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Examination of two bacterial strains designated '*Brucella suis* biotype 5'

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SUMMARY

The morphological, cultural, biochemical, serological and pathogenic properties of two bacterial strains of the group designated '*Br. suis* biotype 5' were examined. Both strains were found to be atypical of the genus *Brucella* in many of these characteristics. No serological relationship to known brucella strains could be detected. On the basis of the evidence obtained the two strains examined were classified as *Moraxella duplex* and the status of '*Br. suis* biotype 5' questioned.

INTRODUCTION

Renoux & Philippon (1969) proposed that certain organisms isolated from the reproductive tracts of cattle and sheep and from abortion material should be included in the classification scheme for the genus *Brucella* as examples of a fifth biotype of *Brucella suis*. These organisms differed from other *Br. suis* strains in their resistance to Safranine O and in their oxidative metabolic pattern. According to Renoux & Philippon (1969) all isolates were rough and thus could not be characterized by phage typing nor by agglutination reactions with mono-specific antisera to *Br. abortus* and *Br. melitensis*. Recently the opportunity occurred to examine two of the isolates of Renoux and Philippon and the results obtained indicated that the inclusion of these strains in a new brucella biotype should be re-examined.

MATERIALS AND METHODS

Bacterial strains

The two strains designated '*Br. suis* biotype 5' were provided by Dr A. Philippon of I.N.R.A., Station de Pathologie de la Reproduction, Nouzilly, France, as freeze-dried cultures B58 and 4607. Strain B58 was of bovine origin and strain 4607 was of ovine origin. Both were studied by Renoux & Philippon (1969) and some of their characteristics reported in their published results. Brucella and other bacterial strains used as controls in this study were from the culture collection maintained at this laboratory.

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Morphological examination

The general morphology of heat-fixed organisms was studied in smears stained by Gram's method or with Loeffler's methylene blue. Wayson's method was used for demonstrating bipolar staining, the Ziehl-Neelsen method and the modified Köster's method (Christoffersen & Ottosen, 1941) for detecting acid-fast staining and the method of Lechtman, Bartholomew, Phillips & Russo (1965) for demonstrating spores. Flagella staining was done according to Leifson (1951) and capsule staining according to Howie & Kirkpatrick (1934). Negative staining with indian ink was done according to Duguid (1951). Electron-microscopic examination of negatively stained preparations was done as described by Corbel & Phillip (1972).

Bacteriological examination

Standard bacteriological procedures for the identification of bacteria were used (Cowan & Steel, 1965). Identification of non-fermentative organisms was done using the medium described by Sellers (1964). Sterile 50% (w/v) lactose was added to some tubes in place of D(+) glucose. The methods used for the typing of *Brucella* species were as recommended by Morgan & Gower (1966) and Alton & Jones (1967). Measurements of oxidative metabolic rate with various substrates were made using a Gilson differential respirometer.

Phage sensitivity tests

Bacterial strains were tested for susceptibility to lysis by the Tbilisi brucella-phage and by the phages A422, M51 and S708 of Moreira-Jacob (1968). Phage suspensions were used at routine test dilution (RTD $\simeq 5\times10^4$ p.f.u./ml.) and $10,000\times$ routine test dilution (10,000 RTD $\simeq 5\times10$ p.f.u./ml.) according to procedures recommended by Alton & Jones (1967).

Antibiotic sensitivity determination

This was done by the multiple disk method using confluent growths of the organism on trypticase soy agar. Zones of inhibition were measured after 24 hr. incubation at 37°C. Multodiscs (Oxoid, London) were used for the assays.

Serological examination

Rabbit and bovine antisera to *Brucella* spp. and *Y. enterocolitica* IX were prepared according to Corbel & Cullen (1970). Antisera to *Francisella tularensis* and *Mima polymorpha* were obtained from Difco Laboratories, Detroit.

The serum agglutination test (SAT), the complement fixation test (CFT), the Rose Bengal plate test and disulphide reduction and antiglobulin tests were done by procedures described by Morgan *et al.* (1971). Immunodiffusion and indirect immunofluorescence tests were performed on extracts of ultrasonically disrupted organisms according to Corbel & Cullen (1970).

Agglutination tests for antibodies to rough organisms were done according to Diaz, Jones & Wilson (1967).

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The serological response of animals to inoculation with B58 and 4607 was examined by intramuscular injection of guinea-pigs with doses of *ca.* 10^{11} heat-killed organisms and intravenous injection of rabbits with 5×10^{10} heat-killed organisms. The responses of animals receiving live organisms by the intraperitoneal and subcutaneous routes were also examined. Serum samples were tested for antibodies to smooth and rough brucella strains using *Br. abortus* strain 99, *Br. canis* RM 6-66 and *Br. ovis* as antigens. Antibodies to the homologous bacterial strains were also detected by agglutination, CFT and precipitin tests using suspensions of B58 and 4607 as antigens. The anamnestic response to brucella antigens was examined by intravenous injection of rabbits inoculated with *Br. abortus* strain 19 some 6 months previously, with suspensions of B58, 4607 and *Br. abortus* 45/20 organisms standardized turbidimetrically to contain *ca.* 10^{10} organisms/ml. Blood samples were collected daily for 7 days after injection and twice weekly thereafter.

Determination of virulence

Virulence was assessed by intravenous inoculation of pairs of adult rabbits with *ca.* 10^{11} viable B58 or 4607 organisms. Male and female weaned albino guinea-pigs were injected by the intraperitoneal route with doses of 5×10^{11} viable organisms. Six female guinea-pigs were given similar doses of these organisms at about the fortieth day of pregnancy. Three of these animals were killed and examined 3 weeks after inoculation and the other three were allowed to proceed to parturition before killing and autopsy of themselves and their offspring. All animals were examined *post mortem* for macroscopic signs of disease and in addition smears were made of the viscera for microscopic examination. The spleens of all animals were emulsified and cultured on sheep blood agar, serum dextrose agar, MacConkey bile salt agar and Levine eosin-methylene blue agar for up to 14 days at 37°C. Fetuses and membranes recovered from pregnant animals were treated similarly.

Albino mice of *ca* 30 g. weight were inoculated intraperitoneally with *ca.* 5×10^{10} viable B58 or 4607 organisms. Six similar mice were also inoculated by the intracerebral route with *ca.* 10^7 viable organisms. All mice were killed 14 days after inoculation and smears and cultures prepared from the internal organs, including the brain.

RESULTS

Morphological examination

Gram-stained smears of B58 and 4607 showed small Gram-negative cocci or cocco-bacilli arranged mainly in pairs or short chains with a proportion of single cells. Methylene blue stained preparations showed mainly diplococcal forms. No indication of bipolar staining was evident in smears stained by Wayson's method and no spores or flagella were observed in preparations stained by the relevant method. The organisms were not acid-fast and stained poorly by Köster's method. No capsules could be seen in indian ink preparations but were visible in preparations made according to Howie & Kirkpatrick (1934) as stained areas surrounding

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the bacterial cells (Pl. 1, figs. 1–4). Diplococcal and chain formations were also readily demonstrated by this method which does not involve heat fixation. Electron-microscopic examination confirmed these results and the capsules were visible as distinct layers surrounding the bacterial cells. The pairs of cells were 1·25–1·45 μm. in length by 0·6–0·70 μm. in width with capsules 0·15–0·17 μm. thick for the B58 strain. For the 4607 strain the pairs of cells were 1·15–1·25 μm. in length, 0·50–0·72 μm. in breadth and with capsules 0·10–0·30 μm. thick (Pl. 2, figs. 1, 2). Because of the capsules the phosphotungstic acid stain penetrated poorly into the surface of the organisms and subcapsular structures could not be visualized. It was difficult in most cases to distinguish the intersections between diplococci. No capsules were observed in preparations of smooth and rough brucella strains stained and examined by the same methods.

Bacteriological examination

Cultural characteristics

Both strains grew on nutrient agar, serum dextrose agar, trypticase soy agar and Albimi brucella agar, producing visible growth in 24 hr. at 37° C. Growth also occurred, but more slowly, at 20° C. No growth occurred at 4° or 50° C. The growth of both strains was enhanced on serum-containing media. On all media strain B58 grew more rapidly than 4607. Initially colonies on these media resembled morphologically those of brucella strains, but differed in their more rapid growth rate. On prolonged incubation, however (6–7 days at 37° C., 10 days at 20° C.) large umbonate colonies, 7–8 mm. in diameter for the B58 strain and 5–6 mm. in diameter for the 4607 strain, were produced.

On sheep, horse and rabbit blood agar both strains produced small white hemispherical colonies in 24 hr. at 37° C. No haemolysis was observed. Similar growth was produced on lysed and heated blood media.

Both strains also produced colonies on MacConkey bile salt agar, deoxycholate citrate agar, Levine eosin–methylene blue agar and sheep blood thioglycollate agar. The colonies were lactose-negative and on bile salt media similar in appearance to the growth on nutrient agar. On eosin–methylene blue agar lavender-coloured colonies with a transparent entire margin were produced in 2–3 days at 37° C. B58 also grew on Wilson and Blair medium producing transparent drop-like colonies 0·1 mm. in diameter after 7 days incubation, but 4607 did not grow on this medium. Neither strain grew anaerobically and growth was neither enhanced nor inhibited in the presence of added CO_2. Both strains grew readily in the presence of the brucella dyes, basic fuchsin at 1/50,000, thionin at 1/50,000, pyronin Y at 1/100,000, safranin O at 1/5000 and thionin blue at 1/100,000. B58 grew well in the presence of 1/50,000 methyl violet but 4607 did not grow in the presence of this dye. *meso*-Erythritol at concentrations of 10 mg./ml. did not significantly affect growth. B58 grew readily on serum dextrose agar containing 10 units of penicillin G per ml. but 4607 was inhibited on this medium. Both strains grew readily on the serum dextrose agar antibiotic medium of Kuzdas & Morse (1956). In semi-solid thioglycollate media both strains produced a uniform disk of growth in the aerobic layers and no growth in the deeper layers.

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Table 1. *Biochemical reactions of strains B58 and* 4607

Test	B58	4607
H_2S	–	–
Urea	+ (8 hr.), + + + (24 hr.)	– (8 hr.) – (24 hr.) – (21 days)
KCN	+	–
Methyl Red	–	–
Voges Proskauer	–	–
Indole	–	–
Nitrate reduction	–	–
Gelatin stab	Surface growth. No liquefaction in 21 days	Surface growth. No liquefaction in 21 days
Litmus milk	No acid or digestion. Reduction at 21 days	No change
Loeffler's serum slope	Growth. No liquefaction	Growth. No liquefaction
Brewer's thioglycollate medium	Discoid surface growth	Discoid surface growth
Catalase	+	+
Oxidase	+	+
Motility 37° C.	–	–
Motility 20° C.	–	–
Decarboxylase, arginine	–	–
Decarboxylase, lysine	–	–
Decarboxylase, ornithine	–	–
Anaerobic growth	–	–
Microaerophilic growth (10 % CO_2)	+	+

Biochemical properties

Both B58 and 4607 showed very limited biochemical activity in the conventional tests as shown in Table 1. Strain B58 possessed strong urease activity but 4607 had no urease activity. Neither strain produced H_2S detectable with lead acetate papers or on Kligler's medium. Both strains showed very limited fermentative activity towards carbohydrates in peptone water (Table 2), but in Hugh and Leifson's medium acid was very slowly produced from D(+)-glucose. Both strains grew on Seller's medium, without production of gas, fluorescent pigment or substantial fermentation of sugars. B58 slowly released acid from D(+)-glucose on this medium although 4607 did not. In the presence of lactose, B58 produced an alkaline slant but 4607 produced no change. Both strains reacted positively in tests for catalase and oxidase. In oxidative metabolism tests conducted in the Gilson differential respirometer, both strains oxidized a number of substrates including arginine, dextrose, asparagine and ribose.

Where the cultural and biochemical tests performed coincided with those done by Renoux & Philippon (1969), the results obtained were generally consistent with those reported.

Phage susceptibility

B58 and 4607 were both resistant to lysis by the four brucella phages tested. Smooth cultures of B58 were used for the test and these were quite refractory to lysis by any phage at RTD or 10,000 RTD, including the *Br. suis* phage S708.

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Table 2. *Carbohydrate reactions of strains B58 and 4607*

	B58 Day number						4607 Day number					
Substrate	1	3	7	10	14	21	1	3	7	10	14	21
Adonitol	–	–	–	–	(+)	+	–	–	–	(+)	+	+
Aesculin	–	+	+	+	+	+	–	–	–	–	–	–
Amygdalin	–	(+)	+	+	+	+	–	(+)	+	+	+	+
Arabinose	–	–	–	–	–	–	–	–	–	–	–	–
Cellobiose	–	(+)	+	+	+	+	–	(+)	+	+	+	+
Dextrin	–	–	–	–	–	–	–	–	–	–	–	–
Dulcitol	–	–	–	–	–	–	–	–	–	–	(+)	+
Erythritol	–	(+)	+	+	+	+	–	(+)	+	+	+	+
Fructose	–	–	–	–	–	–	–	–	–	–	–	–
Glucose	–	–	–	–	–	–	–	(+)	(+)	+	+	+
Glycerol	–	–	–	–	–	(+)	–	–	–	–	–	–
Glycogen	–	–	–	–	(+)	(+)	–	–	–	–	(+)	+
Inositol	–	–	–	–	–	–	–	–	–	–	–	–
Inulin	–	–	–	–	–	–	–	–	–	–	–	–
Lactose	–	–	–	–	–	–	–	–	–	–	–	–
Maltose	–	–	–	–	–	–	–	–	–	–	–	–
Mannitol	–	–	–	–	(+)	+	–	–	–	–	(+)	+
Mannose	–	–	–	–	–	–	–	–	–	–	–	–
Melezitose	–	(+)	+	+	+	+	–	(+)	+	+	+	+
Raffinose	–	–	(+)	+	+	+	–	–	–	–	–	–
Rhamnose	–	–	–	–	–	–	–	–	–	–	–	–
Salicin	–	–	–	–	–	–	–	–	–	–	–	–
Starch	–	–	–	–	–	–	–	–	–	–	–	–
Sorbitol	–	–	–	–	–	–	–	–	–	–	(+)	–
Sucrose	–	–	–	–	–	–	–	–	–	–	–	–
Trehalose	–	–	–	–	–	–	–	–	–	–	–	–
Xylose	–	–	–	–	–	–	–	–	–	–	–	–
Seller's lactose	ALK	ALK	ALK	ALK	ALK	ALK	(A)	(A)	(A)	(A)	A	A
Seller's glucose	–	(A)	A	A	A	A	–	(A)	(A)	A	A	A
Seller's aerogenesis	–	–	–	–	–	–	–	–	–	–	–	–
Seller's anaerobiosis	–	–	–	–	–	–	–	–	–	–	–	–
Seller's pigment	–	–	–	–	–	–	–	–	–	–	–	–
Hugh & Leifson's O/F medium	–	–	(F)	F	F	F	–	–	(F)	F	F	F
Kligler's medium	–	ALK	ALK	ALK	ALK	ALK	–	–	–	–	(A)	(A)
Kligler's aerogenesis	–	–	–	–	–	–	–	–	–	–	–	–
Kligler's H_2S	–	–	–	–	–	–	–	–	–	–	–	–
Koser's citrate	–	–	–	–	–	–	–	–	–	–	–	–
ONPG	–	–	–	–	–	–	–	–	–	–	–	–

– = no change, + = acid production, F = fermentation, O = oxidation, () = slight reaction, A = acid, ALK = alkali.

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Table 3. *Antibiotic sensitivity of strains B*58 *and* 4607

Antibiotic	Concentration	Strain B58	Strain 4607
Ampicillin	2 u.	R	S
Ampicillin	25 u.	S	S
Bacitracin	25 u.	R	R
Chloramphenicol	10 μg.	S	S
Chloramphenicol	50 μg.	S	S
Chlortetracycline	25 μg.	S	S
Chlortetracycline	50 μg.	S	S
Erythromycin	15 μg.	S	S
Furazolidone	15 μg.	R	R
Furazolidone	50 μg.	R	R
Kanamycin	30 μg.	S	S
Mitomycin C	1 μg.	R	R
Mitomycin C	5 μg.	R	R
Neomycin	10 μg.	S	S
Neomycin	30 μg.	S	S
Novobiocin	30 μg.	S	S
Oxytetracycline	30 μg.	S	S
Penicillin G	5 u.	R	S
Penicillin G	10 u.	R	S
Polymyxin B	10 u.	R	R
Streptomycin	10 μg.	R	R
Streptomycin	25 μg.	R	R
Tetracycline	10 μg.	S	S
Tetracycline	50 μg.	S	S
Trimethoprim + sulphamethoxizole	25 μg.	R	R
Triple sulphonamides	50 μg.	R	R
Triple sulphonamides	300 μg.	R	R
Spectinomycin	25 μg.	S	S
Amphotericin B	10 μg.	R	R

R = Not inhibited. S = Inhibited.

Strain 4607 was only available as a rough strain and this was also resistant to the phages at all concentrations tested.

Antibiotic sensitivity

Both strains were resistant to sulphonamides, furazolidone, and trimethoprim even in high concentrations. They were also resistant to streptomycin, polymyxin B and bacitracin but both were sensitive to chloramphenicol, tetracyclines, kanamycin, neomycin, erythromycin, novobiocin and ampicillin. B58 was resistant to penicillin G and only moderately sensitive to ampicillin whereas 4607 was highly sensitive to both antibiotics (Table 3).

Pathogenicity

No evidence of any pathological disturbance was obtained after inoculation of rabbits, mice and guinea-pigs with large numbers of viable B58 or 4607 organisms. Even intracerebral inoculation of mice failed to produce signs of disease. Pregnant

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Table 4. *Effect of strains B58 and 4607 on the anamnestic response to* Br. abortus *antigens*

Animal*	Inoculum	SAT	2ME	Quantitative RBPT	CFT	Immunodiffusion lps	Immunodiffusion ssa	
BS 1	45/20	1/20	1/20	1/2	1/20	0	2	Day 0
BS 2	45/20	4/10	3/10	1/2	1/10	0	2	
BS 3	B58	1/10	1/10	1/1	1/4	0	1+	
BS 4	B58	1/20	4/10	—/1	1/4	0	1+	
BS 5	4607	1/10	1/10	—/1	1/4	0	1+	
BS 6	4607	2/20	3/20	1/2	1/10	0	2	
BS 1	45/20	1/80	3/20	1/8	1/40	0	2	Day 4
BS 2	45/20	3/40	4/20	1/4	1/20	0	2	
BS 3	B58	1/10	1/10	1/1	1/4	0	1+	
BS 4	B58	2/20	4/10	—/1	1/4	0	1+	
BS 5	4607	1/10	—/10	—/1	1/4	0	1+	
BS 6	4607	3/20	2/20	1/2	1/10	0	2	
BS 1	45/20	4/320	3/80	1/32	1/200	1	2+	Day 7
BS 2	45/20	1/320	4/40	1/16	1/200	1	2+	
BS 3	B58	1/10	1/10	1/1	1/4	0	1+	
BS 4	B58	2/20	2/10	—/1	1/4	0	1+	
BS 5	4607	—/10	—/10	—/1	1/4	0	1	
BS 6	4607	3/20	3/20	1/2	1/10	0	2	

2ME = 2-mercapto-ethanol reduction test.
RBPT = Rose Bengal plate test.
lps = lipopolysaccharide antigen of *Br. abortus*.
ssa = sub-surface antigens of *Br. abortus*.
* Rabbits BS 1–6 had been inoculated with *Br. abortus* strain 19 *ca.* 6 months prior to this experiment.

guinea-pigs inoculated at about mid-term did not show evidence of disturbance and they eventually gave birth to healthy offspring. No organisms were recovered *post mortem* from any animal inoculated with these strains and no microscopical evidence was obtained of infection. It must be concluded that these two strains have a very low pathogenic potential for laboratory animals.

Serological properties

On emulsifying suspensions of organisms in 1/1000 acriflavine B58 was found to react as a smooth organism and was not agglutinated, whereas 4607 was immediately agglutinated and hence rough. B58 formed stable suspensions in 0·15 M-NaCl but 4607 auto-agglutinated in this medium. Both organisms were readily agglutinated by many samples of 'normal' rabbit and bovine sera as well as antisera to a variety of Gram-negative and Gram-positive organisms.

No reaction was obtained in CF tests with antisera to *Br. abortus* 544, *Br. abortus* 45/20, *Br. ovis* or *Br. canis* RM 6–66.

Neither B58 nor 4607 absorbed antibodies to rough or smooth brucellas from anti-*Brucella* sera. Similarly extracts of disrupted B58 or 4607 organisms did not react in immunodiffusion tests with antisera to *Brucella* spp. (Pl. 3, fig. 1) although extensive cross-reactions were observed when these sera were tested against

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extracts of various brucella strains. In immunodiffusion tests with homologous antisera, 4607 and B58 showed extensive cross-reactions with each other but not with brucellas (Pl. 3, fig. 2).

Attempts were made to detect low concentrations of masked antigens cross-reacting with brucellas by provoking an anamnestic response to brucella antigens by inoculating *Br. abortus* sensitized rabbits with B58 and 4607 organisms. No evidence of any increase in titre of antibody to *Br. abortus* was detected in these animals although *Br. abortus* 45/20 produced rapid increases in titre within 4–5 days when inoculated into similar animals. That this was anamnestic and not a primary response was shown by the substantial titres of reduction-stable agglutinins in these sera (Table 4).

Extracts of both B58 and 4607 produced precipitin lines on diffusion against polyvalent antiserum to *Mima polymorpha*. A reaction of identity was given by both strains on diffusion against this antiserum which produced no reaction on diffusion against extracts of *Brucella* spp. (Pl. 3, fig. 3). This serum contained antibodies to both *Moraxella* spp. and *Acinetobacter* spp. (*Herellea vaginicola* and *M. polymorpha* respectively). Strains B58 and 4607 produced reactions of identity with the *Moraxella duplex* reference strain but no cross-reaction with *Acinetobacter* spp. (Pl. 4, fig. 4).

These results were confirmed by indirect immunofluorescence and complement fixation tests.

DISCUSSION

The morphological characteristics of the two bacterial strains examined were distinct from those typical of the *Brucella* genus. Thus the growth of most organisms in pairs or short chains and the presence of discrete capsules are not features generally considered typical of brucellas. The lack of acid-fast staining with Köster's stain and the tendency to retain crystal violet with Gram's stain also distinguished these organisms from typical brucellas.

The electron-microscopy results confirmed the presence of capsules and negatively stained preparations were clearly quite different from typical *Brucella* strains which normally show a rugose cell surface with no evidence of capsule formation.

The cultural characteristics of the two strains B58 and 4607 emphasized their differences from typical brucella strains. Their rapid growth on a wide range of media at 20° and 37° C. suggested that B58 and 4607 were not brucellas, and their ability to grow on selective media such as MacConkey, deoxycholate-citrate, Levine eosin–methylene blue agar and, in the case of B58, on Wilson and Blair medium, confirmed this. Furthermore the ability to grow in the presence of safranine O, lack of H_2S production and of nitrate-reducing activity, and, in the case of 4607, absence of urease activity, are all inconsistent with the *Br. suis* group. It should be mentioned, however, that 10 out of the 13 strains studied by Renoux & Philippon (1969) were H_2S producers and in this respect B58 and 4607 were not representative of the group.

Minimal fermentative activity towards carbohydrates, although a property of

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the *Brucella* genus, with the exception of *Br. neotomae*, is by no means exclusive to this group and is in fact typical of the tribe Mimeae (de Bord, 1942).

Similarly the oxidative metabolic pattern, although consistent with that described by Renoux & Philippon (1969) for strains B58 and 4607, did not resemble that of other *Brucella* biotypes. This illustrates the point that unless the genetic relationships of micro-organisms can be established by independent means, physiological tests are of little value in identification.

The other properties of these strains further confined their non-identity with brucellas. Strain B58 was found to be smooth, but even so was quite resistant to lysis by the brucella phages studied. These included one phage, S708, which is lytic for *Br. abortus*, *Br. neotomae* and *Br. suis* biotypes 1, 2, 3 and 4 (J. A. Morris & M. J. Corbel; to be published). Strain 4607 was rough and thus not unexpectedly phage-resistant.

Examination of the phenol-soluble proteins of B58 and 4607 by disk electrophoresis according to Razin & Rottem (1967) has shown that they have very similar electrophoretic mobility distributions which are distinct from those typical of the *Brucella* genus (J. A. Morris, to be published).

The serological studies provided the final indisputable evidence for the absence of any relationship of strains B58 and 4607 to the *Brucella* genus. No serological cross-reactivity between brucellas and these strains could be demonstrated by complement fixation, immunofluorescence, immunoadsorption, ring precipitin or immunodiffusion reactions. The apparent cross-agglutination observed with many serum samples was probably due to natural antibodies coincidentally present in the sera. This was confirmed by the frequent occurrence of agglutinins for B58 in sera from 'normal' animals.

The immunodiffusion results clearly established the absence of serological relationship between B58 and 4607 and brucellas. Furthermore the cross-precipitation obtained with polyvalent antisera to *Mima polymorpha* provided confirmatory evidence for the identification of these two strains. Although B58 and 4607 differed from each other in certain cultural and biochemical properties and in antibiotic sensitivity, the reaction of identity obtained with *M. polymorpha* antiserum in immunodiffusion tests confirmed their relationship. *M. polymorpha* comprises a heterogeneous group of Gram-negative non-fermentative organisms most of which have now been reclassified into the *Acinetobacter* and *Moraxella* genera. As both B58 and 4607 were oxidase-positive they should be classified as *M. polymorpha* var. *oxidans* or *Mor. duplex* strains. Their general morphological, cultural and biochemical properties are consistent with this, although B58 is somewhat atypical in view of its resistance to penicillin G. However, antibiotic sensitivity is essentially a mutable characteristic and certainly not a basis for microbial classification. The biochemical activities described for *M. polymorpha* var. *oxidans* show some variation between isolates and between reports by different authors (Schaub & Hauber, 1948; Brodie & Henderson, 1964; Pickett & Manclark, 1965; Gilardi, 1968).

Similarly the lack of pathogenicity of these strains, although at variance with some studies on *M. polymorpha* (Schaub & Hauber, 1948), is consistent with

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others (Brooke, 1951) and it seems probable that pathogenicity is a variable characteristic which may be lost on repeated subculture.

The pathological significance of strains B58 and 4607 is uncertain. Their isolation from abortion material is no evidence that they caused the abortions and it can only be stated that the veterinary significance of *M. polymorpha* var. *oxidans* (*Mor. duplex*) is at present uncertain. It is the author's impression that organisms of this group are commonly isolated from pathological material but are usually discarded as contaminants. Clearly further study of their pathogenic significance, particularly in relation to reproductive disorders in animals, is indicated.

In view of the results of the present study, identifying two of the strains representative of '*Br. suis* biotype 5' (Renoux & Philippon, 1969) as *M. polymorpha* var. *oxidans,* it is clear that the taxonomic status of this group should be re-examined.

The two strains B58 and 4607 cannot be considered as examples of a fifth *Br. suis* biotype and this suggests that the proposed group of Renoux & Philippon (1969) is of dubious validity.

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EXPLANATION OF PLATES

Plate 1

Fig. 1. Strain B58, showing general morphology. Capsule stain.

Fig. 2. High-power magnification detail of Fig. 1, showing capsules.

Fig. 3. Strain 4607, showing general morphology and similarity to B58. Capsule stain.

Fig. 4. Strain 4607. High-power magnification detail of Fig. 3, showing capsules and chain formation.

Plate 2

Fig. 1. Electron micrograph of a negatively stained preparation of strain B58. The capsules (*C*) are clearly visible.

Fig. 2. Electron micrograph of strain 4607 prepared as for Fig. 1, showing capsules (*C*). The morphological similarity of strains B58 and 4607 is readily apparent.

Plate 3

Fig. 1. Immunodiffusion of ultrasonically disrupted extracts of strains B58 (B58 u/s), 4607 (4607 u/s) and *Br. abortus* 544 (544 u/s) against rabbit antiserum to *Br. abortus* 544 (a Br.ab). Extensive reactions were produced by 544 u/s against its homologous serum but no reaction was given by the other strains.

Fig. 2. Immunodiffusion of B58 u/s, 4607 u/s and 544 u/s against antiserum to B58 (a B58). Extensive cross-reactions were apparent between the B58 and 4607 preparations but there was no evidence of any reaction between 544 u/s and this antiserum.

Fig. 3. Immunodiffusion of B58 u/s, 4607 u/s, 544 u/s against rabbit serum a Br.ab and polyvalent antiserum to *Mima polymorpha* (aMP). Reactions of identity were produced between B58 u/s and 4607 u/s on diffusion against aMP, but no reaction was produced on diffusion against a Br.ab. Similarly 544 u/s reacted only with its homologous antiserum and not with aMP.

Fig. 4. Diffusion of B58 u/s, 4607 u/s and ultrasonically disrupted *Moraxella duplex* and *Acinetobacter anitratum* reference strains against serum aMP. A reaction of identity was produced between the B58, 4607 and *Mor. duplex* preparations, but a reaction of non-identity was given between the *Ac. anitratum* preparation and these strains.

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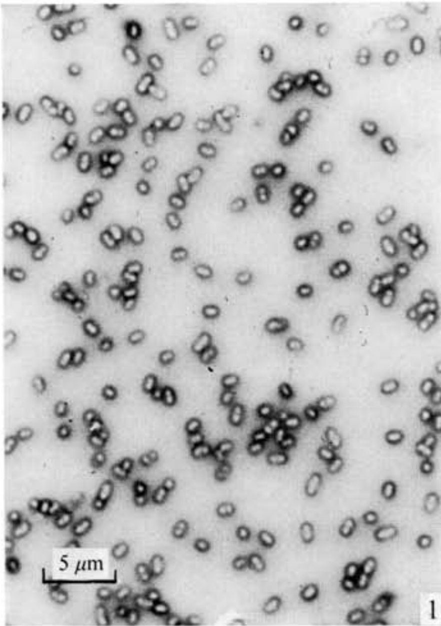

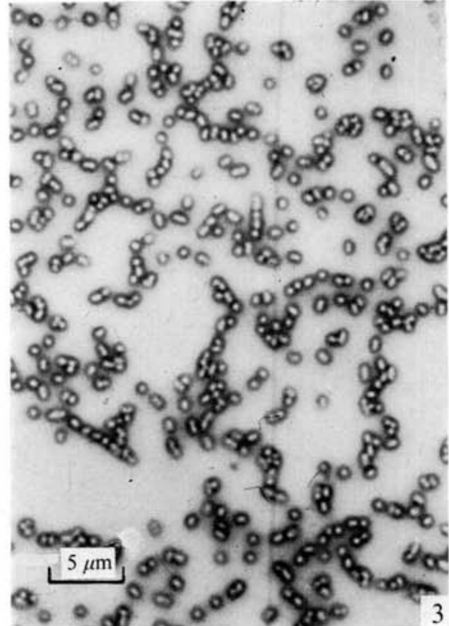

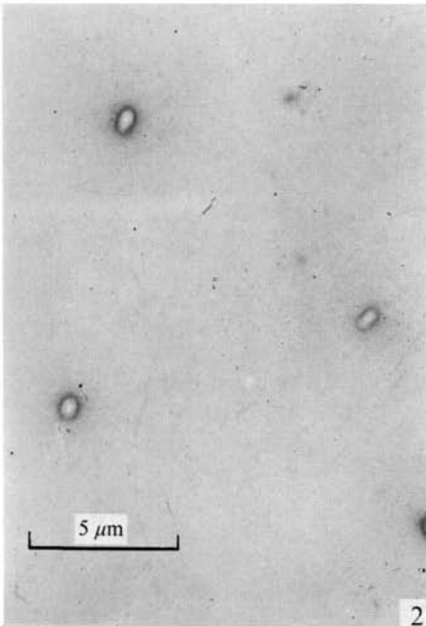

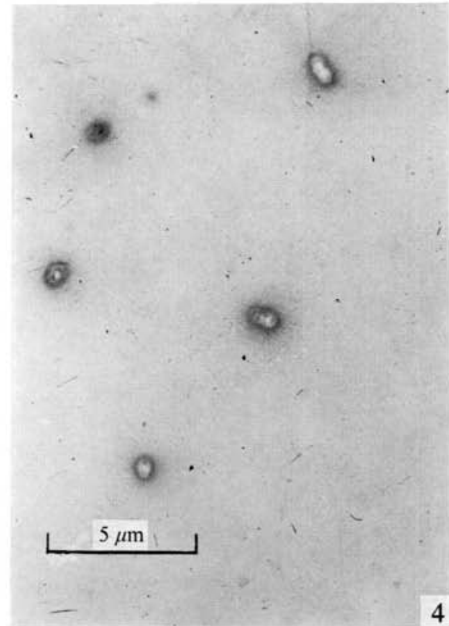

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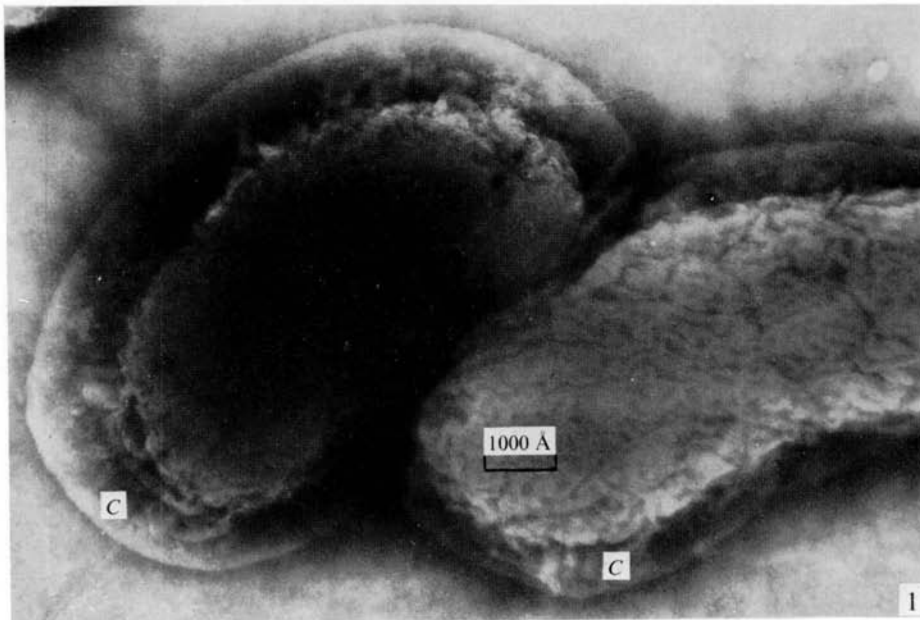

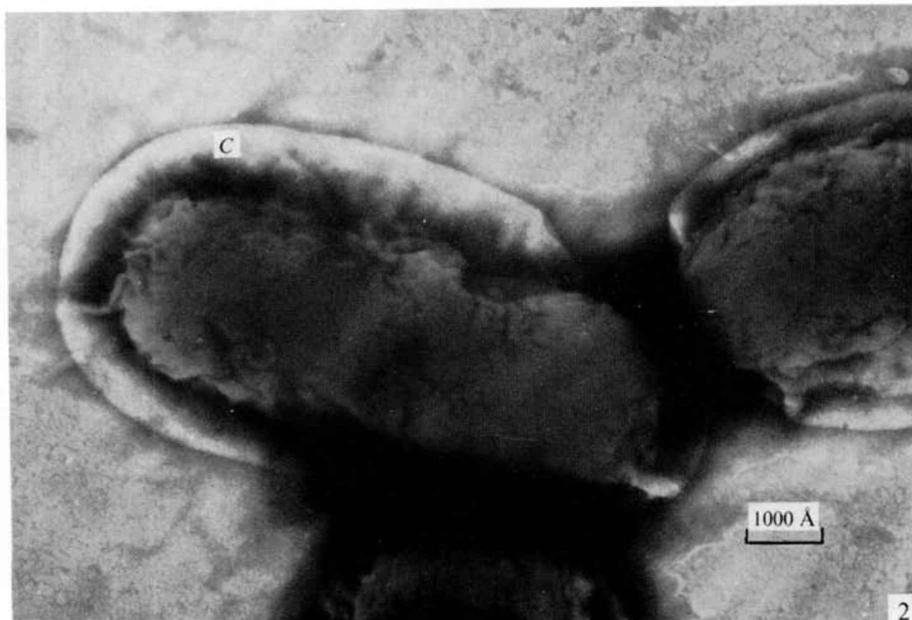

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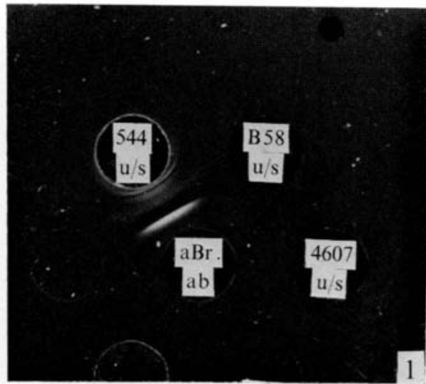

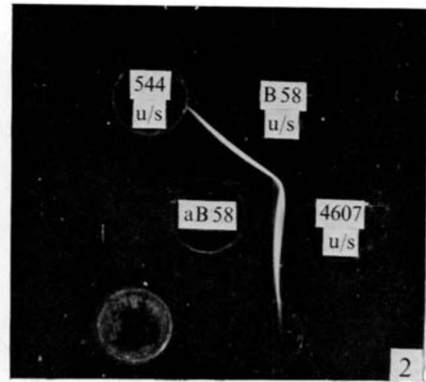

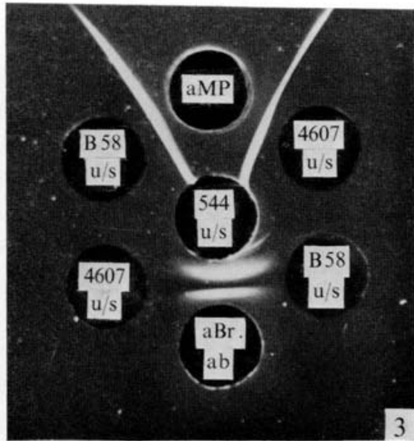

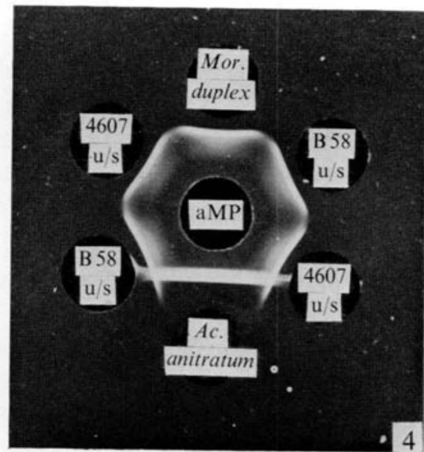

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