FURTHER STUDIES ON BACTERIUM ABORTUS AND RELATED BACTERIA

II. A COMPARISON OF BACTERIUM ABORTUS WITH BACTERIUM BRONCHISEPTICUS AND WITH THE ORGANISM WHICH CAUSES MALTA FEVER

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The similarity between Bacterium bronchisepticus and Bacterium abortus in their growth on potato, and in their inability to attack sugars has been pointed out by Smith.[1] So far as the writer is aware, no further attention has been called to the similarity between the two organisms. From a biologic standpoint the resemblance is of interest; and since, as McGowan[2] has shown, Bact. bronchisepticus causes epizootics among laboratory animals, and during an epizootic may inhabit the organs of normal animals, the resemblance between the two organisms has a practical significance in the possibility of their confusion when isolated from experimental animals.

Certain facts as stated in the literature concerning another pathogenic organism — that causing Malta fever — suggested that a comparison with Bact. abortus might also be of interest. The Malta fever organism was discovered by Bruce.[3] He examined stained smears from the spleen of human subjects in fatal cases, and in every case found minute organisms which he called micrococci. These were later proved to be the causal organism, and the name Micrococcus melitensis was given. In fresh material obtained from infected individuals this organism is said to appear invariably in the coccus form, but under artificial cultivation bacillary forms are reported to be common, and these have been regarded as involution forms. Some of the most recent textbooks, however, have called the organism Bacillus melitensis. The descriptions of the Malta fever organism suggested a similar morphology to that of Bact. abortus which is described as a

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[1] Jour. Med. Research, 1913, 29, p. 291.
[2] Jour. Path. and Bacteriol., 1911, 15, p. 373.
[3] Practitioner, London, 1887, 39, p. 161.

"cocco bacillus," with the coccoid forms occurring commonly in pathologic material, and with distinct bacillary forms commonly found in cultures.

The habitats of Bact. abortus and the Malta fever organism also are very much the same. An extensive investigation was made by the British Commission on Mediterranean Fever,[4] which reported that the disease was propagated by goats. The English investigators were able to cultivate "Micrococcus melitensis" from the milk of 10% of the goats supplying milk to various parts of Malta. Judged by the serum reactions, they concluded that 41% of the goats on that island were infected. They were able to infect monkeys and goats by feeding with cultures isolated from the milk, or by feeding the infected milk itself. Schroeder and Cotton[5] found by inoculating cows' milk into guinea-pigs that about 14% of the samples tested were infected with Bact. abortus. In a recent publication Fleichner and Meyer[6] report that as a result of inoculating guinea-pigs with certified milk they conclude that "Bact. abortus is, for all practical purposes, always present in the certified milk produced in the San Francisco Bay regions." Thus it has been shown that the Malta fever organism is a common infection of goats' milk on the Island of Malta, and Bact. abortus is a common infection of cow's milk in this country.

The three organisms, Bact. abortus, Bact. bronchisepticus, and the Malta fever organism, will be described in detail in order that they may be compared.

BACTERIUM ABORTUS

A large number of strains of Bact. abortus which served for this study were obtained from the Pathological Division of this bureau. They had originally been isolated from pathologic material. A few of the strains were isolated from milk. Only typical pathogenic strains were considered. The criterion for judging a culture as typical was agglutination in a high dilution of Bact. abortus immune serum. Only strains which reacted positive to this test in a 1:1,280 dilution or higher of the serums which served for this study were considered. All strains accepted as typical according to this test agreed in practically all cultural and biochemical tests. The author is indebted to Dr. John M. Buck of the Pathological Division of this Bureau for the inoculation of the animals and the postmortem examinations reported in this paper, and for the antiserums used in the agglutination tests.

Morphology.—Bact. abortus is a short, slender, pleomorphic rod with rounded ends, whose form is influenced by the medium in which it was grown. The cells are sometimes so short as to appear coccoid. The width is about 0.5 mikrons,

[4] Reports of the Commission on Mediterranean Fever, 1906, London.

[5] Twenty-eighth annual report of the Bureau of Animal Industry, Department of Agriculture, 1911, p. 139.

[6] Am. Jour. Dis. Child., 1917, 14, p. 157.

the length varies from this dimension to 2 mikrons. (Mohler and Traum[7] report that it may be as long as 3 mikrons.) The morphology of the organism from the condensation water of a 24-hour culture on agar slope is given in Figure 1. Bact. abortus is nonmotile. It does not form spores.

Staining.—Bact. abortus is readily stained with the ordinary dyes, but it is negative to Gram's stain.

Cultural Characteristics.—The first growth of a strain transferred from pathologic material to artificial media is often difficult to obtain. Growth is favored by incubation in a closed jar with a culture of Bacillus subtilis. Glycerol agar or serum agar serves well for such a strain, but after the strain has become accustomed to artificial conditions growth is abundant on all the ordinary media. An infusion agar slope culture of a readily growing strain shows an opalescent growth after 24 hours' incubation, which becomes heavier during the next day or two. It is a lustrous, moist growth with a sharply defined margin. Crystals begin to form in the agar after 5 or 6 days' incubation.

On agar plates after 2 days' incubation colonies like tiny dew drops appear on the surface of the agar. They gradually become opaque as they continue to increase in size during 10 or 12 days' incubation when finally the largest colonies attain a diameter of about 6 mm. In the depths of the agar there are two kinds of colonies; small, bluish-white, circular colonies about ⅓ mm. in diameter, and opaque, lemon-shaped colonies about ⅓ mm. long.

In agar shake cultures there is an abundant surface growth, but no growth beneath the surface. The agar just beneath the surface growth is rendered white and opaque.

In broth cultures a faint clouding is visible after 1 day's incubation. During the next day or two the clouding becomes heavier, but the broth never becomes heavily clouded. There is no surface ring or pellicle. After several days a sediment begins to precipitate.

In litmus milk the only change is a slight alkalinity apparent after several days' incubation; this reaction never becomes pronounced.

On potato there appears a slight glistening growth of a brownish color. After several days' incubation the potato itself takes on a brownish tinge.

Biochemical Reactions.—Bact. abortus does not attack the sugars nor any of the other commonly used fermentable test substances. In broth cultures there is a reduction of the hydrogen-ion concentration equal to about 0.7 or 0.8 P_H. This reaction is fairly definite and characteristic. The initial hydrogen-ion concentration of the broth may vary over quite a wide range in either direction from the neutral point without affecting the results. Both urea and asparagin are decomposed with the production of ammonia, but the reaction in asparagin medium is often slight. In some cultures there is a slight reduction of nitrates to nitrites; other cultures show no reduction. Indol is not produced in tryptophan medium. Gelatin is not liquefied. The biochemical reactions of two of the strains are given in Table 1. Strain w1 was from an aborted fetus. Strain aap was from milk.

BACTERIUM BRONCHISEPTICUS

Twenty-three strains of Bact. bronchisepticus have come under observation. Twenty-one of the strains were isolated from infected guinea-pigs. One strain obtained from the Pathological Division was isolated from the lung of a dog sick with distemper, and one strain was obtained from Dr. Theobald Smith. All of the 23 strains gave the same reactions to the cultural and biochemical tests.

[7] Twenty-eighth annual report of the Bureau of Animal Industry, U. S. Department of Agriculture, 1911, p. 147.

Morphology.—Bact. bronchisepticus is also a short, slender, pleomorphic rod with rounded ends, whose form is influenced by the medium in which it grows. In the tissues, and sometimes in artificial mediums, it has more or less of a coccal form. McGowan[2] reports that the width is 0.4-0.5 mikrons, and that the length varies from 0.5-2.3 mikrons. The morphology of the organism from the condensation water of agar slope is shown in Figure 2. In the stained smear it cannot be distinguished from Bact. abortus, but Bact. bronchisepticus is motile. No spores are formed.

Staining.—The organism is readily stained with ordinary dyes, but is decolorized by Gram's stain.

Cultural Characteristics.—Bact. bronchisepticus is easily cultivated from infected tissues. McGowan[2] thus describes its growth in agar plate cultures: "After 24 hours, all that is seen is a number of discrete dew points. These enlarge very rapidly during the next 24 hours, becoming as large as pinheads. They are raised above the surface, are regular hemispheres in shape, and have an

TABLE 1

A COMPARISON OF THE BIOCHEMICAL REACTIONS OF BACT. ABORTUS WITH THOSE OF BACT. BRONCHISEPTICUS AND BACT. MELITENSIS

Species	Strain	Reaction in Litmus Whole Milk	Fermentation of				
			Dextrose	Lactose	Saccharose	Maltose	Mannite
Bact. bronchisepticus....	vl	Decidedly alkaline	—	—	—	—	—
Bact. bronchisepticus....	wy	Decidedly alkaline	—	—	—	—	—
Bact. abortus.....	wl	Faintly alkaline	—	—	—	—	—
Bact. abortus.....	aap	Faintly alkaline	—	—	—	—	—
Bact. melitensis.....	yf	Faintly alkaline	—	—	—	—	—
Bact. melitensis.....	aay	Faintly alkaline	—	—	—	—	—

opaque, white, porcelainous look, with an opalescent sheen." The growth is more rapid than that of Bact. abortus, and after several days' incubation the largest colonies attain a diameter of about 8 mm., which is somewhat larger than the largest colonies of Bact. abortus. Otherwise the agar plate cultures of the two organisms cannot be distinguished. Likewise on agar slopes the cultures of Bact. bronchisepticus grow more rapidly, so that at the end of 24 hours their growth is heavier than that of Bact. abortus. Afterward the cultures of the two organisms on agar slope cannot be distinguished. Crystals appear in the agar after several days' incubation, just as they do in agar cultures of Bact. abortus. In agar shake cultures the growth of Bact. bronchisepticus is identical with that of Bact. abortus.

In broth cultures Bact. bronchisepticus can be distinguished from Bact. abortus by a heavier clouding of the medium. This difference is apparent in 24-hour cultures, as well as in cultures of several days' incubation. The Bact. bronchisepticus cultures can also be distinguished by a faint, broken film which covers part of the surface.

In litmus whole milk Bact. bronchisepticus can be distinguished from Bact. abortus by a decidedly greater alkalinity of the medium. The alkalinity appears on the surface of the cream layer in a 24-hour culture. The milk beneath the cream layer becomes alkaline slowly, but in a week-old culture the reaction is pronounced.

On potato there is a glistening growth of a brownish color, somewhat heavier than the growth of Bact. abortus.

Biochemical Reactions.—Like Bact. abortus, Bact. bronchisepticus does not attack the sugars nor the other commonly used fermentable test substances. In broth cultures there is a reduction of the hydrogen-ion concentration equal to about 2.0 P_H. Compared with Bact. abortus the more intense alkaline reaction of Bact. bronchisepticus is a definite point of distinction. But the two organisms cannot be distinguished by their other biochemical reactions. Bact. bronchisepticus decomposes urea and asparagin with the production of ammonia. The majority of strains do not reduce nitrates to nitrites; there is no production of

TABLE 1—*Continued*

A COMPARISON OF THE BIOCHEMICAL REACTIONS OF BACT. ABORTUS WITH THOSE OF BACT. BRONCHISEPTICUS AND BACT. MELITENSIS

Decomposition of		Reaction in			Reduction of Hydrogen-ion Concentration in Broth Cultures Recorded in PH Values
Urea	Asparagin	Nitrate Broth	Gelatin	Tryptophan Medium	
+	+	—	—	—	1.9
+	+	—	—	—	2.2
+	+	—	—	—	0.8
+	+	Slight	—	—	0.7
+	Faint	Slight	—	—	0.7
+	Faint	—	—	—	0.8

indol in tryptophan medium; gelatin is not liquefied. In Table 1 the biochemical reactions of Bact. bronchisepticus can be compared with those of Bact. abortus.

Agglutination Reactions.—The serum used for the agglutination tests was obtained from a cow which had been inoculated with Bact. abortus many times by the investigators of the Pathological Division for the purposes of another experiment. Her serum agglutinated Bact. abortus suspensions in higher dilutions than that of naturally infected cows. Bact. bronchisepticus was agglutinated in low dilutions of this antiserum. In Table 2 the reactions of 6 strains are given, together with the reactions of typical strains of Bact. abortus. Strain yc was the only one of the collection which failed to give an agglutination reaction in low dilutions of the serum. This strain was the one which was obtained from Dr. Theobald Smith. Morphologically, culturally, and biochemically it was identical with our strains. Strain xb, which was isolated from the lung of a dog sick with distemper reacted in the same manner as the strains isolated from guinea-pig organs. The agglutination of Bact. bronchisepticus in dilutions of 1:40 or 1:80 of this Bact. abortus antiserum readily distinguishes that organism from Bact. abortus, which was agglutinated in dilutions of 1:1,280 or higher of the same serum.

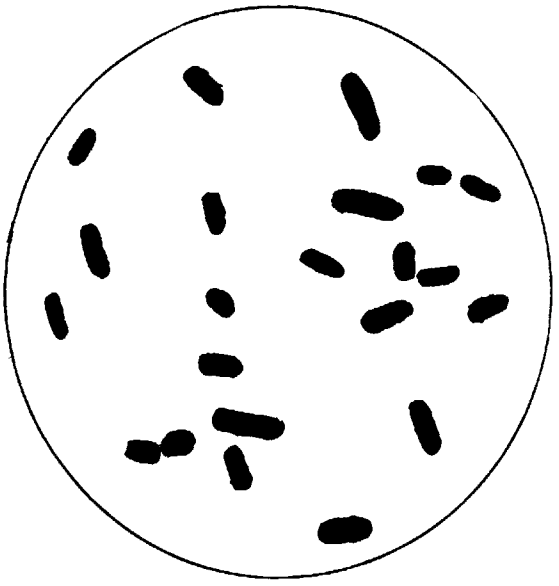

Fig. 1.—Bact. abortus.

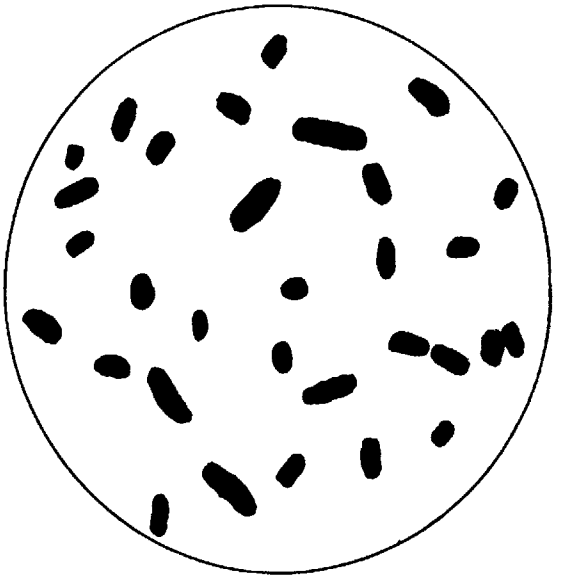

Fig. 2.—Bact. bronchisepticus.

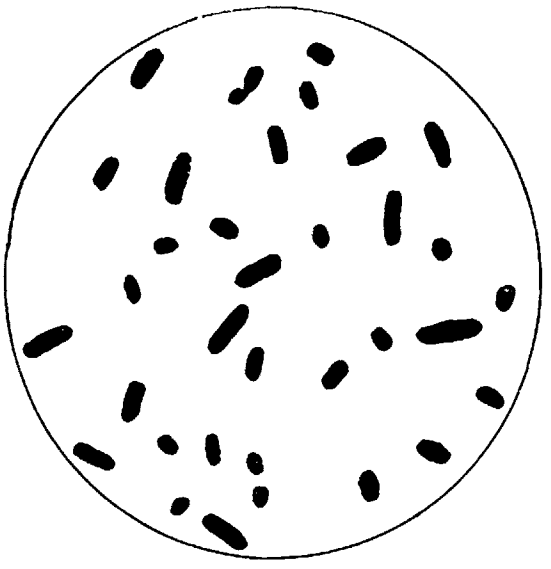

Fig. 3.—Bact. melitensis.

The drawings in Figures 1, 2, and 3 were all made with the aid of a camera lucida from smears of the condensation water from agar slopes after 24-hours' incubation. The smears were stained according to the Gram method without decolorization. × 4,800.

BACTERIUM MELITENSIS

Six strains of the Malta fever organism were available for this study. Strain yf was a strain marked "Micrococcus melitensis (33)" obtained from the American Museum of Natural History. It had been isolated from the arm vein of a human subject in London in 1896. The remaining 5 strains were obtained from the Pathological Division. Their history so far as it could be traced was as follows: Strain aax, marked "M. melitensis Dr. Annett," was obtained in December, 1907, from England; Strain aay, which was marked "M. melitensis Stock 22," and Strain aba, which was marked "M. melitensis Stock," were both received from the Pasteur Institute at Paris some time prior to 1900; Strain aaz, marked "M. melitensis R. A. M. C.," was obtained from the Royal Army Medical College of London, England, in January, 1908; Strain abb, marked "M. melitensis U. S. N.," was obtained from the United States Naval Medical School in March, 1909. Thus all the 6 strains had been cultivated for several years—Strain yf had been cultivated for 21 years—before this study was made. Unfortunately the histories of 5 of the strains gave no information as to whether they were isolated from human subjects or from infected animals. But since some of them were isolated in England, some in France, and one in the United States, and the dates of isolation were distributed throughout a period of a number of years, they may be considered as good representatives of the species. The 6 strains responded alike to all the cultural, biochemical, and serologic tests.

Morphology.—The Malta fever organism like Bact. abortus and Bact. bronchisepticus, is a short, slender rod with rounded ends varying in form according to the conditions under which it was grown. The camera lucida drawings shown in Figure 3 demonstrate that it is unquestionably a rod form, with morphology identical with Bact. abortus. Hence, it should bear the generic name Bacterium. It has a width of about 0.5 mikrons. The longest rod in the drawing is about 1.8 mikrons in length. Bact. melitensis is nonmotile and does not form spores.

Staining.—The organism is easily stained with the ordinary dyes and is decolorized by Gram's method of staining.

Cultural Characteristics.—Bruce[8] states that the first generation on agar slopes required 68 hours at 37 C. to make its appearance. The growth of the old strains on agar slopes, in agar plates, and in agar shake cultures could not be distinguished from the growth of Bact. abortus until after about 7 days' incubation, when the cultures of Bact. melitensis were noticeably browner. The bacterial mass itself was darker and the agar began to take on a brownish tinge which intensified with continued cultivation. Crystals appeared in the agar as in the case of Bact. abortus and Bact. bronchisepticus. The growth on potato also could not be distinguished from Bact. abortus until after about a week's incubation, when it became browner and the potato itself became browner.

Cultures of Bact. melitensis in broth and in whole milk could not be distinguished from cultures of Bact. abortus.

Biochemical Reactions.—Bact. melitensis responded to the biochemical tests exactly in the same manner as Bact. abortus. The identity of the reactions may be compared in Table 1. Most striking is the identity of the reaction to the one quantitative test—the change of hydrogen-ion concentration in broth cultures. Both the Bact. abortus and the Bact. melitensis gave a reduction of the hydrogen-ion concentration equal to about 0.7 or 0.8 P_H.

Agglutination Reactions.—The results obtained by comparing Bact. melitensis with Bact. abortus in respect to agglutination reactions were most surprising. The data are presented in Table 2. The Bact. abortus antiserum was the same as was used for the agglutination of Bact. bronchisepticus. Four of the 6 strains

of Bact. abortus included in Table 2 were partially agglutinated in a dilution of 1:1,280 with complete agglutination in lower dilutions. The other 2 strains of Bact. abortus were partially agglutinated in the 1:2,560 dilution. Four of the 6 strains of Bact. melitensis were partially agglutinated in the 1:1,280 dilution. The other 2 strains were partially agglutinated in the next higher dilution of 1:2,560, with a complete agglutination in the 1:1,280 dilution. Therefore, Bact. abortus and Bact. melitensis were agglutinated by the Bact. abortus antiserum in exactly the same dilutions.

Thus the comparative study showed Bact. abortus and Bact. melitensis to be identical in morphology, in biochemical reactions, and in their reactions in Bact. abortus antiserum. The only distinction found

TABLE 2

AGGLUTINATION REACTIONS IN BACT. ABORTUS ANTISERUM

Species	Name of Strain	Bact. Abortus Antiserum									Normal Cow Serum	
		1:20	1:40	1:80	1:160	1:320	1:640	1:1,280	1:2,560	1:5,120	1:20	1:40
Bact. bronchisepticus..	vl	+	+	0	0	0					0	0
Bact. bronchisepticus..	xb	+	+	0	0	0					0	0
Bact. bronchisepticus..	vp	+	+	+	0	0					0	0
Bact. bronchisepticus..	wn	+	+	+	0	0					0	0
Bact. bronchisepticus..	ww	+	+	+	0	0					0	0
Bact. bronchisepticus..	yc	0	0	0	0	0					0	0
Bact. abortus.....	tq		C*	C	C	C	C	+	0	0	0	0
Bact. abortus.....	wh		C	C	C	C	C	C	+	0	0	0
Bact. abortus.....	wk		C	C	C	C	C	C	+	0	0	0
Bact. abortus.....	wl		C	C	C	C	C	+	0	0	0	0
Bact. abortus.....	aap		C	C	C	C	C	+	0	0	0	0
Bact. abortus.....	wt		C	C	C	C	C	+	0	0	0	0
Bact. melitensis.....	yf		C	C	C	C	C	C	+	0	0	0
Bact. melitensis.....	aax		C	C	C	C	C	+	0	0	0	0
Bact. melitensis.....	aay		C	C	C	C	C	+	0	0	0	0
Bact. melitensis.....	aaz		C	C	C	C	C	+	0	0	0	0
Bact. melitensis.....	aba		C	C	C	C	C	+	0	0	0	0
Bact. melitensis.....	abb		C	C	C	C	C	C	+	0	0	0

* Complete clumping.

in their cultural characteristics was the more intense brown pigmentation of the Bact. melitensis. The next point to be determined was the comparative pathogenic action of the two organisms on guinea-pigs. Four pregnant guinea-pigs were inoculated with a strain of Bact. abortus freshly isolated from an aborted fetus. The inoculated bacteria were from the first growth on agar slope. At the same time 4 pregnant guinea-pigs were inoculated with Bact. melitensis. The strain was yf, which had been isolated from the arm vein of a human subject in London 21 years ago. Within a few days 3 of the animals of each group aborted. Five days after the inoculations were made, one animal from each group was killed and agar slope cultures were made from the organs. In 3 or 4 days the characteristic "dew drop"

colonies made their appearance on both sets of slopes inoculated from the liver, spleen and uterus. No distinction could be found between the growth of the two organisms until the slopes had been incubated for several weeks, when the melitensis slopes showed a more intense brown coloring.

Thirteen days after the inoculations were made one guinea-pig from each group was killed and the serum was obtained for agglutination tests. The results are given in Table 3. The results obtained with the Bact. abortus antiserum from the guinea-pig were the same as with the cow's antiserum. Three of the four tested strains of each species were agglutinated in the 1:2,560 dilution, and the other strain of each species was agglutinated in the next lower dilution of 1:1,280. The reaction of the Bact. melitensis antiserum was less uniform. The

TABLE 3

AGGLUTINATION REACTIONS OF BACT. ABORTUS AND BACT. MELITENSIS IN HOMOLOGOUS AND HETEROLOGOUS SERUMS

Suspension	Strain	Bact. Abortus Antiserum							
		1:40	1:80	1:160	1:320	1:640	1:1,280	1:2,560	1:5,120
Bact. abortus.....	tq	C	C	C	C	C	C	+	0
Bact. abortus.....	wh	C	C	C	C	C	+	0	0
Bact. abortus.....	wk	C	C	C	C	C	C	++	0
Bact. abortus.....	aap	C	C	C	C	C	++	+	0
Bact. melitensis.....	yf	C	C	C	C	C	C	++	0
Bact. melitensis.....	aay	C	C	C	C	C	++	+	0
Bact. melitensis.....	aaz	C	C	C	C	C	C	++	
Bact. melitensis.....	abb	C	C	C	C	++	+	0	0

Bact. abortus suspensions were agglutinated in the 1:320 or 1:640 dilutions, whereas the Bact. melitensis suspensions were agglutinated in considerably higher dilutions. Two strains were agglutinated in the 1:1,280 dilution; one strain in the 1:2,560 dilution; and yf, which was the homologous strain, was agglutinated in the highest dilution of 1:5,120.

Absorption Tests. — The agglutination tests showed that Bact. abortus antiserum reacts in the same manner on suspensions of Bact. abortus and Bact. melitensis, but that Bact. melitensis antiserum reacts in a different manner on the 2 suspensions. The absorption test was, therefore, applied to the 2 antiserums and their antigens, to demonstrate more clearly the relationship between the 2 species of organisms. The results obtained with the 2 antiserums are given in Table 4. The A series shows the original agglutination reaction of the antiserums,

and the B series shows the reaction of the same antiserums after having been absorbed by the suspensions of the A series. The reactions with abortus antiserum show only one slight distinction between the 2 organisms. It is remarkable that Series Ib and IVb reacted alike to abortus antiserum. It is also remarkable that Series IIb and IIIb reacted alike to this antiserum, but that agglutination took place in higher dilutions than in the Ib and IVb series, showing that the absorbed serum reacts in somewhat higher dilutions on the alternate suspension, whichever suspension may have been first absorbed in Series A. But this is an exceedingly fine point of distinction which would be useless in diagnostic work. The conclusion must be drawn that for practical purposes Bact. abortus antiserum cannot differeniate Bact. abortus from Bact. melitensis.

TABLE 3—*Continued*

AGGLUTINATION REACTIONS OF BACT. ABORTUS AND BACT. MELITENSIS IN HOMOLOGOUS AND HETEROLOGOUS SERUMS

Bact. Melitensis Antiserum							
1:40	1:80	1:160	1:320	1:640	1:1,280	1:2,560	1:5,120
C	C	C	C	++	0	0	0
C	C	C	+	0	0	0	0
C	C	C	++	+	0	0	0
C	C	C	+	0	0	0	
C	C	C	C	C	C	C	+
C	C	C	C	++	+	0	0
C	C	C	C	C	++	+	0
C	C	C	C	++	+	0	0

But the agglutination reactions with the Bact. melitensis antiserum indicate that the abortus and melitensis antigens are not identical. Series IVa and b show that the melitensis antiserum acts on the melitensis antigen in the same manner that abortus antiserum acts on the suspensions of both species. Series Ia and b show that melitensis antiserum acts on abortus suspensions in characteristically lower dilutions. Series IIIa and b show that even in low dilutions of the melitensis antiserum the melitensis antigen absorbs all agglutinins which are active toward abortus suspensions. Series IIa and b show that after having been saturated with Bact. abortus the melitensis antiserum reacts in the same manner on the melitensis antigen as it would react without having been thus saturated.

The results of the absorption tests can be explained by assuming that both the abortus and the melitensis antiserums contain more than

one agglutinin, that the agglutinins in the two antiserums are alike in kind, but differ in proportion; and that the corresponding agglutinable substances are present in the bodies of the two species of bacteria in different proportions.

PATHOGENIC ACTION OF BACT. ABORTUS, BACT. BRONCHISEPTICUS, AND BACT. MELITENSIS

A study of the literature shows that there is a similarity between the three organisms under consideration in their choice of location in the animal body.

Bact. abortus takes its name from the most pronounced symptom—the abortion of the fetus, which it produces in cattle. It is known

TABLE 4

ABSORPTION TESTS WITH BACT. ABORTUS AND BACT. MELITENSIS ANTISERUMS

No.	Series	Suspension	Bact. Abortus Antiserum						
			1:80	1:160	1:320	1:640	1:1,280	1:2,560	1:5,120
I	A	Bact. abortus.....	C	C	C	C	C	+	0
	B	Bact. abortus.....	C	C	++	0	0	0	
II	A	Bact. abortus.....	C	C	C	C	C	+	0
	B	Bact. melitensis.....	C	C	C	C	++	0	
III	A	Bact. melitensis.....	C	C	C	C	C	+	0
	B	Bact. abortus.....	C	C	C	++	+	0	
IV	A	Bact. melitensis.....	C	C	C	C	C	++	0
	B	Bact. melitensis.....	C	C	+	0	0	0	

to lead a passive existence in the udders of normal cows which eliminate the organism in their milk. Other organs are affected in other species of animals. Fabyan[8] has shown that in 58 guinea-pigs inoculated with Bact. abortus the spleen was enlarged in 98% of cases; the lymph nodes were enlarged in 95%; the liver was diseased in 75% and lesions were noted in the lungs in 65% of cases. Other organs also were infected in less than 40% of cases, but no lesions of the muscles, heart and digestive tract were found.

Mohler and Eichhorn[9] state: "The most important symptom which is observed among goats affected with Malta fever is the frequency of abortions which result in the course of the disease." Bact. melitensis, like Bact. abortus may lead a passive existence in the bodies of healthy animals. The British Commission[4] found that it was

[8] Jour. Med. Research, 1912, 26, p. 441.

[9] Twenty-eighth Annual Report of the Bureau of Animal Industry, U. S. Dept. of Agriculture, 1911, p. 119.

eliminated in the milk of healthy goats. This Commission made post-mortem examinations of 13 fatal human cases. They isolated the organism from the spleen, liver, and lymphatic glands of 100% of cases in which these organs were examined, and from the kidney of 85% of the cases in which this organ was examined. Mohler and Hart[10] state that in several cases of infected goats the spleen was enlarged, the liver engorged, the kidneys inflamed, and the lymph glands swollen. They also report that in 3 reacting goats the lungs were found to be congested along the borders, and occasionally pneumonic consolidation was present. The British Commission noted that infected goats may suffer from a short hacking cough, indicating an affection of the respiratory tract.

TABLE 4—*Continued*

ABSORPTION TESTS WITH BACT. ABORTUS AND BACT. MELITENSIS ANTISERUMS

Bact. Melitensis Antiserum						
1:80	1:160	1:320	1:640	1:1,280	1:2,560	1:5,120
C +	C +	+ 0	+ 0	0 0	0	0
C C	C C	+ C	+ C	0 C	0 ++	0 0
C 0	C 0	C 0	C 0	C 0	C 0	+ 0
C C	C C	C ++	C +	C 0	C 0	+ 0

Bact. bronchisepticus causes primarily diseases of the respiratory tract. It is easily cultivated from lung lesions of diseased guinea-pigs in which it is present in very great numbers. It may also be cultivated from the trachea of healthy guinea-pigs. Smith[1] found the organism occasionally in the uterine horns of guinea-pigs, and then chiefly in association with dead embryos. In our own investigation Bact. bronchisepticus has been isolated from the spleen, liver, heart blood, kidneys and uterus of infected guinea-pigs, as well as from the lungs and trachea.

Considered together, the 3 organisms under discussion may be said to have a predilection for glandular tissue, the respiratory tract and the uterus, with the primary focus of the disease differing with the species of animal infected with the infecting organism. All 3 organisms may lead a passive existence in infected animals.

[10] Twenty-fifth Annual Report of the Bureau of Animal Industry, U. S. Dept. of Agriculture, 1908, p. 279.

DISCUSSION

It is only with great difficulty that Bact. melitensis can be distinguished from Bact. abortus. They are alike morphologically, and no difference could be found in their biochemical reactions. The 2 organisms produced the same results when inoculated into pregnant guinea-pigs. The only distinction between the 2 organisms in cultural characteristics was a more intense brown pigmentation by Bact. melitensis — an insignificant characteristic, which does not appear until after the cultures have been incubated for a week or more. This distinction can be made only when cultures of the 2 species which have been incubated for the same length of time can be compared. The agglutination reactions in Bact. abortus antiserum do not distinguish the two organisms; and the agglutination reactions in Bact. melitensis antiserum can distinguish Bact. abortus from Bact. melitensis only when the agglutinating strength of the serum for both species is known.

The fact that Bact. abortus and Bact. melitensis are serologically so closely related explains Kennedy's[11] discovery that the milk and the blood serum of a considerable percentage of cows in London contained agglutinins for the Malta fever organism in high dilution. This author was unable to explain his findings, but suggested that agglutination of the Malta fever organism by cows' milk was not necessarily specific, or else that the reaction was indicative of infection with the organism in question — the later alternative being an explanation too alarming to be acceptable, although he states that he has heard of two cases of undulant fever in people who have never been out of England, and he thinks it possible that there are other cases undiagnosed.

The very close relationship between Bact. abortus and an organism pathogenic to human beings adds a new interest to the question of the possible pathogenicity of Bact. abortus to human subjects. Considering the close relationship between the two organisms, and the reported frequency of virulent strains of Bact. abortus in cows' milk, it would seem remarkable that we do not have a disease resembling Malta fever prevalent in this country. A possible explanation can be offered. The data presented in the third paper of this series indicates that although there may be numerous abortus-like bacteria in the milk of cows which have aborted, the actual number of virulent bacteria which persist in the milk is not great, or in all probability it is negligible in many cases in which the milk and blood serum contain agglutinins. But the work

[11] Jour. of the Royal Army Med. Corps, 1914, 22, p. 9.

of the British Commission indicates that Bact. melitensis is very abundant in the milk of infected goats, for those investigators were able by cultural methods to demonstrate the organism in the milk of 10% of the goats of Malta. Since infection is dependent on the amount of infectious material, it may be that this difference in the number of bacteria in the milk of the two species of animals may account for our freedom from disease when cow's milk containing Bact. abortus is consumed. On the other hand, are we sure that cases of glandular disease, or cases of abortion, or possibly diseases of the respiratory tract, may not sometimes occur among human subjects in this country as a result of drinking raw cows' milk? It is certain that the agglutination tests, which have been relied on for the diagnosis of Malta fever, have not proved per se whether the infections were due to Bact. melitensis or Bact. abortus.

SUMMARY

Bact. abortus and Bact. bronchisepticus are related. They resemble each other morphologically, culturaly, biochemically, and serologically. Bact. bronchisepticus can be easily distinguished from Bact. abortus, however, by its motility, by its more rapid and abundant growth in all artificial media, by its more intense alkaline reactions, and by its agglutination in Bact. abortus immune serum only in low dilutions.

The organism which causes Malta fever is unquestionably a rod form and should be called Bact. melitensis.

Bact. melitensis is very closely related to Bact. abortus. The only test which has been found to distinguish these two organisms is the agglutination of Bact. melitensis suspensions in higher dilutions of melitensis serum than will agglutinate suspensions of Bact. abortus.

The agglutination tests as they have been used to diagnose infections of Bact. melitensis in goats and human subjects cannot be relied on to distinguish one infection from the other.