OBSERVATIONS ON BRUCELLA SPECIES BASED ON THE EXAMINATION OF 800 STRAINS*

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(With Plate 4 in the Text)

During the last seven years 800 strains of brucella have been submitted to the Brucella Reference Laboratory for identification. In the course of this work a number of observations of some interest have been made, and it has been considered worth while to make a brief report on certain of the findings.

SOURCE OF THE STRAINS

Most of the strains were submitted from the laboratories of the Public Health Laboratory Service. Many of these were strains about which an opinion was requested because they had given some unusual reaction. On the other hand, some bacteriologists sent in all the strains they had isolated over a period of months or years, thus providing a useful cross-section of the types occurring in their particular areas. Most of these strains were isolated from milk, but some were from cases of undulant fever. When a strain of special significance was identified, for example *Brucella melitensis* from cows' milk, further specimens were usually submitted in the course of the investigation of the infected herd. In addition, a considerable number of strains were obtained from hospital pathologists, and a few from various sources in other countries. The numerous stock strains that have been held in this laboratory for many years are not included. No strains of *Br. suis* were encountered in the course of the survey, but a number from other sources were examined for comparison.

METHODS OF IDENTIFICATION

All cultures were examined morphologically and checked for purity. For serological work the thermo-agglutination and acriflavine tests were used to detect roughness. The strains were then submitted to the following tests.

CO_2 *requirements*

The strains were inoculated on two liver agar slopes, one of which was incubated at 37° C. aerobically, and the other in a jar containing 10 % CO_2. The amount of growth was recorded after 1, 2 and 3 days' incubation.

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Production of H_2S

A strip of filter-paper impregnated with 10% lead acetate was inserted in the neck of the tube of liver agar and the amount of blackening was recorded on each of the first 4 days of incubation, the strip being changed each day.

Growth in the presence of dyes

(*a*) *Routine procedure*

The four dyes used in routine work are thionin, basic fuchsin, methyl violet and pyronin. The conventional method of testing is that described by Huddleson (1929, 1931) in which appropriate concentrations of the dyes are incorporated in liver agar plates, two different concentrations of each dye being used. In the early stages of the work this method was employed, but a simpler and more economical technique was devised. In this procedure, which has been described elsewhere (Cruickshank, 1948), strips of filter-paper impregnated with the dyes and dried are laid in parallel on liver agar plates and overlaid with a standard amount of melted liver agar. After the medium has set the cultures to be tested are inoculated at right angles to the strips, and the degree to which growth is inhibited is measured after incubation for 2 days at 37° C. in CO_2. The strength of the dyes used for impregnation of the strips is: thionin 1/600, basic fuchsin 1/200, methyl violet 1/400, pyronin 1/600.

The dyes were those of the National Aniline Dye Company of New York. Dyes prepared by Messrs G. T. Gurr were tried and these gave the same results as the American dyes with a representative series of strains, except pyronin which did not inhibit *Br. suis* even in a concentration of 1/50 in the filter-paper strips. This was not surprising, in view of the inconsistency of commercial brands of pyronin. However, the firm of G. T. Gurr eventually produced a pyronin labelled BX which gave satisfactory results, in accord with those given by the American dyes.

(*b*) *Comparison of Huddleson plate and filter-paper strip methods*

It was of interest to know whether there was good correspondence between the results obtained by the two methods, and a trial was made with about thirty strains, including a number that had behaved atypically. Sixteen sets of dye-plates were prepared, comprising the two usual concentrations and two higher dilutions of each of the test dyes. The plates were inoculated with a thick suspension of the strains under test over an area of 1 cm. in diameter. The same suspensions were inoculated on filter-paper strip plates prepared in the usual way. Table 1 shows a number of results representative of those given by the thirty strains. They include the reactions of a specially dye-sensitive type of *Br. abortus* to which reference will be made later. It is clear that the filter-paper strip method gives the essential information provided by the more elaborate plate method.

Pickett, Nelson, Hoyt & Eisenstein (1952) have devised a modification of this technique in which tablets incorporating the dyes are used in place of the filter-paper strips.

Agglutination by monospecific antisera

Monospecific antisera were prepared by quantitative absorption of *Br. abortus* and *Br. melitensis* antisera with organisms of the heterologous type. Rabbits were

Table 1. *Dye tests: comparison of plate and filter-paper strip methods*

Organism	No dye	Dye-plate method: Thionin 1/30,000	Thionin 1/60,000	Thionin 1/120,000	Thionin 1/240,000	Basic fuchsin 1/25,000	Basic fuchsin 1/50,000	Basic fuchsin 1/100,000	Basic fuchsin 1/200,000	Methyl violet 1/50,000	Methyl violet 1/100,000	Methyl violet 1/200,000	Methyl violet 1/400,000	Pyronin 1/100,000	Pyronin 1/200,000	Pyronin 1/400,000	Pyronin 1/800,000	Filter-paper strip method: Thionin	Basic fuchsin	Methyl violet	Pyronin
Br. abortus, typical	+++	−	−	+	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	− − −	+++	+++	+++
Br. melitensis	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
Br. suis	+++	+++	+++	+++	+++	−	−	−	±	−	−	−	+	−	−	+++	+++	+++	− − −	− − −	− − −
Br. abortus, thionin-resistant	+++	+	++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+	+++	+++	+++
Br. abortus, dye-sensitive 1	+++	−	−	++	+++	−	−	−	−	−	−	−	−	−	−	++	+++	− − −	− − −	− − −	− − −
Br. abortus, dye-sensitive 2	+++	−	+	++	+++	−	−	−	+	−	−	+	+	+++	+++	+++	+++	− − −	− − −	− − −	++

−, ±, +, ++, +++, degrees of growth.
In the filter-paper strip method, the sign − − − indicates that growth was inhibited over a zone atl east 7 mm. wide.

given the minimum number of injections to produce a homologous titre of about 1/2560 (usually two injections), since multiple injections are liable to stimulate antibodies to the minor antigens in excessive amount. The sera, appropriately diluted, were absorbed with a suspension of the heterologous organism, standardized by opacity to match a 3000×10^6 *Bact. coli* suspension. Equal amounts of serum and suspension were mixed and kept, with occasional agitation, in the incubator at 37° C. for 3 hr., then cleared by centrifugation. The appropriate serum dilution, which is about 1/32–1/64 of the homologous titre, should be determined by trial absorptions at several different levels, the absorbed sera being tested against a number of representative strains of *Br. abortus* and *Br. melitensis*. They should behave specifically with typical strains.

Other tests

Various other tests have been used from time to time, but none has so far given results that seemed to justify its adoption in routine work.

The urease activity of the three brucella species has been studied by various methods by a number of workers (Hoyer, 1950; Pacheco & de Mello, 1950; Sanders & Warner, 1951; Renoux & Quatrefages, 1951; Huddleson, 1952), who have reached similar conclusions with minor divergencies due probably to the use of different techniques and to the existence of a higher proportion of atypical strains in some localities. The consensus of opinion is summed up in the latest report of the Joint FAO/WHO Expert Committee on Brucellosis (Report, 1953) which describes Bauer's method (1949) of performing the test and states that, in general, the urease activity of *Br. suis* strains is detectable in 15–30 min. and of *Br. abortus* in 2 hr. or more, *Br. melitensis* strains behaving either like *Br. suis* or *Br. abortus*. The test thus has a limited value, but may help in distinguishing *Br. suis* and *Br. abortus*; even for this purpose, however, it often yields equivocal results. Huddleson (1952) points out that strains of *Br. melitensis* with low and high urease activity are associated with different geographical localities, and that definition of these groups may help to elucidate spread of this species within a country or from one country to another.

On tryptose agar plates containing triphenyl-tetrazolium salts the different species of brucella and their colonial variants develop characteristic colour patterns which some workers have found of value in identification and specially in the detection of early dissociation (Huddleson, 1952).

Renoux (1952*a*) described a new method of differentiation in which disks of filter-paper soaked in sodium diethyldithio-carbamate were placed on the surface of heavily inoculated Albimi agar plates. On incubation the plates showed, with the different species, a variety of zones of bacterial inhibition and growth around the disks. In a small trial of this method the few available strains of *Br. suis* gave the clear zone of inhibition described by Renoux, but the strains of *Br. abortus* and *Br. melitensis* tested produced a variety of more or less complex zones and rings which could not be readily interpreted. Pickett, Nelson & Liberman (1953) used a modification of the test in which the carbamate was incorporated in tablets, and found it of value for distinguishing *Br. melitensis* from *Br. abortus* and *Br. suis* by

the wide ring of inhibition of growth in the fourth or outer zone. The test is being investigated in this laboratory.

The use of different basic media

The routine culture medium used in this laboratory is liver agar prepared from ox liver. Some workers have found that ox liver is difficult to obtain, and a batch of medium prepared with horse liver was provided by Dr J. M. Croll (Lincoln). Ten strains (four *Br. abortus*, three *Br. melitensis* and three *Br. suis*) were tested in parallel on media prepared from ox liver and horse liver, and on Difco tryptose agar. All three media were satisfactory and gave essentially the same results, though there were differences in amounts of growth and amounts of H_2S produced with the same original inoculum. The horse-liver agar produced distinctly more growth and larger amounts of H_2S than the other two media. It is, of course, appreciated that these may merely be batch differences. Dye tests and agglutination reactions with monospecific sera were equally satisfactory whichever basic medium was used.

CLASSIFICATION OF THE STRAINS

All the strains, 800 in number, were examined by the above tests, and are listed in Table 2 according to their final classification.

Br. abortus, *typical*. These strains, which were far more numerous than any other type, produced H_2S, were inhibited by thionin but not by basic fuchsin, methyl violet or pyronin, and were agglutinated by a *Br. abortus* monospecific antiserum. Most of these strains grew much better, and many grew only in the presence of 10% CO_2, but about 8% of the strains grew equally well in air. Wilson (1933) found that a higher proportion than this (about 44%) of his bovine *abortus* strains grew in air as well as, or better than, in CO_2, but many of these were old cultures some of which had also lost the property of producing H_2S. It has recently been reported that 60% of nearly 600 strains of *Br. abortus* isolated in France grew in primary culture without added CO_2 (Report, 1953). No strain in the present series failed to produce H_2S, but a few produced only traces. Most strains formed fair amounts of H_2S on each of the first 2–4 days of growth.

Br. abortus, *dye-sensitive*. These are strains that were inhibited not only by thionin but also by basic fuchsin, methyl violet and usually pyronin in the ordinary test concentrations. Of the sixty-five strains of this type, four showed the usual resistance of *Br. abortus* to pyronin. Most of the dye-sensitive strains were isolated from milk, sometimes from a number of cows in one herd, but three were from the blood of patients with undulant fever. Table 1 shows that these strains are totally inhibited by the dyes in the filter-paper strip method, and that, in the plate method, they are inhibited by thionin at a dilution of 1/60,000 and partly at 1/120,000, by basic fuchsin at 1/200,000 or higher, and by methyl violet at 1/200,000 or 1/400,000, or even higher. Most of the strains were inhibited by pyronin at 1/200,000 and not at 1/400,000, but a few strains sensitive to the other dyes grew in the usual test concentrations of pyronin.

These strains with high susceptibility to all dyes appear to constitute a fairly

Table 2. *Classification of* 800 *brucella strains*

Place of isolation and source	*Br. abortus*, typical	*Br. abortus*, dye-sensitive	*Br. abortus*, thionin-resistant	*Br. abortus* biochemically, but inagglutinable	*Br. melitensis*, typical	*Br. melitensis* biochemically, but inagglutinable	*Br. abortus*, biochemically; *Br. melitensis*, serologically	*Br. melitensis*, biochemically; *Br. abortus*, serologically	Total
Great Britain:									
Blood of patient	12*	3†	0	0	5‡	0	1§	2‖	23
Cows' milk	628	62	0	0	30	0	18	0	738
Other bovine source	2	0	0	0	0	0	0	0	2
Kenya:									
Blood of patient	3	0	2	2	3	0	0	0	10
Northern Rhodesia:									
Blood of patient	0	0	1	0	0	0	0	0	1
Mauritius:									
Cow	2	0	0	0	0	0	0	0	2
Southern Italy:									
Blood of patient	1	0	2	0	0	3	0	13	19
Israel:									
Milk (goat)	3	0	1	0	0	0	1	0	5
Total	651	65	6	2	38	3	20	15	800

* One infected in Italy, one in Sudan.
† One infected in Spain.
‡ Three infected abroad, two laboratory injections.
§ A seaman infected abroad.
‖ One infected in Southern Italy, the other, a seaman, infected in the Far East.

definite type. They have been noted by other workers. Wilson (1933) reported that six of his ninety-one bovine *abortus* strains (6·6 %) were of this type, and it is of interest that they formed 9·0 % of the *abortus* strains in the present series. Wilson's three most dye-sensitive strains were of bovine origin from the north of France. In Veazie & Meyer's (1936) series of 447 brucella strains from many parts of the world twenty (4·7 %) were ultra-sensitive to all dyes; they would appear to comprise about 8·6 % of the strains identifiable as *Br. abortus*. Huddleson (1952) also examined eighteen strains which failed to grow on either thionin or basic fuchsin agar, and suggests that they should be classified as a subspecies designated *Br. abortus* (Wilson).

Br. abortus, *thionin-resistant*. Only six strains of this type (two from Italy, one from Israel, one from Northern Rhodesia and two from Kenya) were identified. Wilson (1933) studied a number of strains of this type, some of them from Southern Rhodesia where it is common. They grew well without added CO_2 as did four of the six strains in the present series. Strains otherwise like *Br. abortus*, requiring increased CO_2 tension, but growing well on thionin and basic fuchsin agar, were isolated from cattle in Sumatra by Van der Schaff & Rosa (1940), and in India by Polding (1950). Huddleson (1952) proposes that they should be designated *Br. abortus* (Van der Schaff).

Br. abortus, *inagglutinable*. Two strains, both from the blood of cases of undulant fever in Kenya, had all the other characters of *Br. abortus* but were inagglutinable by *Br. abortus* antiserum. They had been subcultured often before they were received and had become rough.

Br. melitensis, *typical*. These strains grew without added CO_2, were not inhibited by any of the test dyes and were agglutinated by *Br. melitensis* monospecific antiserum. Almost without exception they produced no H_2S, or a very slight trace on the first day only. These observations thus support the view generally held that a strain forming H_2S on the second day of growth is not *Br. melitensis*. However, the Expert Committee of WHO (Report, 1953) refer to strains otherwise typical of *Br. melitensis* that produced considerable quantities of H_2S for several days, and recently a few such strains, all isolated from milk samples from the same farm, were examined in this laboratory.

Of the five cases of human brucella infection from which *Br. melitensis* was isolated in this country, two were laboratory infections and three were infected abroad—one in North Africa and two in Malta. One of the latter was a young pregnant woman, who aborted. The occurrence of *Br. melitensis* in the milk of cows in Britain is discussed in a later section.

Br. melitensis, *inagglutinable*. Three inagglutinable strains with the biochemical characters of *Br. melitensis* were obtained from the blood of patients in Italy. They had been isolated some time previously and were probably antigenically degraded.

Strains behaving biochemically like Br. abortus *and serologically like* Br. melitensis. These strains behaved like *Br. abortus* in their production of H_2S and their sensitivity to thionin, and with few exceptions were favoured in their growth by CO_2. However, they were agglutinated to titre by *Br. melitensis* and not by *Br. abortus*

monospecific serum. Of the twenty strains, eighteen were isolated from cows' milk in Britain, one from milk in Israel and one from the blood of a seaman infected abroad.

Strains with these characters have been previously reported by Gilbert (1930) from a cow in Palestine and by Wilson (1933) who found one (of unknown source) in his own main series and two (one of human, and the other of bovine origin) in a series collected from the north-east of France by Lisbonne and Taylor. Veazie & Meyer (1936) described seventeen such strains, all from bovine sources, in their series of 447 brucella cultures; eleven were from the United States, eight being from different cows in the same dairy. The recent WHO Report (1953) refers to strains of this sort, and it is probable that most experienced workers have encountered them.

Strains behaving biochemically like Br. melitensis *and serologically like* Br. abortus. These strains grew well without added CO_2, produced no H_2S (or a trace only on the first day), and were not inhibited by any of the dyes. They were agglutinated by *Br. abortus* and not by *Br. melitensis* monospecific serum.

Wilson (1933) found that this was the commonest type in the Lisbonne-Taylor group of strains; these organisms were isolated from human, bovine, caprine and ovine sources in the north-east, east and south-east of France. Veazie & Meyer (1936) noted this behaviour in a number of cultures specially from Italy and Tunis. Renoux (1952*b*) recently described eighteen strains from Tunisia, comprising two from goats, one from a cow and fifteen from man, and he proposes that they should be called *Br. intermedia* (n.sp.). These strains required added CO_2, grew on thionin and basic fuchsin and produced no H_2S, but they were agglutinated with monospecific *Br. abortus* antiserum and had the urease activity usually observed with strains of *Br. abortus*.

Thirteen of the fifteen strains of this type in the present series belonged to a group sent from Italy or Sicily. One of the others was isolated from the blood of a seaman who fell ill on a voyage to England from India, and the other was isolated from a patient in the Middlesex Hospital, London. It was identified as this type and subsequent inquiries revealed that the patient had recently returned from southern Italy.

Occurrence of the types of brucella in Britain

With the exception of laboratory infections, there have been no reports of undulant fever due to species other than *Br. abortus* in persons infected in Great Britain. The vast majority of brucella infections in cows in this country are due to *Br. abortus*. In both cows and man, a small proportion of the infections are due to *Br. abortus* of the dye-sensitive type. A certain number (2·5 %) of the strains submitted from cows' milk in Britain were of the type that behaved biochemically like *Br. abortus* and serologically like *Br. melitensis* but none of the relatively few strains from persons infected in this country was of this type. The southern Italian type with the reverse characters was not isolated from milk in Britain but, as noted above, was isolated from two patients infected abroad. Strains with all the characters of *Br. melitensis* were isolated from milk on a number of occasions.

Br. melitensis *in cows' milk in Britain*

Br. melitensis was first reported in Britain in 1940 by Menton who identified the organism in the milk from an accredited herd in Staffordshire. The discovery, specially in time of war, was disturbing and led to the introduction of the Brucellosis-melitensis Order 1940, which empowered the authorities to slaughter the infected animals. The herd detected by Menton had been dispersed, but the cows were traced and five found to be excreting *Br. melitensis* were destroyed. From 1947 onwards, the organism has been repeatedly isolated from milk and identified in this and other laboratories.

Up to the present *Br. melitensis* has been isolated from the milk of individual cows or of herds, or, in one instance, from a Tuberculin Tested bulk supply, in the following counties (the Public Health Laboratories in which the strains were isolated are stated within brackets):

1940 Staffordshire (Stafford).
1947 Staffordshire, twice (Stafford); Shropshire (Stafford); East Suffolk (Ipswich).
1950 East Suffolk, twice (Ipswich); Norfolk, three times (Norwich, Ipswich); Staffordshire (Stafford).
1951 East Suffolk (Ipswich); Hertfordshire (Luton); Sussex, four times (Brighton).
1952 Hertfordshire (Luton); Rutland (Leicester); Sussex, twice (Brighton).
1953 Leicestershire, twice (Leicester); Sussex, twice (Brighton).

These reports led to the detailed investigation of the herds by the officers of the Ministry of Agriculture and Fisheries. Apart from Menton's original outbreak, information is available about twelve of the herds. The following list shows the number of cows found infected with *Br. melitensis* (numerator) out of the number of cows tested (denominator): 3/28, 1/47, 1/54, 3/100, 3/44, 1/9, 1/6, 1/12, 1/11, 2/22, 1/13, 2/5. Some herds were infected with both *Br. melitensis* and *Br. abortus*. It is emphasized that the list given above is very unlikely to reflect the actual distribution of *Br. melitensis* in England. More probably it indicates those areas in which the bacteriologist is specially interested in brucella work, and notes particularly any deviation of his strains from the typical behaviour of *Br. abortus*. This is supported by the fact that one or more strains of *Br. melitensis* have been identified among the cultures submitted by each of the laboratories contributing large numbers of strains to the present investigation (Ipswich, Luton, Leicester) or known to be examining large numbers of milk samples for brucella (Norwich, Brighton).

These strains of *Br. melitensis* behave typically in all the laboratory tests and cannot be distinguished from strains isolated from cases of Malta fever or from type strains. It is important that this should be noted, for it has sometimes been stated, for example in the report of a recent joint meeting of medical and veterinary workers (Dalrymple-Champneys, 1953), that these are curious or aberrant strains of an intermediate type. Although there is no record of human undulant fever due to *Br. melitensis* arising from milk in this country, no grounds

exist for assuming that these strains are of low virulence. Their isolation in fact depended on their ability to infect guinea-pigs. Although 10–15 % of the herds in England and Wales produce milk containing *Br. abortus*, the annual number of cases of undulant fever probably does not exceed 1000. The minute proportion of herds yielding *Br. melitensis* would therefore not be expected to give rise to more than a very occasional case and, specially in these days of antibiotic therapy, it would be easy both for the clinician and for the routine laboratory relying on serological identification with unabsorbed antisera to regard the infecting organism as *Br. abortus*. It would thus not be surprising to find that human infection due to *Br. melitensis* does arise as a very rare occurrence in this country.

OTHER OBSERVATIONS

Identification of Br. abortus *strain* 19

Br. abortus strain 19 is an organism of low virulence widely used as a living vaccine for the immunization of cattle. According to the available evidence, it does not appear in the milk after subcutaneous inoculation in the normal way in non-pregnant animals, and it has not been established that cases of undulant fever in man have been caused by the milk of inoculated cows. However, some mild infections have been reported due to accidents with loaded syringes. A ready means of recognition of strain 19 would be useful. It grows without added CO_2 and is of relatively low virulence for guinea-pigs, a character that is of little value for identification since it can only be determined by the use of large groups of animals. The most useful differential character has been found to be the special sensitivity of strain 19 to the dye thionin blue (McLeod, 1944; Levine & Wilson, 1949). The value of this method was estimated by the filter-paper strip technique with the use of strips impregnated with 1/600 thionin blue, strain 19 and a number of other brucella strains being inoculated across the strip. A typical result is shown in Pl. 4; the wide zone of inhibition with strain 19 contrasts strikingly with the unimpeded growth of the other eleven strains, a random collection of cultures of *Br. abortus*. Strain 19 was consistently inhibited over a zone 25–26 mm. wide. Of thirty-nine strains of *Br. abortus*, typical except that a number grew well without CO_2, thirty-seven showed no inhibition by thionin blue; the other two showed zones 8–9 mm. wide. Three strains of the type sensitive to all the usual test dyes were also sensitive to thionin blue, giving zones 11, 32 and 35 mm. wide. Of seven strains of *Br. melitensis* and three aberrant strains, none was inhibited. Of five strains of *Br. suis* four were inhibited, showing zones 11, 13, 15 and 20 mm. wide.

It thus appears that, of the *Br. abortus* strains tested, the only ones inhibited by thionin blue to an extent approaching strain 19 are those sensitive to all the dyes. Strain 19 is not inhibited by basic fuchsin or methyl violet. The justifiable conclusion seems to be that, if a suspected strain of *Br. abortus* is not inhibited by thionin blue, it is not strain 19; if it grows without added CO_2 and is strongly inhibited by thionin blue and not by basic fuchsin or methyl violet, it *may* be strain 19.

Absorption tests with patient's serum

A serological diagnosis of undulant fever has been made on a number of occasions in patients infected abroad, and the serum has been sent with a request for information about the probable infecting organism. Absorption tests have given surprisingly clear-cut results. The method, described by A. C. Evans and quoted by Huddleson (1943), is to absorb quantities of the serum with thick suspensions of *Br. abortus* and *Br. melitensis* respectively, and to test the absorbed sera for agglutinins to each organism. One such serum tested in dilutions from 1/50 upwards gave the following results:

	Br. abortus	*Br. melitensis*
Patient's serum, unabsorbed	1/2560	1/1280
Patient's serum, absorbed with *Br. abortus*	0	0
Patient's serum, absorbed with *Br. melitensis*	1/1280	0

Br. abortus thus removed from the serum the agglutinins for both organisms, whereas absorption with *Br. melitensis* left the serum still capable of agglutinating *Br. abortus*. It was inferred that the patient was infected with *Br. abortus* and this was later confirmed by the isolation of that organism from the blood.

DISCUSSION

The differences between the three species of brucella are quantitative rather than qualitative, and no single test can serve to distinguish them. However, by the use of the four usual tests— CO_2 requirement, production of H_2S, sensitivity to dyes and agglutination with monospecific antisera—it is possible to classify strains satisfactorily for epidemiological purposes. The performance of these tests by accepted methods gives consistent results, a fact confirmed during the present survey, in which numerous typical and atypical strains were exchanged with Dr A. W. Stableforth of the Ministry of Agriculture and Fisheries Veterinary Laboratory at Weybridge, without the observation of any significant discrepancy. It may be desirable and possible to standardize some of the methods. For example, Clarke (1953) refers to the irregularity of tests for H_2S production due to differences in the content and availability of sulphur in the medium, the sensitivity of the method used for detection of H_2S and the degree of bacterial growth; she recommends for some organisms the use of 0·01 % cysteine hydrochloride in Lemco broth to provide a medium with a standard source of sulphur. Another useful step is the designation by the Joint FAO/WHO Expert Committee on Brucellosis (Report, 1953) of type strains of the three species for comparison: *Br. abortus* 544 (Weybridge), *Br. melitensis* 16 M (Beltsville) and *Br. suis* 1330 (Minneapolis). These are available from the Weybridge Centre.

Most of the strains in the series now reported were easy to identify and behaved typically in all the tests. Others departed very slightly from the typical, for example strains of *Br. abortus* not requiring added CO_2; and others more widely, for example *Br. abortus* strains of special sensitivity to all dyes. The behaviour of a small proportion of strains was so atypical that they could not be confidently assigned to any of the known species, the biochemical characters being those of *Br. abortus* or *Br. melitensis* and the serological behaviour that of the other species.

It has already been noted that these atypical strains have been observed by all experienced workers in this field (Wilson, 1933; Huddleson, 1952; Renoux, 1952*b*; Report, 1953). Veazie & Meyer (1936) encountered twenty-four in their series of 427 strains, and Rita & Levi Della Vida (1951) twenty-two in their series of 176. Picket *et al.* (1953), in their series of 232 smooth strains, found that eighteen could not be satisfactorily classified. They found that the urease and carbamate tests, which correlated well with the dye tests and serology, correctly defined all of 197 strains from the United States and Mexico. They conclude that, with the usual four dyes, the urease and the carbamate tests, the great majority of strains can be identified, leaving only a few for which the use of monospecific sera is necessary.

Renoux (1952*c*) has recently reported the classification of 2598 strains by the biochemical tests but not by serological methods, which he considers of little value. He found that 5 % of his strains, and 11 % of those of bovine origin, could not be classified as members of the three recognized species. While admitting the practical value of such classification, he would prefer to include all the organisms under discussion in a single species, *Br. brucei*, with five varieties, *melitensis*, *abortus*, *suis* (the American type), *thomseni* (the Danish type of *Br. suis*) and *lisbonnei* (thionin-resistant strains producing H_2S). The proposed new species *Br. intermedia*, of Renoux (1952*b*), and the subspecies *Br. abortus* (Wilson) and *Br. abortus* (Van der Schaff), of Huddleson (1952), have been referred to above. Recently, van Drimmelen (1953) has proposed the name *Br. melitensis* var. *karakul* for strains, isolated from Karakul sheep in south-west Africa, which did not require added CO_2, produced constant but moderate amounts of H_2S, were not inhibited by any of the test dyes and showed some antigenic differences both from *Br. abortus* strain 19 and *Br. melitensis*. A strain kindly sent by Dr van Drimmelen was examined in this laboratory. Its biochemical characters were confirmed and it was found to agglutinate with a *Br. melitensis* monospecific antiserum and not with a *Br. abortus* monospecific antiserum; that is, it behaved like *Br. melitensis* except that it produced considerable amounts of H_2S on each of the first three days of growth. It is clearly desirable that more information about the characters and distribution of these atypical strains should be collected and considered, so that they may be given an agreed designation where it is thought appropriate. Observations on variation and stability of the types are also indicated, particularly in view of the reports by Renoux & Carrère (1952), who picked resistant colonies from dye plates or recovered strains after passage through animals, and found that they differed in their biochemical behaviour from the parent strain and would by these tests be classified as a different type.

SUMMARY

An account is given of the examination by biochemical and serological methods of 800 strains of brucella sent for identification to the Brucella Reference Laboratory of the Public Health Laboratory Service.

Most of the strains were isolated from milk in Great Britain, but strains from other countries, and from cases of undulant fever, were also examined.

Of 738 strains from milk in Britain, 680 were *Br. abortus*, of which sixty-two

(9·1 %) were of a type inhibited by all the usual test-dyes in their customary concentrations. Thirty strains of *Br. melitensis*, behaving typically in all laboratory tests, were obtained from milk from individual cows, herds or bulk sources. *Br. melitensis* has now been identified from twenty-four separate farms and one Tuberculin Tested bulk supply in this country. Eighteen strains behaved like *Br. abortus* in biochemical tests, but serologically like *Br. melitensis*.

Of the relatively small number of strains isolated from patients with undulant fever in Britain, all those from persons infected in this country were *Br. abortus*, with the exception of two laboratory infections with *Br. melitensis*. The organisms isolated in Britain from patients infected abroad comprised three strains of *Br. melitensis*, one strain behaving biochemically like *Br. abortus* and serologically like *Br. melitensis*, and two behaving biochemically like *Br. melitensis* and serologically like *Br. abortus*. One of the latter was from a patient infected in southern Italy, and the examination of nineteen strains from that country showed that thirteen were of this type. The thionin-resistant type of *Br. abortus* was identified several times, from undulant fever cases in Rhodesia, Kenya and Italy. No strains of *Br. suis* were observed.

I wish to thank the numerous bacteriologists who sent cultures and provided details of their isolation. A few submitted large numbers of strains and enabled a general picture of the prevailing types to be obtained. I am grateful to these and to the other bacteriologists who provided information about the herds from which *Br. melitensis* was isolated, in particular Dr P. H. Martin and Dr J. A. Sykes (Ipswich), Dr L. M. Dowsett (Norwich), Dr J. Kennedy (Newcastle, then at Stafford), Dr J. H. C. Walker (Luton), Dr E. H. Gillespie and Dr N. S. Mair (Leicester) and Dr J. E. Jameson (Brighton). I have also to acknowledge the help of Dr A. W. Stableforth, Director of the Ministry of Agriculture and Fisheries Veterinary Laboratory, who provided much bacteriological and veterinary information about the infected herds.

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EXPLANATION OF PLATE 4

Test of the sensitivity to thionin blue, by the filter-paper strip method, of twelve strains of *Br. abortus*, including strain 19.

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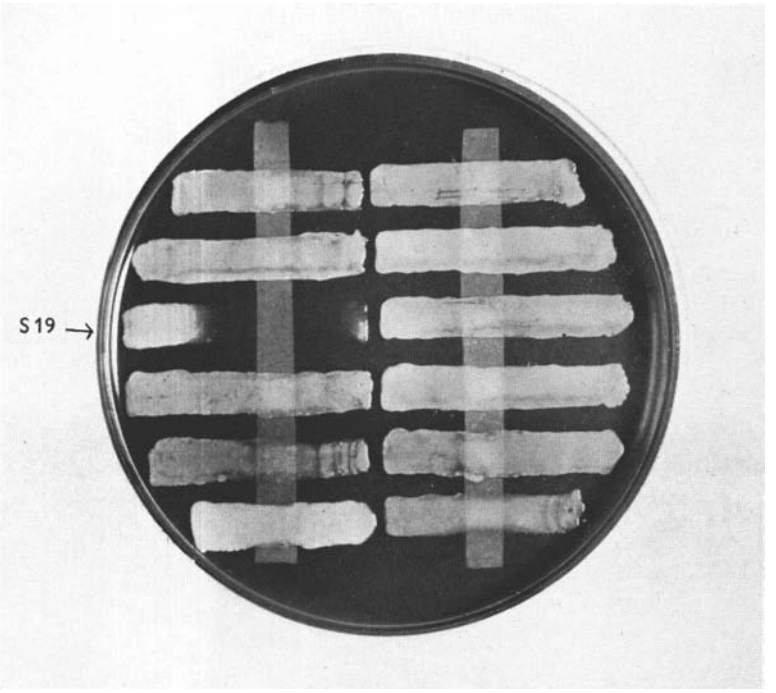

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