A SEROLOGICAL STUDY OF UNDULANT FEVER IN SOUTHERN RHODESIA.

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The discussion which has so recently arisen regarding the part played by *Brucella abortus* in the etiology of undulant fever was to a large extent initiated by the observations of investigators in Southern Rhodesia.

Bevan (1922) was the first to call attention to the possible danger to man arising from the ingestion of dairy products from a herd infected with contagious abortion, and Orpen (1924) followed this up by being amongst the first to report the actual isolation of an organism, serologically indistinguishable from *Br. abortus*, from the blood of patients suffering from undulant fever in Southern Rhodesia. Duncan (1924) has also reported the serological characteristics of an organism isolated by him from an undulant fever patient coming from this colony. This organism, he found, was more closely related to *Br. abortus* than to *Br. melitensis*.

Since the publication of the Rhodesian observations, cases in which *Br. abortus* has been isolated from patients suffering from typical undulant fever have been reported mainly from America and Italy. Keefer (1924), prior to Orpen, stated that he had isolated *Br. abortus* from a case of undulant fever in Baltimore, while Huddleson (1926), Carpenter (1926), and Carpenter and Merriam (1926), have published similar results regarding 3, 5 and 2 cases respectively. In Italy Ficai and Alessandrini (1925) claim also to have isolated this organism from cases of undulant fever. In addition to these cases, in which serological evidence pointed to *Br. abortus* as the cause of the infection, several have been reported in which undulant fever has developed in patients who had been in intimate connection with animals suffering from contagious abortion and in which this seemed the only possible source of infection, but in which no satisfactory bacteriological or serological proof exists as to the exact nature of the infecting organism. Ignoring such cases, however, it would seem that there is some evidence that *Br. abortus* may be responsible for a type of undulant fever in man.

Regarded from the epidemiological point of view, the fact that contagious bovine abortion is so widely distributed over areas where undulant fever is practically non-existent appears to militate against the relationship of the two diseases. Theobald Smith (1926) has drawn attention to this and also to the great infectivity of *Br. melitensis* towards laboratory workers handling this

organism. While making no statement he implies that such is not the case with *Br. abortus*, but it should be noted that in all three of Huddleson's cases there was a distinct possibility of infection having been acquired in the laboratory. The results of the enquiry in France by Aublant, Dubois, Lafenêtre and Lisbonne (1926) laid stress on the circumstance that while contagious abortion prevailed throughout France, excepting areas in which epizootic abortion was endemic in sheep and goats, it was only in these latter areas that endemic undulant fever was present. The apparent lack of correlation between the bovine and the human disease is emphasised by the very definite relationship between the caprine and human disease in regions where *Br. melitensis* is prevalent, but this affords no proof that *Br. abortus* is not an etiological factor in undulant fever. It must be remembered that if both *Br. melitensis* and *Br. abortus* are proved capable of producing the symptom complex known in man as undulant fever, it does not necessarily follow that both possess a similar epidemiology.

We know little about the infectivity of *Br. abortus* in man and probably less about the immune state of the populations exposed to infection by it. This is not surprising because it is only since 1924 that the question of *Br. abortus* being the cause of undulant fever has arisen. Again the belief that the disease could be traced invariably to goats has undoubtedly led to the assumption that it could not exist endemically in countries where goat products were not in use. Thus in Great Britain the diagnosis of undulant fever in febrile cases which have never been out of the country is practically never made. Few if any laboratories lay stress upon their facilities for the bacteriological and serological diagnosis of undulant fever, and such facilities are seldom made use of by practitioners.

Contagious abortion is common amongst the bovine population in England: Wilson and Nutt (1926) in a routine examination of milk from different herds in Cheshire, Lancashire, Derbyshire, Staffordshire, and Cumberland found 5·7 per cent. of single milks infected with *Br. abortus*. If therefore *Br. abortus* is a cause of undulant fever it is probably incorrect to say that the latter does not occur in England. It is recognised that contagious abortion occurs but a diagnosis of undulant fever is never considered possible by the vast number of diagnosticians because the possibility of *Br. abortus* being a cause of this disease is not sufficiently recognised. While it cannot be asserted positively that endemic undulant fever exists in Britain, there seems to be reason in refusing to accept the assumption of its non-existence without further investigation.

Experience in Rhodesia shows how essential it is, in the diagnosis of undulant fever, to abandon the idea that it is only *Br. melitensis* that can cause the disease, and that the vehicle of infection is goat's milk or one of its products. Since relatively few Europeans consume goat's milk, undulant fever was practically never diagnosed until Bevan pointed out the possibility of *Br. abortus* being also a causal organism. The serological diagnosis of the first case

was made in 1920 and since then each year's record shows an increasing number of tests and an increasing number of positive results.

Leaving aside, however, for the moment, the puzzles of the epidemiology of the two diseases, an enquiry into the validity of the results of those who have claimed to have isolated *Br. abortus* from man and the criteria on which they base their identification of the organism seems necessary.

Since Evans (1918) pointed out the close morphological, biological, and serological relationship between *Br. melitensis* and *Br. abortus*, subsequent work has but served to confirm her results. Observers now agree that on morphological and biological grounds there can be no serious attempt made at the separation of the two organisms, but serological results are sometimes confusing. Here again it is more or less generally agreed that either organism agglutinates to an equal degree with an antiserum prepared against the homologue or heterologue; but while some claim that differentiation between the two can be made by the absorption of agglutinin technique, there are others who deny this. Evans (1918) stated that *melitensis* antiserum, absorbed with *Br. melitensis*, lost all agglutinins for both cultures but absorption with *Br. abortus* removed only agglutinins for itself and left those for *Br. melitensis* present. Feusier and Meyer (1920) studied 14 strains of the *melitensis-abortus* group serologically and found that by the agglutinin-absorption test they could differentiate four groups. Group I was composed of all the *abortus* strains tested and one *melitensis* strain, Group II of nine *melitensis* strains, Group III of one *melitensis* strain, and Group IV of two *paramelitensis* strains. They emphasised the close relationship of Groups I and II, and contrasted therewith the wide difference between these groups and Group IV composed of *paramelitensis* strains. It must be noted, however, that in Group I there is a so-called *melitensis* strain included with all the *abortus* strains.

Khaled (1921) studied 30 strains of *Br. melitensis*, *Br. abortus*, and *Br. paramelitensis* serologically, and found, like previous observers, that by direct agglutination no marked difference could be made out between *melitensis* and *abortus*, which both reacted with anti-melitensis and anti-abortus serum, but that *paramelitensis* exhibited more specific agglutination. When tested by the agglutinin-absorption method he found that absorption of:

(1) Anti-melitensis serum by *Br. melitensis* removed all agglutinins for *Br. melitensis* and *Br. abortus*.

(2) Anti-melitensis serum by *Br. abortus* removed all agglutinins for *Br. melitensis* and *Br. abortus*.

(3) Anti-abortus serum by *Br. abortus* also removed all agglutinins.

(4) Anti-abortus serum by *Br. melitensis* removed all agglutinins for *melitensis* but not for *Br. abortus*.

Therefore he concludes that *Br. melitensis* is a sub-strain of *Br. abortus*, but it must be remarked that the result (2) is curious and does not agree with Evans's finding previously quoted; nevertheless a difference between *Br. melitensis* and *Br. abortus* has been made out.

Later Evans (1926) described eight serological groups, four being varieties of *abortus* and the remainder *para-abortus*, *melitensis* A, *melitensis* B, and *paramelitensis*.

Others have reported similar findings, but the authors I have cited, who made the most important and detailed serological study of the group are united in concluding that serological differences exist between members of the *abortus-melitensis* group.

In contrast, however, to the opinion of these authors, it must be acknowledged that certain investigators deny the possibility of separating *Br. melitensis* from *Br. abortus* by serological methods. Thus Burnet (1925) states that while it is possible to state positively that an organism is *Br. melitensis*, it is impossible to prove that a strain is *Br. abortus*. Contrary to other workers he asserts that *Br. abortus* is closely related to *Br. paramelitensis*; he remarks, however, that it is premature to assume that the strains are identical. Favilli (1926) and Béguet (1926) also failed to confirm the results of the above-mentioned authors. This divergence of opinion demands an analysis of the methods whereby such results have been obtained and a recent paper by Orcutt (1926) is most valuable in that it may in part explain the failure of other observers to differentiate between the organisms. Orcutt studied two bovine abortus strains, two swine *abortus* strains and two strains which she names *Br. melitensis* isolated from cases of undulant fever in man in America. The first, *Br. melitensis* 2 (Blake), was isolated from a patient who handled fresh pork in a slaughter house, and the second was the strain isolated by Keefer and was actually described by him as *Br. abortus*. Orcutt was unable to find any serological difference between the six strains and comes to the conclusion that *Br. melitensis* 2 and *Br. melitensis* 3, the human strains, are identical with the bovine and swine *abortus* strains as judged by agglutination and agglutinin-absorption tests. This conclusion demands critical analysis.

To classify the two human strains as *Br. melitensis* seems unwarrantable since no comparison was made with *Br. melitensis* strains of caprine origin, and since in one case the so-called *Br. melitensis* had been previously described as *Br. abortus*. A far more legitimate conclusion would have been that since the American strains were identical serologically with *Br. abortus*, Keefer's assumption that his organism was not *Br. melitensis* but *Br. abortus* was correct, and that this assumption was strengthened by the isolation of a second strain possessing the same characteristics. Orcutt's erroneous premise, that since the strains were isolated from cases of undulant fever they must be *Br. melitensis*, illustrates the pitfall into which other investigators may have fallen unless great care was taken to trace the origin of the strains employed.

Up to 1924 the question of the etiological significance of *Br. abortus* in undulant fever had not arisen. Any micro-organism isolated from patients suffering from undulant fever before that date, provided it possessed the characteristics of the *alcaligenes* group, would naturally be assumed to be *Br. melitensis* or *Br. paramelitensis*. There is agreement that by serological

methods and other characteristics *Br. melitensis* can be distinguished fairly easily from *Br. paramelitensis*.

Therefore any organism possessing the morphological, cultural and agglutination reactions of *Br. melitensis* would naturally be classified as that organism. We now know that these criteria are insufficient to enable us to distinguish *Br. melitensis* from *Br. abortus*. In the past, however, the need for differentiation was not recognised and hence it is quite likely that certain strains have been labelled *Br. melitensis* which in reality are *Br. abortus*. If this be true it explains the failure of some observers to differentiate between the two organisms and it certainly emphasises the need for a most thorough investigation of all *Br. melitensis* strains which are used as type strains and with which recently isolated strains are compared.

Scope of present investigation.

In the present investigation an attempt was made to confirm the serological differences which have been stated to exist between *Br. melitensis* and *Br. abortus* and to obtain information regarding the nature of the strains isolated from cases of undulant fever in Southern Rhodesia. The first step was to obtain strains of *Br. melitensis*, *Br. paramelitensis*, and *Br. abortus* from various sources to act as type strains and to investigate their morphological, cultural and serological characteristics before adopting them as types to which the Rhodesian strains might be referred. The following strains were obtained:

Br. melitensis.

Br. M. 1. Isolated by Bassett-Smith.
Source: Lister Institute.

Br. M. 2. Isolated by Arkwright.
Source: Lister Institute.

Br. M. 3. Isolated in South Africa.
Source: South African Institute of Medical Research.

Br. M. 4. Origin unknown.
Source: Director Veterinary Research, S. Rhodesia.

Br. M. 5. Origin unknown.
Source: Public Health Laboratory, Salisbury.

Br. abortus.

Br. A. 1. Isolated by Prof. Leslie Sheather from bovine.
Source: Royal Veterinary College, England.

Br. A. 2. Isolated by Prof. Leslie Sheather from bovine.
Source: Royal Veterinary College, England.

Br. A. 3. Isolated in South Africa from bovine.
Source: Veterinary Research Institute, Onderstepoort, Transvaal.

Br. paramelitensis.

Br. Pm. 1. Isolated by Bassett-Smith.
Source: Lister Institute.

Therefore the collection included strains from English and South African sources.

Morphological and cultural characters of type strains.

This examination was conducted mainly to ensure that the strains possessed the characteristics of the *alcaligenes* group as defined by Bergey (1926)[1], and no attempt at differentiation was made along these lines. Microscopically there was the usual difficulty in deciding whether to classify a strain as bacillary or coccal, owing to variations of the same strain according to the age of the culture and the nature of the medium.

As regards cultural characteristics all behaved as typical members of the *alcaligenes* group, producing no indol, giving a negative Voges-Proskauer reaction and producing no acid in the presence of the following carbohydrates: glucose, lactose, laevulose, mannite, dulcite, salicin, inulin, galactose, raffinose, adonitol, and xylose. No liquefaction was produced in gelatine. Nitrates were not reduced. It was therefore concluded that the organisms could be regarded as fulfilling the requirements necessary to place them in the *melitensis-abortus* group of *alcaligenes*.

Serological investigation of type strains.

Agglutination tests.

Immune sera were prepared by the intravenous inoculation of rabbits with saline suspensions of Br. M. 1, Br. A. 3, and Br. Pm. 1. Suspensions killed by heating to 60° C. for 30 minutes were used. In the case of Br. M. 1, and Br. A. 3 the inoculation of 1 c.c. of suspension on three successive days resulted in the production of an immune serum of high titre in from 7 to 9 days, but in the case of Br. Pm., it was only after repeated courses that satisfactory serum was obtained. The maximum titre obtainable, however, was only 1/200. Direct agglutination tests were then performed with these sera against the test organisms. The results of these tests are best shown in tabular form (Table I).

Table I.

Organism	Titre of Br. M. 1 serum against	Titre of Br. A. 3 serum against	Titre of Br. Pm. 1 serum
Br. M. 1	1 in 1600	1 in 1600	Nil.
Br. M. 2	1 in 1600	1 in 1600	Nil.
Br. M. 3	1 in 1600	1 in 1200	Nil.
Br. M. 4	1 in 1600	1 in 1600	Nil.
Br. M. 5	1 in 200	1 in 100	1 in 200
Br. A. 1	1 in 1600	1 in 1600	Nil.
Br. A. 2	1 in 100	1 in 100	1 in 200
Br. A. 3	1 in 1600	1 in 1600	Nil.
Br. Pm. 1	1 in 100	1 in 100	1 in 200

Technique. 0·25 c.c. of serum dilution was tested with 0·25 c.c. of a four thousand million (4 T.M.) suspension of organism. Incubation was at 37° C. and results were read after 18 hours.

[1] The generic name *Brucella* seems by general accord to apply to the members of the alcaligenes group producing disease in man.

Table I shows that the organisms can be separated into two categories by agglutination alone. There are those organisms which agglutinate to titre with Br. M. 1 and Br. A. 3 sera, comprising four of the melitensis and two of the *abortus* strains. The three remaining strains Br. Pm. 1, Br. M. 5, and Br. A. 2 are only agglutinated to a slight degree by Br. M. 1 and Br. A. 3 sera, but are agglutinated to titre by Br. Pm. 1 serum, and can reasonably be regarded as being serologically distinct from the first group.

While it is thus possible to separate the organisms into two groups, such separation is obviously incomplete since the first group includes both *melitensis* and *abortus* strains, while the second contains *melitensis*, *abortus* and *paramelitensis* strains. In order to obtain further differentiation the agglutinin-absorption test was employed.

Agglutinin-absorption tests.

(*a*) *Technique.* The method employed was as follows. To 1 c.c. of bacterial suspension, standardised to contain 16,000,000,000 organisms per c.c., was added an equal quantity of immune serum in a strength of 16 times the titre strength (T.). The actual "absorbing" dilution of serum was therefore 8 times titre strength. The mixture of serum and organism was incubated at 37° C. overnight, was centrifuged the following morning and the supernatant fluid distributed for the second phase of the test, in which three sets of four tubes were employed. The absorbed serum was distributed into the second and third sets of tubes in each series, in 0·25 c.c. amounts, and in such dilutions that strengths of 8 T., 6 T., 4 T., and 2 T. were obtained. In the first set of four tubes, in similar dilutions, was distributed unabsorbed serum which had also stood overnight at 37° C. To the first and third sets of tubes was then added 0·25 c.c. of a 4 T.M. suspension of the organism under investigation, while to the second set was added the same amount of a similar suspension of the organism which had been employed as antigen in the production of the serum. The tubes, plugged to prevent evaporation, were incubated at 37° C. for 18 hours and results recorded at the end of this period. By this system it was possible to determine by means of the three sets of tubes:

Set 1. Whether the organism was agglutinated or not by the unabsorbed serum.

Set 2. Whether all agglutinins for the homologue were removed or not by the absorption of the serum with the test organism.

Set 3. Whether absorption had been complete and had removed from the serum all agglutinins, if any were present, for the test organism. In the presence of an organism which agglutinates with the unabsorbed serum, this set of tubes must show no agglutination before any importance can be attached to agglutination of the homologue after absorption.

In addition, the suspension tested was also put up against normal rabbit serum in a dilution of 1 in 25, and in saline, in order to recognise if auto-agglutination occurred with any strain.

To include such controls is most important since it is occasionally found that strains develop the property of agglutinating spontaneously, or in the presence of normal serum. Izar (1926) notes that after cultivation on glucose-glycerine agar a strain of *Br. melitensis* became readily agglutinable with normal as well as pathological sera. This finding has been corroborated by Bevan (personal communication), and during the course of the present investigation. It was found, however, that repeated subculture in broth and subsequently upon Torrey-Buckell hormone agar restored specificity to the agglutinin reaction. The strain Br. Pm. 1, when received from the South African Institute of Medical Research, was accompanied by a note to the effect that it was useless for agglutination purposes as it agglutinated spontaneously, but this characteristic was removed by repeated subculture by the method described and ultimately a stable suspension was obtainable. This was achieved without disturbance of its thermo-agglutination properties, for at 90° C. this strain showed agglutination. In the present investigation both control tubes had to show no agglutination before the results were included in the tables.

(*b*) *Absorption of* Br. M. 1 *immune serum.* The result of the absorption of Br. M. 1 immune serum by the various strains is shown in Table II.

Table II.

	Unabsorbed serum: simple agglutination				Absorbed serum v. homologue				Absorbed serum v. test organism			
Organism	4 T.	3 T.	2 T.	1 T.	4 T.	3 T.	2 T.	1 T.	4 T.	3 T.	2 T.	1 T.
Br. M. 1	+	+	+	+	−	−	−	−	−	−	−	−
Br. M. 2	+	+	+	+	−	−	−	−	−	−	−	−
Br. M. 3	+	+	+	+	−	−	−	−	−	−	−	−
Br. M. 4	+	+	+	+	(±)	−	−	−	(±)	−	−	−
Br. M. 5	−	−	−	−	+	+	+	+	−	−	−	−
Br. A. 1	+	+	+	+	+	+	+	+	−	−	−	−
Br. A. 2	−	−	−	−	+	+	+	+	−	−	−	−
Br. A. 3	+	+	+	+	+	+	+	+	−	−	−	−
Br. Pm. 1	(±)	−	−	−	+	+	+	+	−	−	−	−

Note. In this and subsequent tables
+ = complete agglutination. + = partial agglutination
(±) = doubtful agglutination. − = no agglutination.

Table II shows that with Br. M. 1 immune serum:

(*a*) Br. M. 1, Br. M. 2, Br. M. 3, Br. M. 4 agglutinate to titre and on absorption remove the agglutinin for the homologue.

(*b*) Br. A. 1, Br. A. 3 agglutinate to titre, but on absorption do not remove the agglutinin for the homologue.

(*c*) Br. M. 5, Br. A. 2 and Br. Pm. 1 do not agglutinate and on absorption do not remove the agglutinin for the homologue.

Before commenting further on the results it is interesting to compare the results recorded in the next section.

(*c*) *Absorption of* Br. A. 3 *immune serum.* This is shown in Table III.

Table III shows the following results:

(*a*) Br. A. 1, and Br. A. 3 agglutinate to titre and on absorption remove the agglutinin for the homologue.

Table III.

Organism	Unabsorbed serum: simple agglutination				Absorbed serum v. homologue				Absorbed serum v. test organism			
	4 T.	3 T.	2 T.	1 T.	4 T.	3 T.	2 T.	1 T.	4 T.	3 T.	2 T.	1 T.
Br. M. 1	+	+	+	+	+	+	+	+	–	–	–	–
Br. M. 2	+	+	+	+	+	+	+	+	–	–	–	–
Br. M. 3	+	+	+	+	+	+	+	+	–	–	–	–
Br. M. 4	+	+	+	+	+	+	+	+	–	–	–	–
Br. M. 5	–	–	–	–	+	+	+	+	–	–	–	–
Br. A. 1	+	+	+	+	–	–	–	–	–	–	–	–
Br. A. 2	–	–	–	–	+	+	+	+	–	–	–	–
Br. A. 3	+	+	+	+	–	–	–	–	–	–	–	–
Br. Pm. 1	–	–	–	–	+	+	+	+	–	–	–	–

(*b*) Br. M. 1, Br. M. 2, Br. M. 3, Br. M. 4 agglutinate to titre, but on absorption do not remove the agglutinin for the homologue.

(*c*) Br. M. 5, Br. A. 2, and Br. Pm. 1 do not agglutinate and on absorption do not remove the agglutinin for the homologue.

Ignoring class (*c*) for the moment it will be seen that in Tables II and III the results are exactly opposite. Those organisms which remove the agglutinin for the homologue in Table II do not do so in Table III and *vice versa*. Both classes agglutinate to titre with either serum. The conclusion was, therefore, drawn that they could be accepted, first, from the results of simple agglutination, as the predominant *melitensis-abortus* group, and second, that those which removed the agglutinin for the homologue for Br. M. 1 serum and failed to do so with Br. A. 3 serum could be regarded as *Br. melitensis*, while those that reacted in exactly the opposite fashion could be classified as *Br. abortus*. The first important point then to emerge is that by the technique employed serological differences did exist between *Br. melitensis* and *Br. abortus*.

Further investigation, however, was necessary in the case of those organisms which failed to agglutinate with either serum. This result as far as Br. Pm. 1 was concerned was expected, but the inclusion with it of what were described as a *melitensis* strain and an *abortus* strain respectively was unlooked for. That their serological characteristics resembled those of Br. Pm. 1 in these first experiments at once prevented them from being regarded as type *melitensis* or *abortus* strains, because my investigations and those of previous observers have shown that a wide gap separates the true *melitensis* and *abortus* strains from *paramelitensis* strains. In order to establish their serological identity immune serum against each of these two strains was prepared by the inoculation of rabbits, and compared with the Br. Pm. 1 serum already obtained. I would note that the same difficulties in preparing a serum of adequate titre, which have been described as occurring when Br. Pm. 1 was the organism inoculated, were met with, when Br. M. 5 and Br. A. 2 were similarly employed. A titre of 1/400 was eventually obtained with Br. A. 2 serum and of 1/200 with Br. M. 5 serum.

(*d*) The results of the experiments with these three sera are shown grouped together for convenience in Table IV.

It may be noted that the quality of agglutination obtained with all these sera, whether absorbed or unabsorbed, was very different from that obtained with the organisms which agglutinated with either Br. M. 1 or Br. A. 3 serum. In contrast to the granular precipitate (broken up with difficulty), seen in the latter group, the precipitate obtained with the group under discussion was very fine and seemed to result from sedimentation rather than from actual

Table IV.

	Unabsorbed serum: simple agglutination				Absorbed serum v. homologue				Absorbed serum v. test organism			
Organism	4 T.	3 T.	2 T.	1 T.	4 T.	3 T.	2 T.	1 T.	4 T.	3 T.	2 T.	1 T.
(*a*) Br. Pm. 1 serum:												
Br. Pm. 1	+	+	+	+	–	–	–	–	–	–	–	–
Br. M. 5	+	+	+	+	+	+	+	–	–	–	–	–
Br. A. 3	+	+	+	+	+	+	+	+	–	–	–	–
(*b*) Br. M. 5 serum:												
Br. Pm. 1	+	+	+	+	+	+	+	(±)	–	–	–	–
Br. M. 5	+	+	+	+	–	–	–	–	–	–	–	–
Br. A. 3	+	+	+	+	+	+	+	+	–	–	–	–
(*c*) Br. A. 2 serum:												
Br. Pm. 1	+	+	+	+	+	+	+	+	–	–	–	–
Br. M. 5	+	+	+	+	+	+	+	+	–	–	–	–
Br. A. 3	+	+	+	+	–	–	–	–	–	–	–	–

clumping. The precipitate was easily shaken into a homogeneous suspension, indistinguishable from the untreated suspension, and a positive result was best judged by the clarity of the supernatant fluid as compared with controls. The results of these tests indicate the serological independence of the three strains from each other. It may be said that this conclusion was reached only after repeated experiment in the case of Br. Pm. 1 and Br. M. 5, for in both cases absorption of the homologous serum with the heterologue did not leave the agglutinin for the homologue absolutely untouched. In neither case after absorption did the homologue agglutinate to titre and the quality of agglutination in the higher titres seemed impaired. In the case of Br. A. 2 however, results were definite and in all three tests it stood out as distinct from Br. Pm. 1 and Br. M. 5. Br. Pm. 1 and Br. M. 5 can be regarded as representing *paramelitensis* strains which differ slightly from each other. Br. Pm. 1 was recognised as a *paramelitensis* strain previously, but the inclusion of Br. M. 5 in this group shows the need for the careful study of strains before accepting them as types in any experiment. Br. A. 2 was isolated from a case of bovine contagious abortion, and in view of its distinct difference from true *melitensis* and *abortus* strains, and its close similarity to the two *paramelitensis* strains, it can best be regarded as a *para-abortus* strain. Thus not only can *Br. melitensis* be distinguished from *Br. abortus* by the serological methods employed, but also *Br. paramelitensis* from *Br. para-abortus*.

The results of the preliminary examination of the type strains employed is best illustrated by reference to Table V.

It is pertinent here to emphasise that the relationship between members of

Group I and between members of Group II is much closer than the relationship of Group I to Group II. Thus *Br. abortus* is much more closely related to *Br. melitensis* than is *Br. paramelitensis*.

Table V.

Group I.	Melitensis	Br. M. 1 Br. M. 2 Br. M. 3 Br. M. 4
	Abortus	Br. A. 1 Br. A. 3
Group II.	Paramelitensis	(*a*) Br. M. 5 (*b*) Br. Pm. 1
	Para-abortus	Br. A. 2

Investigation of strains isolated from undulant fever cases in Southern Rhodesia.

Source of strains.

The strains investigated were all obtained from patients undergoing treatment in Salisbury Hospital, and exhibiting the typical symptoms of undulant fever. The diagnosis of the disease was generally made by the agglutination reaction. In the whole series there was no indication that the disease could have been derived from goats. None of the patients drank goat's milk or came into contact with goats. Three of the patients were town and five country residents. All were males and under 30 years of age.

Technique of isolation.

In all cases, with one exception, the organism was isolated by haemoculture, 2–4 c.c. of blood from a vein being run into a bottle containing 30 c.c. of broth. Incubated at 37° C., it was several days, as a rule, before growth appeared. Subcultures were made daily after the third day but generally it took 7–9 days for growth to occur.on agar.

Strains isolated.

Lockie	Isolated by Dr Orpen, 1922.
Weldon	Isolated February, 1926.
Du Rand	Isolated February, 1926.
Narcee	Isolated November, 1926.
Collinson	Isolated November, 1926.
Williams	Isolated December, 1926.
Gray	Isolated December, 1926.
Kenny	Isolated December, 1926.

Microscopical and cultural characters of isolated strains.

The first subculture after 48 hours' incubation showed the majority of the strains to be bacillary in nature, but "Gray," for example, appeared as minute diplococci or as chains of micrococci. The third subculture showed micrococci

only, chain formation being absent. After 2–3 months' cultivation and subculture at weekly intervals it was most difficult to classify the strains. All showed both coccal and bacillary elements, so that no attempt at differentiation on the basis of microscopical appearance was made. Culturally all exhibited the same characteristics as the type strains.

To determine their pathogenicity for laboratory animals, all strains were inoculated into guinea-pigs intraperitoneally and these compared with animals inoculated with Br. M. 1 and Br. A. 3. In no case was death produced in the experimental animals, but at autopsy 2, 5, and 7 months after inoculation, the animals being apparently healthy at the time of death, typical splenic enlargement and in some cases involvement of the liver was found. As animal inoculation was done merely to demonstrate pathogenicity and not as a method of differentiation, the differences found by Smith (1926) between the lesions produced by *Br. melitensis* and *Br. abortus* were not looked for.

Serological investigation of isolated strains.

Since the method employed in the agglutinin-absorption test permits of the demonstration of agglutination with unabsorbed serum, details of results obtained in the preliminary tests by means of agglutination alone will be omitted. In the absorption test each strain was tested in turn against Br. M. 1, Br. A. 3, Br. Pm. 1, Br. M. 5 and Br. A. 2 sera, and in each test, type organisms belonging to each of the groups already demonstrated were included in order to control results. The usual controls with normal serum and saline were also included.

(*a*) *Absorption of* Br. M. 1 *serum with isolated strains.* The results of absorption of Br. M. 1 serum with the Rhodesian strains (Table VI) are remark-

Table VI.

	Unabsorbed serum: simple agglutination				Absorbed serum v. homologue				Absorbed serum v. test organism			
Organism	4 T.	3 T.	2 T.	1 T.	4 T.	3 T.	2 T.	1 T.	4 T.	3 T.	2 T.	1 T.
Br. M. 1	+	+	+	+	–	–	–	–	–	–	–	–
Br. A. 3	+	+	+	+	+	+	+	+	–	–	–	–
Br. Pm. 1	–	–	–	–	+	+	+	+	–	–	–	–
Br. M. 5	–	–	–	–	+	+	+	+	–	–	–	–
Br. A. 2	–	–	–	–	+	+	+	+	–	–	–	–
Lockie	–	–	–	–	+	+	+	+	–	–	–	–
Weldon	–	–	–	–	+	+	+	+	–	–	–	–
Du Rand	+	+	+	+	+	+	+	+	–	–	–	–
Narcee	+	+	+	+	+	+	+	+	–	–	–	–
Collinson	+	+	+	+	+	+	+	+	–	–	–	–
Williams	+	+	+	+	+	+	+	+	–	–	–	–
Gray	+	+	+	+	+	+	+	+	–	–	–	–
Kenny	+	+	+	+	+	+	+	+	–	–	–	–

able in that none of the strains remove the agglutinins for the homologue. This is in striking contrast to the results obtained when the type *melitensis* strains were similarly examined. It is further seen that the strains fall into two classes: those which agglutinate to titre, and those which fail to show agglutination. As regards the first class, their behaviour is similar to Br. A. 3, and points to

the possibility of their serological identification as *Br. abortus*. Discussion on the second group, apart from remarking on their complete separation from *Br. melitensis*, will be deferred.

(*b*) *Absorption of* Br. A. 3 *serum with isolated strains* (Table VII). In consequence of the results obtained with Br. A. 3 serum certain definite conclusions can be drawn. It will be seen that the affinity which was shown to exist

Table VII.

	Unabsorbed serum: simple agglutination				Absorbed serum v. homologue				Absorbed serum v. test organism			
Organism	4 T.	3 T.	2 T.	1 T.	4 T.	3 T.	2 T.	1 T.	4 T.	3 T.	2 T.	1 T.
Br. M. 1	+	+	+	+	+	+	+	+	–	–	–	–
Br. A. 3	+	+	+	+	–	–	–	–	–	–	–	–
Br. Pm. 1	–	–	–	–	+	+	+	+	–	–	–	–
Br. M. 5	–	–	–	–	+	+	+	+	–	–	–	–
Br. A. 2	–	–	–	–	+	+	+	+	–	–	–	–
Lockie	–	–	–	–	+	+	+	+	–	–	–	–
Weldon	–	–	–	–	+	+	+	+	–	–	–	–
Du Rand	+	+	+	+	–	–	–	–	–	–	–	–
Narcee	+	+	+	+	–	–	–	–	–	–	–	–
Collinson	+	+	+	+	–	–	–	–	–	–	–	–
Williams	+	+	+	+	–	–	–	–	–	–	–	–
Gray	+	+	+	+	–	–	–	–	–	–	–	–
Kenny	+	+	+	+	–	–	–	–	–	–	–	–

between Br. A. 3 and certain of the Rhodesian strains in Table VI has been confirmed, and it is now possible to state that these strains are serologically identical with *Br. abortus*. To summarise the evidence which exists in Tables VI and VII for that conclusion, one can say that absorption of an immune serum, specific against a typical *Br. melitensis*, by these strains does not remove the agglutinin for the homologue, whereas absorption of a similar serum specific against a typical *Br. abortus*, does remove the agglutinin for the homologue, and that this type of reaction is that characteristic of *Br. abortus*. This is true of six out of eight strains isolated.

As regards the remaining two strains one can state from these results that they do not resemble either typical *Br. melitensis* or *Br. abortus*, but that their serological reactions link them up with the group of aberrant strains found to exist amongst the type strains, and that their identification demands further tests with sera specific against these type strains.

(*c*) The remainder of this paper deals with the results of such tests, and for simplification the six strains which have been identified as *Br. abortus* will not be included in the tables. These strains were tested against each of the sera employed and reacted in a fashion typical of *Br. abortus*, failing to agglutinate with unabsorbed serum and failing also to remove the agglutinin for the homologue in absorbed serum.

The strains were first tested against Br. Pm. 1 serum in the usual manner and reacted as shown in Table VIII.

Table VIII shows that the strains are not serologically identical with Br. Pm. 1.

When tested against the immune serum specific to the *paramelitensis*

Table VIII.

Br. Pm. 1 *serum.*

Organism	Unabsorbed serum: simple agglutination				Absorbed serum v. homologue				Absorbed serum v. test organism			
	4 T.	3 T.	2 T.	1 T.	4 T.	3 T.	2 T.	1 T.	4 T.	3 T.	2 T.	1 T.
Br. Pm. 1	+	+	+	+	–	–	–	–	–	–	–	–
Lockie	+	+	+	+	+	+	+	+	–	–	–	–
Weldon	+	+	+	+	+	+	+	+	–	–	–	–

strain Br. M. 5, the Rhodesian strains behaved in the same manner as when in the presence of Br. Pm. 1 serum, and were thus distinct from Br. M. 5.

The results of testing against the *para-abortus* serum Br. A. 2 are recorded in Table IX.

Table IX establishes the identity of "Lockie" and "Weldon" and shows them to be serologically indistinguishable from an organism isolated from a case of bovine contagious abortion which has been termed *Br. para-abortus* owing to the wide differences separating it from *Br. abortus* and the slight but

Table IX.

Br. A. 2 *serum.*

Organism	Unabsorbed serum: direct agglutination				Absorbed serum v. homologue				Absorbed serum v. test organism			
	4 T.	3 T.	2 T.	1 T.	4 T.	3 T.	2 T.	1 T.	4 T.	3 T.	2 T.	1 T.
Br. A. 2	+	+	+	+	–	–	–	–	–	–	–	–
Lockie	+	+	+	+	–	–	–	–	–	–	–	–
Weldon	+	+	+	+	–	–	–	–	–	–	–	–

distinct differences which demarcate it from *Br. paramelitensis*. This result was confirmed when similar experiments were conducted with "Lockie" and "Weldon" immune sera. The agglutinins for the homologue were removed on absorption of both sera by Br. A. 2 "Lockie" or "Weldon," whereas absorption by Br. Pm. 1 or Br. M. 5 left the agglutinins for the homologue untouched in each case.

Discussion.

The results indicate that *Br. abortus*, or a mutant type *Br. para-abortus*, is an important factor in the etiology of undulant fever in Southern Rhodesia, and emphasise the necessity for a wider concept of the disease. Certain Rhodesian observers are inclined to the view, that, while the abortus type of undulant fever produces the same type of symptoms as that due to *Br. melitensis*, these symptoms on the whole are milder than in *melitensis* infection, and that infectivity is not so great. No case of accidental infection of hospital or laboratory personnel has yet been reported. As regards treatment, however, the *abortus* variety is as resistant to therapeutic measures as is the classical variety.

Whatever modification of clinical or epidemiological ideas are necessary, there can be no doubt that the bacteriological claim that undulant fever can be caused by an organism distinguishable from *Br. melitensis* but indistinguishable from *Br. abortus*, demands increasing attention.

Apart from the fact that recognisable differences have been shown to exist between *Br. melitensis* and *Br. abortus*, and that the strains isolated from Rhodesian cases of undulant fever are indistinguishable from *Br. abortus* or *Br. para-abortus*, the theoretical interest of the present research lies mainly in the clear-cut differentiation which has been found to exist between the *melitensis-abortus* group on the one hand and the *paramelitensis* and *para-abortus* group on the other. Hadley (1927) in his recent study of microbic dissociation has emphasised the trend in micro-organisms to dissociate from the "S" normal type to the "R" mutant type. This dissociation may be shown in a diversity of ways. In the case of *Br. melitensis* he quotes Burnet (1925) who found that nearly all strains of this organism, whatever the source, when tested against immune type serum, differentiate themselves sharply into two groups; those which agglutinate well and those which agglutinate poorly or not at all. He therefore postulates the existence of two distinct types of *Br. melitensis* which he names *melitensis* I and II. The *paramelitensis* of Bassett-Smith is identical with his *melitensis* II, as is Group IV of Feusier and Meyer. Intermediate forms may also occur, but are infrequent. He also found that rabbits were difficult to immunise with Type II cultures, but that the agglutination of Type II strains usually reached a higher titre than the Type I strains. Hadley regards Burnet's Type II cultures as probably representing the "R" form of culture, Type I being the "S" form.

The present investigation confirms the results of Burnet in the recognisable difference between Types I and II *Br. melitensis*. The characteristics which he describes were present in the *melitensis* and *paramelitensis* strains examined. Burnet, however, regards *Br. abortus* as identical with *Br. melitensis* and states that no *abortus* strains revealed Type II antigen. The results recorded in this communication, however, disagree with this contention. Differentiation has been successfully made between *Br. melitensis* and *Br. abortus* and further certain strains of *abortus* possessed characteristics which have led them to be classified as *Br. para-abortus*. Thus to follow Burnet's nomenclature the existence of Type I and Type II antigens has been recognised in *Br. abortus*. The similarities between *Br. melitensis* I and *Br. abortus* I and between *Br. melitensis* II and *Br. abortus* II are much greater than the similarities between Types I and II of either organism, and there is much to suggest that in Type I organism with its granular precipitate in immune serum we are dealing with the "S" normal type, whereas Type II, with its flocculent and easily disturbed precipitate, represents the "R" mutant type.

Summary.

1. Examination of nine type strains of *Brucella*, collected from various sources, by agglutination and the absorption of agglutinin test revealed distinct serological difference between *Br. abortus* and *Br. melitensis*. Differentiation could only be made by the agglutinin-absorption test, both organisms agglutinating equally with *abortus* or *melitensis* immune sera.

2. By agglutination alone differentiation could be made between a group consisting of *Br. melitensis* and *Br. abortus* strains on the one hand and a group which included one strain labelled *Br. melitensis*, one labelled *Br. abortus*, and *Br. paramelitensis*.

3. The agglutinin-absorption test showed that this *Br. paramelitensis* group comprised two strains of closely allied paramelitensis strains, and that the *Br. abortus* strain could be regarded as a *Br. para-abortus* strain.

4. Serological investigation of eight strains of *Brucella* isolated from patients suffering from undulant fever in Southern Rhodesia showed that six were serologically identical with type *Br. abortus* and two identical with what is regarded as a *Br. para-abortus* strain.

5. It is suggested that the *melitensis-abortus* group represent the "S" normal types, whereas the *paramelitensis-para-abortus* group represent the "R" mutant types of organisms.

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