A COMPARATIVE STUDY OF BOVINE ABORTION AND UNDULANT FEVER, FROM THE BACTERIOLOGICAL POINT OF VIEW.

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(With 2 charts.)

EARLY in 1914 Kennedy (1914) while testing some samples of goat's milk for agglutination of *B. melitensis*, found, to his surprise, that the control cow's milk gave a positive result. Following up this observation he noted that five out of the 13 specimens of cows' milk examined by him contained agglutinins for the organism of Malta fever.

Fabyan and Theobald Smith (1912) had shown that the tuberculous-like lesions produced by inoculating guinea-pigs with raw cow's milk were due to *B. abortus* Bang, the cause of bovine abortion. Since then other workers, notably Zwick and Krage (1913) confirmed the finding of *B. abortus* in the milk of infected cows, this excretion taking place irrespective of any lesion in the udder.

These facts remained uncorrelated until A. E. Evans (1918), in an illuminating piece of work, showed that *B. abortus* and *B. melitensis* were morphologically and serologically (agglutination) indistinguishable. Meyer, Shaw and Feusier (1920), later, corroborated Evans' views by a series of absorption tests.

It was for the purpose of further elucidating the relationship of these two organisms that the present investigation was undertaken.

The cultures used in this research were supplied by the National Collection of Type Cultures and are representative of strains isolated in America, on the Continent and in this country. In all, 13 strains of *B. melitensis*, 10 strains of *B. abortus* and 3 of *B. paramelitensis* were examined.

MORPHOLOGY AND NOMENCLATURE.

These three organisms are morphologically indistinguishable, occurring as small rods 3–5μ in length with somewhat pointed ends. They are non-motile, stain uniformly with basic stains and are gram-negative. They are, however, somewhat pleomorphic and the same strain may show bacillary, cocco-bacillary or coccoid forms from time to time or the three forms may occur together in

the same culture. The age of the culture, the culture medium, and the method of staining have apparently nothing to do with this transformation.

The term "Micrococcus" is, therefore, inexact; and, though the term "Bacterium" would be correct, it would be better still if the generic name "Brucella," in honour of Sir David Bruce who discovered the first species (*melitensis*) in 1886, were adopted as suggested by Meyer and Feusier. Thus we would have *Brucella melitensis*, *Brucella abortus* and *Brucella paramelitensis*.

Biochemical and cultural characters.

The three organisms have the following common cultural and biochemical characters. They grow very slowly and scantily on ordinary agar, preferring glucose agar, on which medium they give a good growth after 36–72 hours' incubation at 37° C. The colonies are small, circular, with a smooth margin 2–3 mm. in diameter and whitish in colour. Later the growth tends to become confluent. In trypsin broth a uniform turbidity is produced after the third day without any surface pellicle. Litmus milk becomes alkaline and is not coagulated.

There is no change (acid or gas) in Hiss' serum with lactose, saccharose, dulcite, mannite or glucose, even after two weeks' incubation.

Mode of infection.

Infection with *B. abortus* may take place during copulation with males who have previously covered infected animals or who are themselves infected and excrete bacilli in their seminal fluid. The bedding may also carry infection, becoming contaminated with the vaginal discharges, amniotic fluid or foetal membranes from infected cows. Apparently the most usual mode of infection, however, is ingestion of food infected with amniotic fluid or afterbirth, as this ensures a considerably larger quantity of virus. After an incubation of 33–230 days a catarrhal condition of the genital passages with some discharge makes its appearance, and the secretion of milk is diminished. This is followed in 3–4 days by abortion accompanied by moderate pains and mild general manifestations. The animal recovers and then either remains sterile or aborts soon after service, generally after two months. The disease is highly infectious and rapidly spreads through whole herds affecting especially young animals. The males apparently act as carriers. The causal organism can be isolated from the spleen, liver, testes, seminal vesicles and uterine discharges as well as from the milk; the foetal membranes, stomach, amniotic fluid and cotyledons as a rule give pure cultures. Isolation and disinfection are very effective measures.

In undulant (Malta) fever, infection may occur through contamination of superficial scratches or pricks, though in the great majority of cases the disease is contracted by the ingestion of infected food—mainly goat's milk. It is well to mention, however, that cases have been reported in which infection was

produced by sexual intercourse, and in this respect it is worthy of note that enlargement of the testes occurs in a great proportion of the cases. The three cardinal signs of this disease are (i) great emaciation, (ii) evening sweats, and (iii) a prolonged, irregular fever continuing for weeks or even months. The disease is not essentially fatal and the mortality is only 2–5 per cent.

In goats the disease may be present without producing any obvious clinical manifestations.

In fatal cases the organism can be recovered from the blood (10 per cent.) from the spleen, liver, enlarged mesenteric glands, kidney, urine, saliva and milk.

Thus the two diseases present the picture of a bacteraemia, with a close similarity in the modes of infection, and of excretion of the causal organism.

IMMUNOLOGY.

Anti-sera were prepared against the three organisms, *B. abortus*, *B. melitensis* and *B. paramelitensis* and the 30 selected strains tested against these sera by means of the agglutination and absorption reactions.

Rabbits were employed in the production of the sera, receiving intravenous inoculations of killed 48-hour cultures (60° C. for 30 minutes). The first dose was 1500 millions and the second 3000 millions at a week's interval. One week after the last inoculation, the serum was tested, and if found satisfactory, the animal was bled out. A satisfactory serum gave complete sedimentation of the homologous strain in a dilution of at least 1 in 6400. Tubes were incubated for 3 hours at 37° C. and read after 12 hours' standing at room temperature.

The various cultures are referred to by their number only in the tables which follow, and for sake of brevity the findings obtained with nine only of the 30 strains are recorded. The results obtained with the other 21 strains differed in no respect from those given below.

Cultures of *B. melitensis*. Nos. 78, 80 and 893.

Cultures of *B. paramelitensis*. Nos. 82 and 84.

Cultures of *B. abortus*. Nos. 624, 830, 895 and 900.

Table I.

Anti-melitensis serum (titre 1 : 6400) tested against the various strains.

	Dilution of serum								
Strain	1 : 100	1 : 200	1 : 400	1 : 800	1 : 1600	1 : 3200	1 : 6400	1 : 12800	Control
78	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +	+	–	–
80	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +	–	–
893	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +	–	–
82	+	–	–	–	–	–	–	–	–
84	+	–	–	–	–	–	–	–	–
624	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +	+ +	–	–
830	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +	+ +	–	–
895	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +	+ +	–	–
900	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +	+	–	–

Table II.

Anti-abortus serum (900) tested against the various strains.

	Dilution of serum								
Strain	1 : 100	1 : 200	1 : 400	1 : 800	1 : 1600	1 : 3200	1 : 6400	1 : 12800	Control
624	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +	+ +	–	–
830	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +	+ +	–	–
895	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +	–	–
900	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +	–	–
78	+ + +	+ + +	+ + +	+ + +	+ + +	+ +	–	–	–
80	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +	–	–	–
893	+ + +	+ + +	+ + +	+ + +	+ + +	+ +	+ +	–	–
82	+ + +	+ + +	+ + +	+ + +	+	–	–	–	–
84	+ + +	+ + +	+ + +	+ + +	+ + +	+	–	–	–

Table III.

Anti-paramelitensis serum (84) tested against the various strains.

	Dilution of strain								
Strain	1 : 100	1 : 200	1 : 400	1 : 800	1 : 1600	1 : 3200	1 : 6400	1 : 12800	Control
82	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +	+ +	–	–
84	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +	+ +	–	–
78	+ +	+	–	–	–	–	–	–	–
80	+ +	+ +	–	–	–	–	–	–	–
893	+	–	–	–	–	–	–	–	–
624	+ + +	+ +	–	–	–	–	–	–	–
830	–	–	–	–	–	–	–	–	–
895	+	–	–	–	–	–	–	–	–
900	+	–	–	–	–	–	–	–	–

It will thus be seen that agglutination alone helps us little in arriving at any valid conclusions as to the serological relationship of *B. melitensis* and *B. abortus*, and a series of absorption tests was made, therefore, to determine this point.

Meyer, Shaw and Feusier (1920), in their paper quoted above, divided the organisms of undulant fever and cattle abortion into four groups serologically, their Group IV containing *B. paramelitensis* only. The results of the following absorption tests, however, do not lend support to this method of classification and justification is therefore felt in mentioning them in some detail.

Technique of absorption used.

Antigens. The 48-hours' growth on 1 per cent. glucose agar slopes was washed off with sterile distilled water, using 0·5 c.c. for each culture.

The antisera, prepared as above were diluted 1 : 25 with salt solution (0·75 per cent.).

To the emulsion obtained from several slopes was added an equal volume of the diluted serum, giving a final dilution of 1 : 50 of the serum. The mixture was then incubated for two hours, centrifuged and the clear serum pipetted off.

The absorption was considered as satisfactory when all agglutinins for the absorbing strain had been removed.

It may be mentioned that broadly speaking, a full agar slope is practically always found sufficient to absorb all the specific agglutinins from 1 c.c. of 1 : 50 serum whose titre is 1 : 6400 (or less) in 2 hours. This obviates the subsequent absorption which is liable to become necessary if a smaller amount of organisms is used.

The technique of the agglutination experiments performed with the absorbed sera was the same as that already described earlier in this communication.

Only the more important results are recorded.

Table IV.

Anti-melitensis serum absorbed with *B. melitensis* (80) and tested against strains 80, 893, 830, 895 and 900.

	Dilution of serum				
Strain	1 : 100	1 : 200	1 : 400	1 : 800	Control
80	–	–	–	–	–
893	–	–	–	–	–
830	–	–	–	–	–
895	–	–	–	–	–
900	–	–	–	–	–

The same result was obtained after absorption with any other strain of *B. melitensis*.

Table V.

Anti-melitensis serum (80) absorbed with *B. abortus* (900) and tested against strains 80, 893, 900, 895 and 830.

	Dilution of serum				
Strain	1 : 100	1 : 200	1 : 400	1 : 800	Control
80	–	–	–	–	–
893	–	–	–	–	–
900	–	–	–	–	–
895	–	–	–	–	–
830	–	–	–	–	–

The same result was obtained after absorption with any other strain of *B. abortus*.

Table VI.

Anti-abortus serum (900) absorbed with strain 900 and tested against strains 78, 80, 893, 830, 895 and 900.

	Dilution of serum				
Strain	1 : 100	1 : 200	1 : 400	1 : 800	Control
78	–	–	–	–	–
80	–	–	–	–	–
893	–	–	–	–	–
830	–	–	–	–	–
895	–	–	–	–	–
900	–	–	–	–	–

The same results were obtained if *abortus* strains other than the homologous were employed or other anti-abortus sera used.

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Table VII.

Anti-abortus serum (900) absorbed with *B. melitensis* (80) and tested against strains 78, 80, 893, 830, 895 and 900.

	Dilution of serum				
Strain	1 : 100	1 : 200	1 : 400	1 : 800	Control
78	–	–	–	–	–
80	–	–	–	–	–
893	+ +	+	–	–	–
830	+ + +	+ + +	+ + +	+ + +	–
895	+ + +	+ + +	+ + +	+ + +	–
900	+ + +	+ + +	+ + +	+ + +	–

Other anti-abortus sera absorbed with this as well as other strains of *B. melitensis* gave similar results.

The salient features of these absorption tests can be summarised as follows:

1. When an anti-melitensis serum is absorbed with *B. melitensis* all agglutinins for *B. melitensis* and *B. abortus* are removed.

2. The same result is obtained if *B. abortus* is used to absorb an anti-melitensis serum.

3. Anti-abortus serum absorbed with any *abortus* strain loses all agglutinins for both *B. melitensis* and *B. abortus*.

4. Anti-abortus serum absorbed with *B. melitensis* has lost its power to agglutinate *B. melitensis* strains but still agglutinates *B. abortus* to full titre.

From these results it would appear that *B. melitensis* is a sub-strain of *B. abortus*, in the sense employed by Schütze (1922).

Absorption experiments on the same lines with *B. paramelitensis* have not so far been carried out.

Pathogenicity.

The close morphological, bio-chemical and serological relationship between *B. melitensis* and *B. abortus* at once raises the question of their relative pathogenicity. This becomes much the more important if we consider the fact that 25 per cent. of the milch cows in this country are infected with *B. abortus*, and this percentage is even higher on the Continent and in America. These bacilli are found even in the "certified milk." A series of animal experiments on guinea-pigs, goats and monkeys was carried out, a few of which will be mentioned.

Guinea-pig 4. Intraperitoneal inoculation of $\frac{1}{4}$-slope culture (48 hrs.) of *B. abortus* (900), 17. ii. 21. In 24 hours the temperature began to rise and reached 104° in 36 hours. The fever continued for three weeks, reaching its highest point (105° F.) at the end of the second week. The thermometer gave a higher reading in the evening (5 p.m.) than in the morning (10 a.m.). The animal looked sluggish and was disinclined to feed during the first week after injection. It was killed on March 16th.

Post-mortem. No marked congestion or exudate in peritoneum. Spleen enlarged and adherent to diaphragm with a small abscess between superior border and the diaphragm. Liver slightly congested. Kidney normal; testes and epididymis normal. Lumbar and mesenteric glands enlarged. No change was noted in heart or lungs. The chief histological finding was an increase of lymphoid tissue in the spleen.

The organism was recovered in pure culture from the spleen, liver, splenic abscess and mesenteric glands.

Guinea-pig 5. Received $\frac{1}{4}$-agar slope of *B. abortus* (strain 830).

This animal gave findings similar to those recorded in the case of Guinea-pig 4, with the exception that the temperature reached 105° F. on the third day after inoculation and that it began to drop to normal about the end of the second week.

Guinea-pig 7. Inoculated with *B. abortus* (strain 895). Similar rise in temperature, reaching 105° F. on the third day and returning to normal on the nineteenth day of the disease.

In both cases (Guinea-pigs 5 and 7) the same post mortem and histological findings were obtained. *B. abortus* was recovered from the kidney in No. 5 and from the bone marrow of No. 7. Cultural examination of the heart blood gave negative results in both cases.

Guinea-pig 8. Inoculated with *B. melitensis* (strain 893) ($\frac{1}{4}$-slope, 48 hours' culture).

The temperature in this case remained high (105–106° F.) for the first ten days, the pyrexia continuing for three and a half weeks.

Post-mortem findings similar to the above three except that splenic and hepatic congestion was more marked.

In order to measure the relative pathogenicity of the two organisms to guinea-pigs, animals of about equal weight were inoculated intraperitoneally with $\frac{1}{4}$, $\frac{1}{2}$ and 1 slope of *B. melitensis* and with 1, 2, $2\frac{1}{2}$ and 3, etc. slopes of *B. abortus*. It was found that $\frac{3}{4}$ of a slope of *B. melitensis* killed a guinea-pig of 240 grms. in 18 hours. To kill a guinea-pig of the same weight in approximately the same time $4\frac{1}{2}$ slopes of *B. abortus* were required. The amount of growth per slope in both cases was practically equal, thus showing that *B. melitensis* is about six times more virulent than *B. abortus* for the guinea-pig. The method is admittedly somewhat crude, since the number of organisms in the M.L.D. has not been determined, but it gives some idea of their comparative pathogenicity.

The following experiment on goats was then carried out:

Two goats *A* and *B* were examined for previous *melitensis* infection. The blood and urine were examined culturally and the serum tested for the presence of agglutinins for *B. melitensis* and *B. abortus*. All tests proved negative.

Goat A then received one 48 hours agar slope culture of living *B. abortus* (900) intravenously (jugular) on 25. iv. 21.

Goat B received a similar dose of *B. abortus* (895) on the same day.

The blood was examined culturally from time to time, a positive result being obtained 48 hours after the injection (only few bacilli). Later, repeated cultural examination on different occasions gave uniformly negative results. The goats showed very little general reaction. They stood the inoculation well, partook of their food as usual and in fact their general health did not apparently suffer. Urine examinations proved negative throughout.

The antibody response to this inoculation was most marked. Thus the agglutinins in the blood of Goat A for *B. abortus* (900) were:

Date	Titre
3. v. 21	1 : 400
10. v. 21	1 : 1600
17. v. 21	1 : 3200
24. v. 21	1 : 6400
4. vi. 21	1 : 6400
11. vi. 21	1 : 12800

Date	Titre
22. vi. 21	1 : 25600
29. vi. 21	1 : 25600
5. vii. 21	1 : 12800
12. vii. 21	1 : 12800
19. vii. 21	1 : 6400

The agglutination titre for *B. melitensis* was somewhat less (1 : 6400–1 : 12800) and least of all for paramelitensis strains (1 : 6400 at most).

Absorption tests carried out with this serum were rather troublesome, as in most cases at least 3 or 4 slopes were required to completely absorb $\frac{1}{2}$ c.c. of 1 : 25 dilution of the serum. However, the same results were obtained with this goat's serum as with rabbits' sera mentioned above (see Tables VI and VII).

Unfortunately the goats available on this occasion were not pregnant and, therefore, the phenomena of abortion, the excretion of bacilli in the milk, and the presence of agglutinins in this latter secretion could not be demonstrated.

Cross immunisation.

An attempt was made to find out whether previous immunisation of monkeys with *B. abortus* could ward off a subsequent melitensis infection. The result is of interest and deserves detailed mention:

1. *Macacus rhesus.* Received three doses ($\frac{1}{4}$, $\frac{1}{2}$ and $\frac{1}{2}$ slope of killed *B. abortus*) intravenously at intervals of ten days. The serum finally agglutinated *B. abortus* 1 : 6400 and *B. melitensis* 1 : 3200.

2. *Macacus sinicus.* Not previously immunised, to act as control.

Both monkeys were inoculated on July 1st with $\frac{1}{2}$-slope of *B. melitensis* intravenously (living culture).

Both stood the infection fairly well for the first 48 hours, and then changes began to appear. The Rhesus (No. 1) continued to take his food and to play as usual, whereas the Sinicus (No. 2 control) became dull, weak, lazy and was inclined to scratch his forehead and to pull some of his crown hair, as if suffering from headache.

At the end of the first week the serum of No. 1 showed a higher titre for *melitensis* (1 : 6400) and *abortus* (1 : 10000) and the blood culture was negative. The second monkey gave agglutination in 1 : 800 and the blood culture was positive (scanty growth).

The accompanying temperature charts show the febrile reaction of *M. sinicus* (No. 2 control) reaching as high as 105·5° F., whereas *M. rhesus* (No. 1) did not show any rise beyond 103·5° F., and this on one occasion only (sixth day).

As regards weight the following table shows that, whereas both monkeys lost weight immediately after the inoculation, the Rhesus monkey soon returned to normal, whereas the control monkey continued to lose weight:

	M. rhesus (No. 1)	*M. sinicus* (No. 2 control)
Before inoculation	2500 gms.	1950 gms.
3. vii. 21	2480	1910
6. vii. 21	2450	1900
9. vii. 21	2330	1800
20. vii. 21	2500	1700
1. viii. 21	2530	1560

The experiment shows quite clearly that the immunisation of Monkey No. 1 (Rhesus) with *B. abortus*, had been able to protect that monkey against an infecting dose of *B. melitensis*, which in the control non-immunised Monkey No. 2 (Sinicus) produced a quite definite febrile illness.

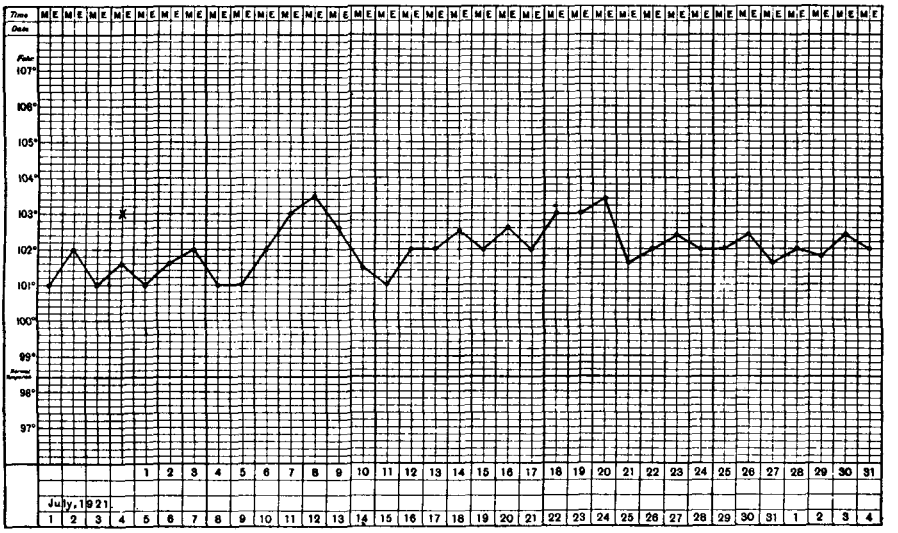

Chart 1. *M. rhesus* ♀ immunised with *B. abortus* and afterwards infected with *B. melitensis*.

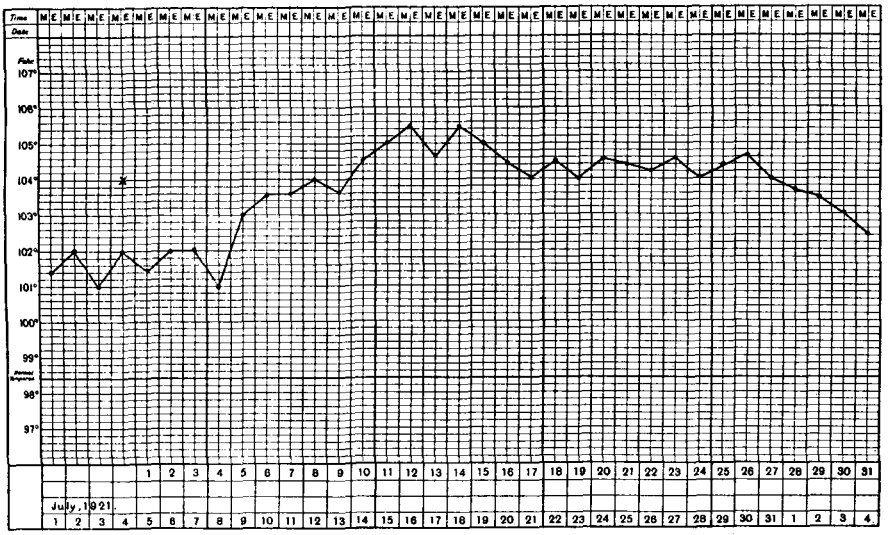

Chart 2. *M. sinicus* ♂ control (non-immunised) infected with *B. melitensis*.

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Pathogenicity to man.

Naturally the question crops up whether or not *B. abortus* being so closely related to *B. melitensis*, is capable of producing an undulant or other form of fever in man. In this connexion I repeat the words of Kennedy (1914): "I think the possibility of melitensis infection of cows in this country should not be lightly thrust aside. I have heard of two cases of undulant fever in people who have never been out of England and it is possible that there are others undiagnosed." I have myself seen cases in Egypt which have never had a chance of ingesting goat's milk and yet suffered from typical melitensis fever as confirmed by laboratory diagnosis.

Meyer and Fleichner (1920) were able to produce in monkeys an undulant-like fever with, in some cases, a fatal result, by means of *B. abortus* (feeding and inoculation).

Cooledge (1916) found anti-abortus bodies in the serum of human beings fed on milk from cows which were suffering from contagious abortion.

There is, however, the fact that undulant fever is unknown in countries where goat's milk is not an important article of food, even though contagious abortion may be widespread. This seems to me more apparent than real. The geographical distribution of undulant fever has been steadily widening since 1886 when it was first known to be a definite clinical entity with a specific organism. Before that time it was often mistaken for a transient fever, for typhoid or for early phthisis, having a very variable symptomatology. The low virulence of *B. abortus* as compared with *B. melitensis* brought out in the experiments on guinea-pigs, would indicate that a larger dose would be required to infect; but, apart from this, there is no reason why *B. abortus* should not produce a febrile condition. In this respect the possibility of the ingestion of large quantities of milk producing a passive immunity to *B. abortus* might require consideration.

Whether the two organisms are one and the same or not and whether the lowered virulence and the different behaviour of *B. abortus* in the absorption tests are produced by passage through cows, I am not ready, at present, to say. It may be that *B. abortus* bears the same relation to *B. melitensis* as cow-pox to small-pox. The fact that cross immunisation of monkeys is successful seems to enhance this supposition, but it is, of course, inadvisable to draw conclusions from a single experiment.

SUMMARY.

1. Morphologically *B. abortus* and *B. melitensis* are identical. The "coccoid" form is not a constant feature and a more satisfactory generic name would be "Brucella."

2. The organisms cannot be differentiated by cultural, bio-chemical, or staining methods, or by the agglutination reaction.

3. From absorption experiments, it would appear that *B. melitensis* is a sub-strain of *B. abortus*.

4. Dose for dose *B. abortus* is much less virulent for the guinea-pig than *B. melitensis*, approximately about 1 : 6.

5. Immunisation of monkeys (one experiment only) with killed suspensions of *B. abortus* protected against subsequent infection with *B. melitensis*.

In conclusion I wish to thank Professor Ledingham for much valuable advice throughout the investigation, and Dr R. St John Brooks for supplying me with cultures from the National Collection.

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Only works to which I have referred in the text of the paper are included in this list.

The Reports of the Royal Commission for the study of Mediterranean Fever (1905–1907) as well as the reports of the Departmental Committee of the Board of Agriculture and Fisheries on Epizootic abortion (1909–1910) contain a great deal of useful information.