	—	+	+	+	+
<i>B. hypernegas</i>	+	+	+	+	+	+	+	—	+	—	+	+	+	+
<i>B. thetaiotaomicron</i>	+	+	+	+	+	+	+	—	+	—	+	+	+	+
<i>B. vulgatus</i>	+	+	+	+	+	+	+	—	+	—	+	+	+	+
<i>Sp. varians</i>	—	—	+	+	+	+	+	—	+	—	+	+	+	+
<i>Sp. necrophorus</i>	—	—	—	+	—	—	—	—	—	—	+	—	—	—
<i>B. necrogenes</i>	—	—	—	+	+	—	+	+	+	—	+	+	—	—
<i>B. melanogenicus</i>	+	—	—	+	+	—	+	+	+	—	+	+	—	—
<i>H. influenzae</i>	—	—	—	—	—	—	—	—	—	—	—	—	—	—
<i>H. parainfluenzae</i>	—	—	—	—	—	—	—	—	—	—	—	—	—	—
<i>H. aphrophilus</i>	+	—	—	+	—	+	+	—	—	—	—	—	—	—
<i>H. paraprothophilus</i>	—	—	—	—	—	—	—	—	—	—	—	—	—	—
<i>H. haemolyticus</i>	—	—	—	—	—	—	—	—	—	—	—	—	—	—
<i>H. paraprothaeomolyticus</i>	—	—	—	—	—	—	—	—	—	—	—	—	—	—
<i>H. aegyptius</i>	—	—	—	—	—	—	—	—	—	—	—	—	—	—
<i>H. suis</i>	—	—	—	—	—	—	—	—	—	—	—	—	—	—
<i>Bord. pertussis</i>	—	—	—	—	—	—	—	—	—	—	—	—	—	—
<i>Bord. parapertussis</i>	—	—	—	—	—	—	—	—	—	—	—	—	—	—
<i>Bord. bronchiseptica</i>	—	—	—	—	—	—	—	—	—	—	—	—	—	—
<i>Acinetobacter Iwoffi</i>	—	—	—	—	—	—	—	—	—	—	—	—	—	—
<i>Streptobacillus moniliformis</i>	—	—	—	—	—	—	—	—	—	—	—	—	—	—
<i>M. liquefaciens</i>	—	—	—	—	—	—	—	—	—	—	—	—	—	—
<i>M. nonliquefaciens</i>	—	—	—	—	—	—	—	—	—	—	—	—	—	—
<i>M. osloensis</i>	—	—	—	—	—	—	—	—	—	—	—	—	—	—
<i>M. Kingii</i>	—	—	—	—	—	—	—	—	—	—	—	—	—	—
<i>M. lacunata</i>	—	—	—	—	—	—	—	—	—	—	—	—	—	—

* Key: see Methods.

TABLE III(b)
Results* of carbohydrate-fermentation tests

Species	Acid production in the presence of							Acetoin production	Methyl-red reaction
	sucrose	maltose	lactose	trehalose	cellobiose	raffinose	dextrin		
<i>B. corrodens</i> NC-1 group	—	—	—	—	—	—	—	—	—
<i>B. corrodens</i> NCL-20 group	—	—	—	—	—	—	—	—	—
<i>E. corrodens</i> AC-5 group	—	—	—	—	—	—	—	—	—
<i>B. fragilis</i>	+	+	+	+	+	+	+	—	—
<i>B. hyemagras</i>	+	+	+	+	+	+	+	—	—
<i>B. thetaiotaomicron</i>	+	+	+	+	+	+	+	—	—
<i>B. vulgatus</i>	—	+	+	—	+	+	+	—	—
<i>Sp. varians</i>	+	+	+	—	+	—	—	—	—
<i>Sp. necrophorus</i>	—	—	—	—	—	—	—	—	—
<i>F. necrogenes</i>	—	—	+	+	—	—	—	—	—
<i>B. melaninogenicus</i>	+	+	+	+	+	—	+	—	—
<i>H. influenzae</i>	—	—	—	—	—	—	—	—	—
<i>H. parainfluenzae</i>	—	—	—	—	—	—	—	—	—
<i>H. aphrophilus</i>	+	+	+	+	—	—	—	—	—
<i>H. paraphrophilus</i>	—	—	—	—	—	—	—	—	—
<i>H. haemolyticus</i>	—	—	—	—	—	—	—	—	—
<i>H. paraphrohaemolyticus</i>	—	—	—	—	—	—	—	—	—
<i>H. aegyptius</i>	—	—	—	—	—	—	—	—	—
<i>H. suis</i>	—	—	—	—	—	—	—	—	—
<i>Bord. pertussis</i>	—	—	—	—	—	—	—	—	—
<i>Bord. parapertussis</i>	—	—	—	—	—	—	—	—	—
<i>Bord. bronchiseptica</i>	—	—	—	—	—	—	—	—	—
<i>Acinetobacter Iwoffi</i>	—	—	—	—	—	—	—	—	—
<i>Streptobacillus moniliformis</i>	—	+	+	—	—	—	+	—	—
<i>M. liquefaciens</i>	—	—	—	—	—	—	—	—	—
<i>M. nonliquefaciens</i>	—	—	—	—	—	—	—	—	—
<i>M. osloensis</i>	—	—	—	—	—	—	—	—	—
<i>M. kingii</i>	—	—	—	—	—	—	—	—	—
<i>M. lacunata</i>	—	—	—	—	—	+	+	—	—

* Key: see Methods.

TABLE IV(a)
Results* of tests of amino-acid metabolism

Species	Decarboxylation of									
	arginine	lysine	ornithine	citrulline	aspartic acid	glutamic acid	creatine	phenylalanine	tyrosine	histidine
<i>B. corrodens</i> NC-1 group	-	-	-	-	-	-	-	-	-	-
<i>B. corrodens</i> NCL-20 group	-	+	+	-	-	-	-	-	-	-
<i>E. corrodens</i> AC-5 group	-	-	-	-	-	-	-	-	-	-
<i>B. fragilis</i>	-	-	-	-	-	-	-	-	-	-
<i>B. hypermegas</i>	-	-	-	-	-	-	-	-	-	-
<i>B. thetaiotaomicron</i>	-	-	-	-	-	-	-	-	-	-
<i>B. vulgatus</i>	-	-	-	-	-	-	-	-	-	-
<i>Sp. varians</i>	-	-	-	-	-	-	-	-	-	-
<i>Sp. necrophorus</i>	-	-	-	-	-	-	-	-	-	-
<i>F. necrogenes</i>	-	-	-	-	-	-	-	-	-	-
<i>B. melaninogenicus</i>	-	-	-	-	-	-	-	-	-	-
<i>H. influenzae</i>	+	-	-	+	-	-	-	-	-	-
<i>H. parainfluenzae</i>	+	-	+	-	-	-	-	-	-	-
<i>H. aphrophilus</i>	+	-	-	-	-	-	-	-	-	-
<i>H. paraphrophilus</i>	-	-	-	-	-	-	-	-	-	-
<i>H. haemolyticus</i>	-	-	-	-	-	-	-	-	-	-
<i>H. paraphrohaemolyticus</i>	-	-	-	-	-	-	-	-	-	-
<i>H. aegyptius</i>	-	-	-	-	-	-	-	-	-	-
<i>H. suis</i>	-	-	-	-	-	-	-	-	-	-
<i>Bord. pertussis</i>	+	+	+	+	+	+	+	+	+	+
<i>Bord. parapertussis</i>	+	+	+	+	+	+	+	+	+	+
<i>Bord. bronchiseptica</i>	+	+	+	+	+	+	+	+	+	+
<i>Acinetobacter Iwoffi</i>	-	-	-	-	-	-	-	-	-	-
<i>Streptobacillus moniliformis</i>	-	-	-	-	-	-	-	-	-	-
<i>M. liquefaciens</i>	-	+	+	+	+	+	+	+	+	+
<i>M. nonliquefaciens</i>	-	+	+	+	+	+	+	+	+	+
<i>M. osloensis</i>	-	+	+	+	+	+	+	+	+	+
<i>M. kingii</i>	-	+	+	+	+	+	+	+	+	+
<i>M. lacunata</i>	-	+	+	+	+	+	+	+	+	+

* Key: see Methods.

TABLE IV(b)
Results* of tests of amino-acid metabolism

Species	Deamination of phenylalanine	Hydrolysis of arginine	Production of	
			indole	H ₂ S
<i>B. corrodens</i> NC-1 group	—	—	—	—
<i>B. corrodens</i> NCL-20 group	—	—	—	—
<i>E. corrodens</i> AC-5 group	—	—	—	—
<i>B. fragilis</i>	—	+	—	+
<i>B. hypermegas</i>	—	—	—	—
<i>B. thetaiotaomicron</i>	—	—	+	—
<i>B. vulgatus</i>	—	++	—	+
<i>Sp. varians</i>	—	++	+	+
<i>Sp. necrophorus</i>	—	—	++	+
<i>F. necrogenes</i>	—	—	—	—
<i>B. melaninogenicus</i>	—	++	V	—v
<i>H. influenzae</i>	—	—	V	—
<i>H. parainfluenzae</i>	—	—	—	—
<i>H. aphrophilus</i>	—	—	—	—
<i>H. paraphrophilus</i>	—	—v	—v	—
<i>H. haemolyticus</i>	—	—	—	—
<i>H. paraphrohaemolyticus</i>	—	—	—	—
<i>H. aegyptius</i>	—	—	—	—
<i>H. suis</i>	—	—	—	—
<i>Bord. pertussis</i>	—	+	—	—
<i>Bord. parapertussis</i>	—	+	—	—
<i>Bord. bronchiseptica</i>	—	++	++	—
<i>Acinetobacter lwoffii</i>	—	—	—	—
<i>Streptobacillus moniliformis</i>	—	+	—	—
<i>M. liquefaciens</i>	—	++	—	—
<i>M. nonliquefaciens</i>	—	+	—	—
<i>M. osloensis</i>	—	—	—	—
<i>M. kingii</i>	—	—	—	—
<i>M. lacunata</i>	—	+	V	—

* Key: see *Methods*.

In the presence of brilliant green 0.1% (w/v), all "corrodens" bacteria grew. With basic fuchsin, however, only *NC-1* grew at this concentration, the *NCL-20* and *AC-5* groups growing only at 0.02% (w/v). On the other hand, the *AC-5* group tolerated crystal violet at a concentration of 0.01% (w/v), whereas the *NC-1* and *NCL-20* groups grew only at the low concentration of 0.0005% (w/v) crystal violet.

All organisms grew in the presence of optochin (ethyl hydrocuprein hydrochloride).

DISCUSSION

Although all "corrodens" bacteria could be classified as "nutritionally exacting" organisms, the facultatively anaerobic strains seem to be the more fastidious. The strictly anaerobic *NC-1* and *NCL-20* groups can be cultivated

TABLE V(a)
Results* of tests for hydrolytic enzymes

Species	Results obtained with the stated substrate grouped according to hydrolases concerned				
	urease	hippuric-acid hydrolase	glycosidases		
			aesculin	2-naphthyl- β -D-glucopyranoside	ortho-nitrophenyl- β -D-galactopyranoside
<i>B. corrodens</i> NC-1 group	++	—	—	—	—
<i>B. corrodens</i> NCL-20 group	++	—	—	—	—
<i>E. corrodens</i> AC-5 group	—	—	—	—	—
<i>B. fragilis</i>	—	—	++	—	—
<i>B. hypermegas</i>	—	—	++	+	—
<i>B. thetaiotaomicron</i>	—	—	++	+	—
<i>B. vulgatus</i>	—	—	+	—	—
<i>Sp. varians</i>	—	+	—	—	—
<i>Sp. necrophorus</i>	—	+	—	—	—
<i>F. necrogenes</i>	—	—	—	++	—
<i>B. melaninogenicus</i>	—	+v	—	—	—
<i>H. influenzae</i>	—	—	—	—	—
<i>H. parainfluenzae</i>	—v	—	—	—	—
<i>H. aphrophilus</i>	—	—	—	—	++
<i>H. paraphrophilus</i>	—	—	—	—	—v
<i>H. haemolyticus</i>	—	—	—	—	—
<i>H. paraphrohaemolyticus</i>	V	—	—	—	—
<i>H. aegyptius</i>	—	—	—	—	—
<i>H. suis</i>	—	—	—	—	—
<i>Bord. pertussis</i>	—	—	—	—	—
<i>Bord. parapertussis</i>	+	—	+	—	+
<i>Bord. bronchiseptica</i>	++	+	—	—	—
<i>Acinetobacter lwoffii</i>	—	—	—	—	—
<i>Streptobacillus moniliformis</i>	—	—	—	—	—
<i>M. liquefaciens</i>	++	—	—	—	—
<i>M. nonliquefaciens</i>	—	—	—	—	—
<i>M. osloensis</i>	—	—	—	—	—
<i>M. kingii</i>	—	—	—	—	—
<i>M. lacunata</i>	—	—	—	—	—

* Key: see *Methods*.

in the absence of whole blood, plasma, haematin or NAD, although growth is enhanced by whole blood or plasma. Growth of the facultatively anaerobic group AC-5 is stimulated by both haematin and NAD as well as by whole blood or plasma.

Whereas the addition of horse or human plasma improves the growth of all "corrodens" bacteria, neither horse nor human serum does so and this indicates that one or more of the components concerned in blood coagulation provides this growth stimulus.

All of the "corrodens" bacteria, and most of the other organisms tested, grew equally well in the presence of horse, human or sheep blood; the

TABLE V(b)
Results* of tests for hydrolytic enzymes

Species	Phosphatases				Lipases or phospholipases			Macromolecular hydrolases		
	phenolphthalein diphosphate	sodium 1-naphthyl phosphate	sodium 2-naphthyl phosphate	4-nitrophenyl disodium orthophosphate	2-naphthyl stearate	1-naphthyl acetate	2-naphthyl acetate	gelatin (liquefaction)	gelatin (hydrolysis)	deoxyribo- nucleic acid
<i>B. corrodens</i> NC-1 group	—	—	+++	—	—	++	++	—	—	—
<i>B. corrodens</i> NCL-20 group	—	—	+++	—	—	+	++	—	+	—
<i>E. corrodens</i> AC-3 group	—	—	+++	—	—	—	++	—	+	—
<i>B. fragilis</i>	+	+	+	—	—	++	++	—	—	+
<i>B. hypermegas</i>	—	+	+	—	—	++	++	—	—	+
<i>B. thetaiotaomicron</i>	+	+	+	—	—	++	++	—	—	+
<i>B. vulgatus</i>	+	+	+	—	—	++	++	—	—	+
<i>Sp. varians</i>	+	+	+	—	—	++	++	—	—	+
<i>Sp. necrophorus</i>	—	—	+	—	—	++	++	—	—	+
<i>F. necrogenes</i>	+	+	+	—	—	++	++	—	—	+
<i>B. melaninogenicus</i>	—	+	+	—	—	++	++	—	—	+
<i>H. influenzae</i>	+	+	+	—	—	V	V	—	—	—
<i>H. parainfluenzae</i>	+	+	+	—	—	—	V	—	—	—
<i>H. aphrophilus</i>	+	—	+	+	—	—	+	—	—	—
<i>H. paraphrophilus</i>	+	—	+	+	—	—	+	—	—	—
<i>H. haemolyticus</i>	+	—	+	+	—	—	+	—	—	—
<i>H. paraphrohaemolyticus</i>	+	—	+	+	—	—	+	—	—	—
<i>H. aegyptius</i>	—	—	—	—	—	V	+	—	—	—
<i>H. suis</i>	—	—	—	—	—	—	+	—	—	—
<i>Bord. pertussis</i>	+	—	+	—	—	+	+	—	—	—
<i>Bord. parapertussis</i>	—	—	+	—	—	—	—	—	—	—
<i>Bord. bronchiseptica</i>	—	—	—	—	—	—	—	—	—	—
<i>Acinetobacter lwoffi</i>	—	—	—	+	—	—	+	—	—	—
<i>Streptobacillus moniliformis</i>	—	—	—	+	—	—	+	—	—	—
<i>M. liquefaciens</i>	—	—	+	+	—	+	+	—	—	+
<i>M. onninaefaciens</i>	—	—	V	+	—	+	+	—	—	+
<i>M. osloensis</i>	—	—	+	+	—	+	+	—	—	—
<i>M. kingii</i>	—	—	+	+	—	+	+	—	—	—
<i>M. lacunata</i>	V	—	+	+	—	V	+	—	V	—

* Key: see Methods.

TABLE VI

Results of carboxylic-acid-utilisation tests*

Species	Result obtained with	
	sodium citrate	sodium malonate
<i>B. corrodens</i> NC-1 group	—	—
<i>B. corrodens</i> NCL-20 group	—	—
<i>E. corrodens</i> AC-5 group	—	—
<i>B. fragilis</i>	—	—
<i>B. hypermegas</i>	—	—
<i>B. thetaiotaomicron</i>	++	—
<i>B. vulgatus</i>	—	—
<i>Sp. varians</i>	—	—
<i>Sp. necrophorus</i>	—	—
<i>F. necrogenes</i>	—	—
<i>B. melaninogenicus</i>	—	—
<i>H. influenzae</i>	—	—
<i>H. parainfluenzae</i>	—	—
<i>H. aphrophilus</i>	—	—
<i>H. paraphrophilus</i>	—	—
<i>H. haemolyticus</i>	—	—
<i>H. paraphrohaemolyticus</i>	—	—
<i>H. aegyptius</i>	—	—
<i>H. suis</i>	—	—
<i>Bord. pertussis</i>	—	—
<i>Bord. parapertussis</i>	—	—
<i>Bord. bronchiseptica</i>	+	+
<i>Acinetobacter lwoffii</i>	—	—
<i>Streptobacillus moniliformis</i>	—	—
<i>M. liquefaciens</i>	—	—
<i>M. nonliquefaciens</i>	—	—
<i>M. osloensis</i>	—	—
<i>M. kingii</i>	—	—
<i>M. lacunata</i>	—	—

* Key: see *Methods*.

Haemophilus genus was a striking exception because most strains failed to grow in the presence of sheep blood.

It is possible that differentiation between the strictly anaerobic and facultatively anaerobic groups of "corrodens" bacteria could be made on the basis of a few simple biochemical tests—the reduction of nitrite and the hydrolysis of urea and 1-naphthyl acetate by strictly anaerobic strains, and the decarboxylation of lysine and ornithine by facultatively anaerobic strains.

Such differentiation could be confirmed by certain equally unsophisticated tolerance tests. We find that only strictly anaerobic strains are able to grow in the presence of the following agents: sodium deoxycholate (1.0%, w/v), potassium tellurite (0.035%, w/v), sodium azide (0.05%, w/v), merthiolate (0.005%, w/v) and phenol (0.1%, w/v); and all facultatively anaerobic strains grow in the presence of crystal violet (0.01%, w/v).

TABLE VII(a)
Results* of tolerance tests

Species	Growth observed in the presence of the given percentage concentration of											
	sodium chloride		phenol		ethylhydrocuprein hydrochloride	basic fuchsin		brilliant green		crystal violet		
	1.0	5.0	0.05	0.1		0.02	0.1	0.02	0.1	0.0005	0.01	
<i>B. corrodens</i> NC-1 group	++	+	++	++	++	+	+	++	++	++	-	
<i>B. corrodens</i> NCL-20 group	+	-	++	-	++	+	-	++	++	++	+	
<i>E. corrodens</i> AC-3 group	+	-	++	+	++	+	+	++	++	++	-	
<i>B. fragilis</i>	++	-	++	++	++	++	++	++	++	++	-	
<i>B. hypermegs</i>	++	-	++	++	++	++	+	++	++	++	-	
<i>B. thetaiotaomicron</i>	++	-	++	++	++	++	-	++	++	++	-	
<i>B. vulgatus</i>	++	-	++	++	++	++	-	++	++	++	-	
<i>Sp. varians</i>	++	-	++	++	++	++	-	++	++	++	-	
<i>Sp. necrophorus</i>	-	-	++	+	++	++	-	++	++	++	-	
<i>F. necrogenes</i>	-	-	++	++	++	++	-	++	++	++	-	
<i>B. melanogenicus</i>	+	-	++	++	++	++	-	++	++	++	-	
<i>H. influenzae</i>	+	-	++	++	++	++	++	++	++	++	+	
<i>H. parainfluenzae</i>	-v	-	++	++	++	++	+	++	++	++	+	
<i>H. aphrophillus</i>	+	-	++	++	++	++	+	++	++	++	+	
<i>H. paraphrophillus</i>	-v	-	++	++	++	++	-v	++	++	++	+	
<i>H. haemolyticus</i>	+	-	++	++	++	++	+	++	++	++	+	
<i>H. paraphrohaemolyticus</i>	V	-	++	++	++	++	V	++	++	++	-	
<i>H. aegyptius</i>	+	-	++	V	++	++	-	++	++	++	V	
<i>H. suis</i>	V	-	++	+	++	++	-	++	++	++	-	
<i>Bord. pertussis</i>	++	-	++	++	++	++	+	++	++	++	+	
<i>Bord. parapertussis</i>	++	+	++	++	++	++	++	++	++	++	+	
<i>Bord. bronchiseptica</i>	++	-	++	++	++	-	-	++	++	++	-	
<i>Acinetobacter lwoffi</i>	+	-	++	+	++	-	-	++	++	++	-	
<i>Streptobacillus moniliformis</i>	+	-	++	-	++	-	-	++	++	++	-	
<i>M. liquefaciens</i>	++	-v	+	+	++	-v	-v	++	++	++	-	
<i>M. nonliquefaciens</i>	++	V	-	-	++	-	-	++	++	++	-	
<i>M. osloensis</i>	++	-	-	-	++	-	-	++	++	++	-	
<i>M. Kingii</i>	++	-	-	-	++	-	-	++	++	++	-	
<i>M. lacunata</i>	++	-	-	-	++	-	-	++	++	++	-	

* Key: see Methods.

TABLE VII(b)
Results* of tolerance tests

Species	Growth observed in the presence of the given percentage concentration of											
	sodium deoxycholate			sodium taurocholate			potassium cyanide		potassium tellurite		sodium azide	
	0.5	1.0		0.5	1.0		0.0075	0.075	0.035		0.05	0.1
<i>B. corrodens</i> NC-1 group	+	+	+v	+	+	+	+	+	+	+	+	+
<i>B. corrodens</i> NCL-20 group	+	+	+	+	+	+	+	+	+	+	+	+
<i>E. corrodens</i> AC-5 group	+	+	+	+	+	+	+	+	+	+	+	+
<i>B. fragilis</i>	+	+	+	+	+	+	+	+	+	+	+	+
<i>B. hypermegs</i>	+	+	+	+	+	+	+	+	+	+	+	+
<i>B. thetaiotaomicron</i>	+	+	+	+	+	+	+	+	+	+	+	+
<i>B. vulgatus</i>	+	+	+	+	+	+	+	+	+	+	+	+
<i>Sp. varians</i>	+	+	+	+	+	+	+	+	+	+	+	+
<i>Sp. necrophorus</i>	+	+	+	+	+	+	+	+	+	+	+	+
<i>F. necrogenes</i>	+	+	+	+	+	+	+	+	+	+	+	+
<i>B. melaninogenicus</i>	+	+	+	+	+	+	+	+	+	+	+	+
<i>H. influenzae</i>	+	+	+	+	+	+	+	+	+	+	+	+
<i>H. parainfluenzae</i>	+	+	+	+	+	+	+	+	+	+	+	+
<i>H. aphrophilus</i>	+	+	+	+	+	+	+	+	+	+	+	+
<i>H. paraprofitus</i>	+	+	+	+	+	+	+	+	+	+	+	+
<i>H. haemolyticus</i>	+	+	+	+	+	+	+	+	+	+	+	+
<i>H. paraproteolyticus</i>	+	+	+	+	+	+	+	+	+	+	+	+
<i>H. aegyptius</i>	+	+	+	+	+	+	+	+	+	+	+	+
<i>H. suis</i>	+	+	+	+	+	+	+	+	+	+	+	+
<i>Bord. pertussis</i>	+	+	+	+	+	+	+	+	+	+	+	+
<i>Bord. parapertussis</i>	+	+	+	+	+	+	+	+	+	+	+	+
<i>Bord. bronchiseptica</i>	+	+	+	+	+	+	+	+	+	+	+	+
<i>Acinetobacter Iwofii</i>	+	+	+	+	+	+	+	+	+	+	+	+
<i>Streptobacillus moniliformis</i>	+	+	+	+	+	+	+	+	+	+	+	+
<i>M. liquefaciens</i>	+	+	+	+	+	+	+	+	+	+	+	+
<i>M. nonliquefaciens</i>	+	+	+	+	+	+	+	+	+	+	+	+
<i>M. osloensis</i>	+	+	+	+	+	+	+	+	+	+	+	+
<i>M. kingii</i>	+	+	+	+	+	+	+	+	+	+	+	+
<i>M. lacunata</i>	+	+	+	+	+	+	+	+	+	+	+	+

* Key: see Methods.

We feel that the evaluation of degree and nature of tolerance as a means of classification has a special application in the study of bacteria such as the "corrodens" group and *Haemophilus*; it seems that the relatively low metabolic activity of these organisms limits the value of our standard biochemical tests.

Much more information is required regarding the reaction sequences by which "corrodens" bacteria derive energy to support their growth and reproduction before such isolated biochemical reactions as are described here can be related to the complete metabolic pattern. In our studies with the "corrodens" bacteria, biochemical reactions leading to an increase in alkalinity appear consistently, e.g., lysine and ornithine decarboxylase and urease activity. The presence of peroxidase and oxidase indicates a potential for aerobic metabolism although this is expressed in the facultatively aerobic forms only where it is supported by a requirement for NAD and low tolerance to cyanide and azide which presumably act on the cytochrome oxidase system. The total lack of activity against carbohydrates is unusual and is borne out by the negative glycosidase and Voges-Proskauer reactions.

The low order of demonstrable enzymic activity in these organisms suggests that other means of characterisation will have to be found before the taxonomic status can be positively established. Towards this end, the DNA-base composition studies of Hill *et al.* (1970) and those of Jackson and Goodman (1972), and the cellular fatty acid studies of Prefontaine and Jackson (1972), are valuable contributions.

SUMMARY

Tests carried out on strictly anaerobic and facultatively anaerobic strains of "corrodens" bacteria, showed that although these organisms are relatively inactive biochemically, differentiation can be made on the basis of tests that demonstrate reduction of nitrite, hydrolysis of urea and 1-naphthyl acetate, decarboxylation of lysine and ornithine, and sensitivity to certain selective agents included in culture medium.

Plasma was found to be superior to serum in supporting the growth of all "corrodens" bacteria, and a combination of heated and unheated blood added to a nutrient base was shown to yield good growth.

Comparative studies are reported with various species of *Bacteroides*, *Haemophilus*, *Bordetella* and related genera.

REFERENCES

- BERGMEYER, H. U. 1963. Methods of enzymatic analysis, *New York*, p. 823.
COWAN, S. T. AND STEEL, K. J. 1966. Manual for the identification of medical bacteria, *London*.
EIKEN, M. 1958. Studies on an anaerobic, rod-shaped, Gram-negative micro-organism: *Bacteroides corrodens* n. sp. *Acta. path. microbiol. scand.*, **43**, 404.
HENRIKSEN, S. D. 1948. Studies on Gram-negative anaerobes. II. Gram-negative anaerobic rods with spreading colonies. *Acta. path. microbiol. scand.*, **25**, 368.
HENRIKSEN, S. D. 1969. Corroding bacteria from the respiratory tract. 2. *Bacteroides corrodens*. *Acta. path. microbiol. scand.*, **75**, 91.

- HILL, L. R., SNELL, J. J. S. AND LAPAGE, S. P. 1970. Identification and characterisation of *Bacteroides corrodens*. *J. med. Microbiol.*, **3**, 483.
- JACKSON, F. L., GOODMAN, Y. E., BEL, F. R., WONG, P. C. AND WHITEHOUSE, R. L. S. 1971. Taxonomic status of facultative and strictly anaerobic "corroding bacilli" that have been classified as *Bacteroides corrodens*. *J. med. Microbiol.*, **4**, 171.
- JACKSON, F. L. AND GOODMAN, Y. E. 1972. Transfer of the facultative anaerobic organism *Bacteroides corrodens* Eiken to a new genus, *Eikenella*. *Int. J. syst. Bact.*, **22**, 73.
- KHAIRAT, O. 1967. *Bacteroides corrodens* isolated from bacteraemias. *J. Path. Bact.*, **94**, 29.
- PREFONTAINE, G. AND JACKSON, F. L. 1972. Cellular fatty acid profiles as an aid to the classification of "corroding bacilli" and certain other bacteria. *Int. J. syst. Bact.*, **22**, 210.
- REINHOLD, L. 1966. Untersuchungen an *Bacteroides corrodens* (Eiken 1958). *Zentbl. Bakt ParasitKde*, Abt. 1 Orig., **201**, 49.
- ROBINSON, D. 1956. The fluorimetric determination of β -glucosidase: its occurrence in the tissues of animals, including insects. *Biochem. J.*, **63**, 39.
- ROBINSON, J. V. A. AND JAMES, A. L. 1973. Some serological studies on *Bacteroides corrodens*. *J. gen. Microbiol.*, **78**, 193.
- SAUNDERS, B. C., HOLMES-SIEDLE, A. G. AND STARK, B. P. 1964. Peroxidase, *Washington*, p. 27.
- WILSON, G. S. AND MILES, A. A. 1964. Topley and Wilson's Principles of bacteriology and immunity, 5th ed., *London*, p. 493.