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This issue completes Volume 9

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BRUCELLA ABORTUS SHEDDER CONDITIONS IN TWENTY COWS[1,2]

B. S. HENRY,[3] C. M. HARING,[4] AND J. TRAUM[5]

SINCE SCHROEDER AND COTTON,[1] and Smith and Fabyan[2] first demonstrated the presence of *Brucella abortus* in the milk of cows, there have been numerous reports of investigations carried on to determine the number of *Br. abortus* organisms excreted, the proportion of infected animals which eliminate the organism in their milk, the duration of this shedder condition, and its relation to blood-serum and whey tests. In most cases these reports have been based upon single tests of a large or small number of infected animals, and as would be expected the results have been exceedingly variable.

Fitch and Lubbehusen[3] found that 29.1 per cent of the cattle which were positive to the agglutination test were shedders of *Brucella abortus* in their milk, but that none of these organisms were found in the milk of animals whose blood-serum titers were less than 1–100 at the time of the test.

Results comparable with those of Fitch and Lubbehusen were obtained by Sheather,[4] who found that 34 per cent of the positive animals were shedders of *Brucella abortus* in their milk, but that 14 per cent of the samples of milk containing the organism gave negative results to the whey agglutination test.

In a group of cows with blood titers of 1–200 or over, Schroeder and Cotton[5] found that 83.3 per cent shed *Brucella abortus* in their milk. In a more recent study, Mitchell and Humphreys[6] reported 75 per cent of the animals in an infected herd to be shedders. However, if those animals in the herd whose blood titers were less than 1–100 were omitted, the percentage of reacting animals which were shedders would have been about 83. These last figures agree rather well with those obtained at this station, as shown later (page 551).

Pröscholdt[7] has stated that only 3 per cent of cows with blood-serum titers less than 1–100 eliminate *Brucella abortus* in the milk, but that cows with whey titers of 1–80 or higher almost certainly are shedders. He states that a whey titer of 1–10 to 1–40 is less certain evidence of infection.

Viridén[8] reports 50 per cent shedders among cows having a blood titer of 1–50, and 56 per cent among cows with a titer of 1–70.

Gwatkin[9] found 52 shedders among 102 cows showing blood-serum titers of 1–100 or greater. *Brucella abortus* was isolated from 3 out of 10 cows whose blood serums gave complete agglutination at the 1–50 dilution. One of these cows aborted and the agglutination titer never exceeded 1–50. What appeared to be a "vaccinal strain" of *Br. abortus* was isolated from the milk of a cow, the blood serum of which gave negative agglutination tests.

McNutt and Walsh[10] have described a cow that apparently was infected with *Brucella* for 21 months before she reacted, and a heifer which evidently carried infection for 13 months before reacting significantly.

Gill,[11] after inoculating a cow with *Brucella abortus* in the mammary vein, reported that the organism was shed from all 4 quarters for several months, and from 3 of the quarters for a complete lactation period, but no agglutinins appeared in the whey, although the blood titer became 1–800.

Thompson[12] found the inoculation of guinea pigs more efficient than direct cultures for detecting *Brucella abortus* in the milk. He considers that each quarter of the udder should be examined separately.

The observations of Hayes and Barger,[13] printed in this issue of Hilgardia, contribute important data on the relation between shedder conditions and the agglutination tests. They observed 3 cases in which shedder conditions existed for several months before a positive agglutinin titer developed in the blood.

METHODS

In this investigation the animals studied were in a so-called "infected group" which consisted of approximately 160 lactating animals, most of which were, or had been, reactors to the agglutination test, and had been segregated from a herd of approximately 800 animals. Some of the cows in this "infected group" had no history of *Brucella* infection. The entire herd was regularly tested for tuberculosis, and reactors removed.

After preparations for the experiment were completed, the first 23 animals which calved or aborted were taken for use in this study, so that no selection might be made which would influence the results. However, data from only 20 cows are presented in this paper; 3 of the 23 cows were removed from the herd because of reaction to tuberculin before representative data were obtained.

Blood and milk were taken from each animal within a few days after parturition, again 14 days after the first samples, and at approximately

monthly intervals thereafter throughout the lactation period. Except in the first part of the work, when the mixed milk from all quarters was used, the milk from each quarter was treated as a separate sample. After the udder had been thoroughly washed with germicidal soap and dried, and each teat immersed in an alcohol-iodine solution, about 150 cc of milk was drawn from each quarter and brought to the laboratory on ice.

A period of from 4 to 14 hours elapsed between the drawing of the samples and the inoculation of the guinea pigs. For these inoculations 70 to 80 cc of the milk was centrifuged at high speed for 20 minutes, the skimmed milk drawn off, and the cream and sediment thoroughly mixed. Two to 3 cc of this mixture was inoculated intraperitoneally into guinea pigs. The guinea pigs were killed 6 weeks later, and the presence or absence of *Brucella abortus* infection determined by spleen cultures, agglutination tests, and gross lesions.

Agglutination tests[6] by the tube method were made on all samples brought to the laboratory, using the serum of the blood and of the milk. To prepare the milk for the agglutination test, one drop of rennet was added to 10 cc of the decanted skimmed milk, which was then incubated at 37° C for 2 hours. The test was performed by adding 0.2 cc of the whey to 2.3 cc of antigen, and removing 1.0 cc of this mixture to a second tube containing 1.0 cc of antigen. This procedure was continued until 12 tubes had been used, giving dilutions of 1–12½, 1–25, 1–50, etc., up to a final dilution of 1–25,600. The initial dilution of 1–12½ was chosen for whey tests in order that subsequent dilutions would be comparable with those of the blood test, which was performed in the same manner except that 0.1 cc of serum was added to 2.4 cc of antigen, giving an initial dilution of 1–25.

For the purposes of this experiment, animals were classed as positive to the blood-serum agglutination test when there was a clumping of 50 per cent or more of the organisms in the 1–100 dilution tube. Animals were classed as negative when agglutination in the 1–25 tube was less than complete. Intermediate titers were classed as suspicious.

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[6] The titers of the tests will be referred to in this paper as "blood titer" or "whey titer." Furthermore, for ease in stating the results of the tests, traces of agglutination in the terminal tube are disregarded, while 50 per cent or greater agglutination in a terminal tube is considered as complete agglutination in that tube. Therefore in a test where dilutions of 1–25, 1–50, 1–100, 1–200, etc. were used, a blood titer of + + ∓ — is herein called 1–50, while + + ± — is considered a titer of 1–100.

The antigen for both the blood and whey tests was prepared with *Brucella abortus* strain 80, isolated by Meyer and Fleischner, a strain now widely used in agglutination testing. It was suspended at a density of 3.5 cm on the Gates' opacimeter (2.5 on the McFarland). In all tests the antigen was sensitive but free from self-agglutinating organisms as shown by control tests with proved negative serums and with positive serums of known titer.

TABLE 1

MAXIMUM AND MINIMUM BLOOD AND WHEY TITERS AND BRUCELLA ISOLATIONS FROM MILK

Result of attempts to isolate Brucella abortus from milk by culture and guinea-pig inoculation

Note: In the date-result columns the symbols are grouped by quarter (quarters 1–4, top to bottom). "+" = positive, "|" = negative, "o" = doubtful. A single "+" in the early columns indicates a blood-sample result.

Cow No.	Blood titer Max.	Blood titer Min.	Quarters*	Whey titer Max.	Whey titer Min.
A-166	1-25600	1-400	1	1-800	1-200
			2	1-1600	1-100
			3	1-800	1-100
			4	1-400	1-50
A-880	1-25600	1-800	1	1-800	1-200
			2	1-1600	1-400
			3	1-1600	1-800
			4	1-3200	1-200
3419	1-25600	1-3200	1	1-400	1-50
			2	1-3200	1-200
			3	1-200	1-50
			4	1-3200	1-200
3191	1-12800	1-1600	1	1-400	1-50
			2	1-800	1-100
			3	1-800	1-100
			4	1-1600	1-200
6958	1-12800	1-800	1	1-1600	1-50
			2	1-800	1-50
			3	1-3200	1-100
			4	1-400	1-50
H-10	1-6400	1-200	1	1-200	1-12½
			2	1-400	1-12½
			3	1-100	1-12½
			4	1-50	1-12½
512	1-6400	1-200	1	1-100	1-25
			2	1-100	1-12½
			3	1-200	1-25
			4	1-400	1-25
H-218	1-3200	1-200	1	1-50	1-12½
			2	1-50	0
			3	1-100	1-25
			4	1-100	1-25
2504	1-3200	1-400	1	1-3200	1-200
			2	1-200	1-25
			3	1-400	1-100
			4	1-200	1-50

Results of isolation attempts by date (grouped by quarters 1–4; "+" positive, "|" negative, "o" doubtful):

Cow No.	Apr 23	Apr 30	May 7	May 21	June 4	June 18	July 2	July 16	July 30	Aug 13	Aug 27	Sept 10	Sept 23	Sept 29	Oct 9	Oct 31	Nov 5	Nov 14	Nov 21	Dec 3	Dec 9	Dec 15	Jan 6	Jan 16	Feb 12	Mar 23	Apr 27	May 25
A-166	+	+	+			+	+		++++		++++			++++			++++					++++	++++		++++			
A-880				+	+			|+++	|+++		|+++		|+++			|+++	|+++	|+++	|+++		|+++	|+++		|+++			
3419								|+|+		|+|+		|+|+			|+|+										|+|+			
3191				+		+	+	|+++		|+++		|+++			|+++		|+++						|+++		|+++		
6958	+		+				+++|	|||+	||o+	|||+		|||+					|||+								||o+	||+|	||+|	||+|
H-10					+		+++|		+++|		+++|		+++|							+++|			+++|					
512									|||+				+++|		||o+									++o+			
H-218				+	+			||+|		|+++	|||+				|++|		|+|+				|+++		|+||	||||	|||+	||+|	||+|
2504	+	+	+		+		+		+|++		+|++			+|++			||++					||++	+|++		+|++			

The symbol readings in the date columns are a best-effort transcription of a densely printed matrix and may contain minor inaccuracies.

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				I I I I	I I I I					
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+		+		I		I	I	I	I	I
	+	+		+						
+	I					I		I	I	I
+						I		I		I
1-100 1-50 1-50 1-50	0 25 0 0	1-12½ 0 25 0	1-12½ 0 0 0	0 0 0 1-12½	0 0 1-12½ 0	0 0 0 0	0 0 0 0	0 0 0 0	0 0 0 0	0 0 0 0
1-400 1-200 1-200 1-100	1-100 1-100 1-100 1-100	1-200 1-25 1-100 1-25	1-400 1-50 1-50 1-50	1-12½ 1-100 1-200 1-100	1-25 1-50 1-50 1-50	1-12½ 1-12½ 1-12½ 1-12½	0 0 0	0 0 0	0 0 0	0 0 0
1 2 3 4	1 2 3 4	1 2 3 4	1 2 3 4	1 2 3 4	1 2 3 4	1 2 3 4	1 2 3 4	1 2 3 4	1 2 3 4	1 2 3 4
1-400	1-50	1-100	1-50	1-50	1-50	1-25	0	0	0	0
1-3200	1-3200	1-1600	1-800	1-400	1-100	1-200	1-25	1-25	1-25	1-25
7692	23295	126	4337	A-828	1009	3325	A-632	F-835	2262	2542

c = contaminated.

Until July 16 mixed milk from the four quarters was tested.

* 1, Right front; 2, left front; 3, right rear; 4, left rear.

RELATION OF BLOOD TITER TO SHEDDER CONDITION

While the elimination of *Brucella abortus* in the milk cannot be considered the sole criterion of active infection, any classification for the separation of positive and negative animals based on an agglutination test that does not remove the highest possible percentage of shedder cows from the negative group, is obviously inadequate. For this reason it is of interest to compare results of the periodic examination of milk with the corresponding blood titers.

From table 1 (p. 548–549) it will be seen that of the 20 animals considered in this investigation, 14 were proved to be shedders. In practically all of these shedder cows, the blood titers were well above 1–100 most of the time, the titer dropping as low as 1–50 in only 3 of the cows, and then only for a short time.

Brucella abortus was never isolated from the remaining 6 cows. The blood titers of these nonshedders were always below 1–100, except for cow 1009, which had 2 tests showing 1–100; and cow 3325, which on the first 2 tests had blood titers of 1–200 and 1–100 respectively. (See figs. 10, and 11, p. 566.)

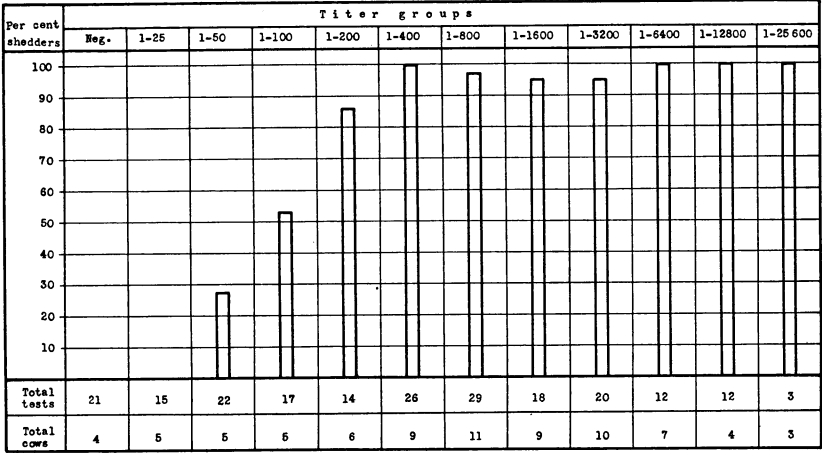| Per cent shedders	Neg.	1-25	1-50	1-100	1-200	1-400	1-800	1-1600	1-3200	1-6400	1-12800	1-25 600
Total tests	21	15	22	17	14	26	29	18	20	12	12	3
Total cows	4	5	5	6	6	9	11	9	10	7	4	3

Fig. 1.—Percentage of *Brucella* isolations from milk in repeated tests of 20 cows, grouped according to corresponding blood titers.

The total number of blood agglutination tests made in this study are grouped in figure 1 according to the number of tests falling into each titer division. Figure 1 shows that no isolations of *Brucella abortus* were made when the blood titer was below 1–50, but that when the titer was 1–50 the organism was obtained from the milk of one or more quarters

in 27.3 per cent of the 22 udder tests. When the blood titer was 1–100, 52.9 per cent of the tests yielded the organism. With a titer of 1–200, there were isolations in 86 per cent of the udder tests. At a blood titer of 1–400 or above, the organism was found in the milk of one or more quarters in from 95 to 100 per cent of the tests. In fact, of the 134 udder tests when the corresponding blood titers were 1–200 or over, *Br. abortus* was isolated in 96.3 per cent of the cases.

Although broad generalizations cannot be drawn from a group of 20 animals, it is obvious from the above that, considering only elimination of *Brucella abortus* in the milk, a standard which classifies animals as positive when the blood titer is 1–100 or over is by no means too severe.

In further support of the view that any cow which has a blood titer sufficiently high to cause any agglutination in a dilution of 1–100 or higher is potentially a shedder of *Brucella abortus* in her milk, the results of guinea-pig inoculations with milk from 210 cows in another project are presented. These animals all had a blood titer of 1–100 or higher. The mixed milk from all 4 quarters of each animal was used to inoculate the guinea pigs. The results were as follows:

> 10 cows had 4 tests; 10 (100 per cent) shed *Br. abortus* 1 or more times.
> 37 others had 3 tests; 34 (91.9 per cent) shed *Br. abortus* 1 or more times.
> 68 others had 2 tests; 55 (80.9 per cent) shed *Br. abortus* 1 or more times.
> 95 others had 1 test; 72 (75.8 per cent) shed *Br. abortus*.

Of the 210 cows, a total of 171 (81.4 per cent) were found to be shedders when tested from 1 to 4 times.

In summarizing the relation of blood titer to shedder condition, the data presented seem to indicate that (1) cows with a blood titer showing any agglutination at 1–100 or higher are actual or potential shedders of *Brucella abortus* in their milk; (2) cows with a titer of 1–50 may shed the organism at times.

RELATION OF WHEY TITER TO SHEDDER CONDITION

In recent years the use of whey rather than the blood serum for the agglutination test has been advocated from time to time. That this method has noteworthy advantages in ease of collection and in lessened disturbance of the animals from which the samples are being drawn, cannot be denied. However, there is still considerable disagreement concerning the delicacy of this test, and no agreement as to what constitutes a positive test.

Fitch and Lubbehusen[3] concluded that the titer of the whey was unsatisfactory as a means of determining the shedder condition, but on the other hand, Torrey[14] concluded that it is a comparatively simple mat-

ter to apply the rapid agglutination test to the whey from each quarter and thus determine the presence or absence of *Brucella abortus* infection.

From studies on 113 cattle, Gilman[15] concluded that milk to be used for agglutination work must be from individual quarters and not a composite sample from all 4 quarters. He found some correlation between the agglutination titer of the whey and the presence of *Brucella abortus* in the milk, and concluded that quarters showing agglutinins at a titer of 1–80 or higher are usually actively infected, but quarters with

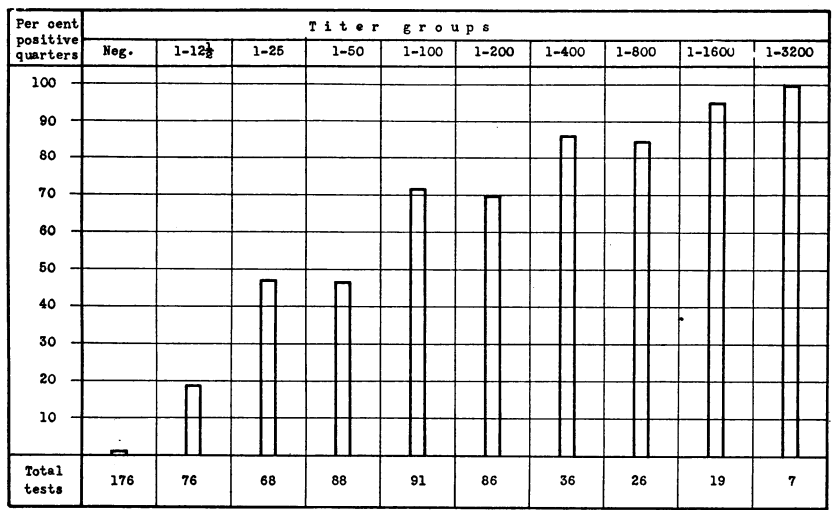| Per cent positive quarters	Titer groups									
	Neg.	1–12½	1–25	1–50	1–100	1–200	1–400	1–800	1–1600	1–3200
Total tests	176	76	68	88	91	86	36	26	19	7

Fig. 2.—Percentage of *Brucella* isolations from milk of individual quarters of twenty cows repeatedly tested, grouped according to corresponding whey titers.

titers lower than 1–80 contain the organism only in rare instances. He found that of 108 cows which showed agglutinins at 1–80 or higher in one or more quarters, 78 per cent were infected.

Prouty[16] found 13 shedders in 18 cows that showed agglutination with 0.02 cc or less of blood serum by the rapid plate method (equivalent to 1–100 or higher by the tube method). All samples from quarters giving negative agglutination reactions with amounts of whey less than 0.08 cc (equivalent to 1–25 by the tube method) gave negative cultural findings for *Brucella abortus*.

Caldwell, Parker, and Medlar[17] are led to believe that the presence of agglutinins in whey is more reliable as an indication of udder infection than is their presence in the blood serum.

Beach and Humphrey[18] observed in a few cases that the colostrum whey of so-called "ceased reactor" cows showed agglutinin concentra-

tion of 1–400 or higher, but after the animals had been milked a few
days the whey titer largely disappeared.

Figure 2 shows that isolations of *Brucella abortus* were made from 1
per cent[7] of the quarters showing no agglutinins in a whey dilution of
1–12½. The percentage of isolations increased in a relatively uniform
line as the titer increased, until 100 per cent of the quarters with a whey
titer of 1–3,200 were proved to be shedding the organism.[8]

Unlike the condition found in blood-titer groups, the percentage of
shedders in the whey-titer groups did not abruptly ascend with the in-

TABLE 2

RELATION OF MAXIMUM MILK AGGLUTINATION TITER OF ANY QUARTER OF UDDER TO
BRUCELLA ABORTUS SHEDDER CONDITION IN SAME UDDER

Titer of highest quarter in udder	Number of udders tested	Number of positive udders	Per cent of positive udders
Negative at 1–12½	35	0	0
1–12½	8	2	25.0
1–25	18	14	77.7
1–50	25	19	76.0
1–100	21	20	95.2
1–200	26	24	92.3
1–400	17	17	100.0
1–800	7	7	100.0
1–1600	9	9	100.0
1–3200	7	7	100.0
Total 1–25 or over	130	117	90.0

crease in titer above a certain point, and as may be seen from figure 2,
it would be impossible in these tests to draw a line dividing the shedder
animals from the nonshedders, based on the agglutination titer of the
milk from individual quarters.

If, however, the whey titer from the quarter showing the highest con-
centration of agglutinins is taken as an index to the shedder condition in
the entire udder, a rapid increase in the percentage of shedders occurs
between the titers of 1–12½ and 1–25, as is shown in table 2. No *Brucella*
organisms were found in any of the 35 udders tested when all quarters
were negative. When the highest titer in any quarter of the udder was
1–12½, 25 per cent of the udders were found to be infected, while 77.7
per cent were infected when the highest titer of any quarter was 1–25.

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[7] The 1 per cent represents 2 cows (fig. 7, p. 564, and fig. 9, p. 565), each of which
had temporarily a negative whey titer in a quarter shedding *Brucella abortus,* but one
of the other quarters in each cow showed a high titer at the same time and was also
shedding the organism.

[8] 1–3,200 was the highest whey titer found in this study, although much higher
titers have been encountered from time to time in connection with other projects. The
highest titer found in whey tested at this laboratory was 1–204,800.

By an inspection of figures 3 to 9 (p. 561–565), the correlation between whey titer and shedder condition can be seen. In most cases the quarters shedding *Brucella abortus* from an udder had higher whey titers than the quarters not shedding the organism, as is also indicated in table 1. This is in agreement with the conclusion of Smith, Orcutt, and Little[19] that at least some of the agglutinins for *Br. abortus* are elaborated in the udder itself.

The mixed milk from all 4 quarters was tested on 16 occasions when the mixed whey showed no agglutinins in the 1–12½ dilution, and *Brucella abortus* was isolated 3 times. This indicates that mixed milk is not dependable for the detection of infected cows by the whey agglutination test, as a titer of 1–50 in a single quarter may easily be masked when the remaining quarters are negative or nearly so.

In summarizing the relation of milk titer to shedder condition, it is concluded from the above that a cow whose milk from any quarter shows, in repeated tests, a whey titer of 1–25 or over is probably eliminating *Brucella abortus* in her milk.

RELATION OF WHEY TITERS TO BLOOD TITERS

Although the blood titers of some of the animals fluctuated over a rather wide range, as may be seen from table 1, most of these cows maintained their status of positive or negative throughout the entire period. The fluctuation is far more marked in the whey titers, and changes from positive to negative occur in several cases. The most rapid change in the agglutination titer of the whey usually occurs within a short period after calving. A drop from a titer of 1–25,600 to 1–200 within 14 days, as shown in the case of cow 3191 (fig. 6, p. 563) has frequently been observed in infected animals when the initial test is made with milk drawn within a few hours after calving.

The blood titer was usually higher than the whey titer of any quarter. In some cases the whey titer is higher for a short period immediately after parturition (see figs. 3, 6, and 8, p. 561, 563, and 564, respectively). Twice a sample of milk from a single quarter showed a slightly higher titer than did the blood serum, but in both cases the next test showed a lower whey titer. Figure 9 (p. 565) shows one of these cases.

From the results of the repeated tests of the milk and blood in this group of animals, it is necessary to conclude that no cow would have been classed as negative by the result of the blood-serum agglutination test at any time when the whey titer was sufficiently high to cause the animal to be classed as positive according to our standard, with the possible exception of the short period immediately after calving.

CONSTANCY OF SHEDDER CONDITION

In the 14 infected cows studied, 44 quarters were found to be shedders of *Brucella abortus*. At the numerous times that the milk from each quarter was tested, 38 of these quarters were shedding the organism in from 33 to 100 per cent of the tests, while the organism was isolated only once from each of the other 6 quarters. The positive findings in these 6 quarters are difficult to explain on any grounds except errors in technic. Two of these (cows 6958 and 23295) were apparently due to switching of labels, as in each case an adjoining quarter, otherwise consistently positive, showed a negative test on the same date.

Of the 38 quarters repeatedly proved to be infected, 23 eliminated *Brucella abortus* at every test throughout the period of lactation. Milk from the remaining 15 quarters failed to infect guinea pigs on more or less irregular occasions, as is shown in table 1.

Further evidence that an infected udder may shed *Brucella abortus* continuously for months has been obtained in connection with an experiment carried on for other purposes. During this work the milk of a cow naturally infected with *Br. abortus* was inoculated into guinea pigs at an average of every 2 days for 78 days. All except 2 of these guinea pigs showed definite infection with *Br. abortus*. The failure to infect these 2 animals was probably due to some cause other than the absence of the organism from the milk. Although the milk from all 4 quarters of the udder of this cow was pooled for these inoculations, it was shown at the time the cow was autopsied that probably one quarter only was responsible for this continuous elimination of *Br. abortus*.

SPREAD OF INFECTION FROM QUARTER TO QUARTER

The ease with which cows are infected with *Brucella abortus* via the teat canals, and the permeable nature of the tissues separating the cisterns of the front and rear quarters on the same side of the udder, would suggest that infection established in one quarter would be apt to spread rather rapidly to the noninfected quarter of the same side of the udder. That such spread of infection did not occur to any extent is shown in table 1. In the 14 infected cows, there were 20 quarters which were negative at the first three tests, and which were therefore classed as negative quarters. At the termination of the study, 12 were found to have remained negative throughout the tests, 6 were positive on one occasion only, and only 2 had become definitely infected. Both of these had adjoining quarters on the same side of the udder which were always nega-

tive except for an isolation of *Brucella abortus* at one test only. The majority of quarters which remained negative during the entire period had adjoining quarters on the same side of the udder which were infected.

NUMBER OF BRUCELLA ABORTUS ORGANISMS EXCRETED IN MILK

While considerable data are now available concerning the number of *Brucella abortus* organisms found in the milk of infected cows at any one time, little has been done in determining the numerical regularity of the elimination. Unfortunately, the regular examinations of the whole milk from the separate quarters was not begun in this experiment until lactation had continued for a period of one or two months in most of the animals used.

The number of organisms in the milk was determined by culturing 0.1 cc of the sample in a petri dish containing solidified cooked-blood agar, to which had been added sufficient gentian violet to give a dye dilution of 1–208,000. Several investigators have reported the number of *Brucella abortus* per cc of milk to be very few. Similar results have been obtained in our work. The greatest number of organsims found in these studies was 3,020 per cc, and it was common to find only 100 to 200 or less per cc. Also, many samples which failed to show growth in cultures made from 0.1 cc of milk, contained sufficient number to cause growth when the sample was first concentrated by centrifuging.

A total of 186 counts of organisms were made, using whole milk from quarters which were shown by guinea-pig inoculations or cultures to be shedding *Brucella abortus* at the time. In 59 cases (31.7 per cent) infected quarters failed to show growth of the organism in the culture; 61 others (32.8 per cent) were found to contain from 10 to 100 *Br. abortus* organisms per cc; 40 others (21.5 per cent) contained from 101 to 500 per cc; 10 cultures (5.4 per cent) had between 501 and 1,000 per cc; 14 (7.5 per cent) showed between 1,001 and 2,000 per cc; the remaining 2 counts were 2,560 and 3,020 organisms per cc, respectively.

A comparison of the occurrence of colonies on cooked-blood agar plates with the blood and whey titers of the animals, shows that as the titers increase, *Brucella abortus* was isolated more frequently. For example, 5 counts were made of the organisms in infected milk from quarters with a whey titer of 1–25 while the blood titer was 1–200, and these samples showed 0, 0, 10, 40, and 350 per cc, respectively. The 5 counts made when the whey titer was 1–800 for each quarter and the blood titer 1–1,600, showed 20, 20, 50, 60, and 80 organisms per cc, respectively. Except that the number of organisms was greater in some cases, these

two examples are typical of the results in plating 186 samples from animals with whey titers ranging from 1–12½ to 1–3,200, and blood titers from 1–25 to 1–25,600. These examples also illustrate what was found to be true in general: namely, that when colonies did occur on the plates, the number of organisms per cc gave no evident index to the corresponding whey or blood titers.

In view of the constant nature of the shedder condition as shown in preceding sections, and the regularity in the number of organisms excreted by many animals, the 14 shedder cows studied may be considered as continual contributors of infection to the mixed milk of the herd.

STAGE OF LACTATION IN RELATION TO AGGLUTINATION TITERS

As previously stated, the agglutination titer of the whey was found to be high immediately after parturition, and usually dropped very rapidly in the first two weeks thereafter. After this sharp drop, the titer tended to remain relatively constant, but with occasional abrupt rises and falls until the end of the lactation period was approached. At that time a gradual increase in whey titer often occurred, which continued until the animal ceased to lactate.

The course followed by the agglutination titer of the blood was similar to that of the whey, except that no definite decline usually occurred after calving, nor was there any very marked rise in the blood titer at this time. Throughout the period the fluctuations were usually less than in the whey titers. Gradual increase in blood titers toward the close of lactation occurred in less than half of the animals.

The charts of the blood titers of the animals studied indicate that a diagnosis of shedder condition, based on several consecutive blood-serum agglutination tests, was possible and feasible at any time during the period of the tests.

OBSERVATIONS ON DISSOCIATION

In connection with a study of dissociation in the *Brucella* group, the *Br. abortus* strains isolated from the 14 infected cattle used in this project were systematically examined to determine whether or not dissociation played a part in the spread or maintenance of infection in brucelliasis of cattle.

For this purpose, 10 colonies (or all, if less than 10 were present) were picked at random from each positive culture during the period of the work. A total of 1,038 colonies of *Brucella abortus* thus picked were transferred and examined as to type. In addition, an equal, if not greater, number of colonies which appeared on the milk plates, and

which resembled but were not typical of *Br. abortus* colonies, were examined in the hope of detecting variant forms other than those obtained by the laboratory methods.

No *Brucella abortus* strains were obtained which were other than "S" in character and no atypical colony examined proved to be *Br. abortus.* From these data it seems probable that dissociation does not play an important rôle in the carrier condition in *Br. abortus* infection in cattle. This conclusion is in accord with the results obtained by Henry[20] with cultures from the milk of 114 cows in all stages of lactation.

SUMMARY

The blood and whey titers for *Brucella abortus* of 20 cows were studied at monthly intervals during a complete lactation period, and correlated with the shedder condition as determined by guinea-pig inoculations and cultures.

No milk sample was found to contain *Brucella abortus* when the blood titer of that cow at the time the milk was collected was below 1–50.

When the corresponding blood titer was 1–200 or over, *Brucella abortus* was isolated from the milk of one or more quarters in 96.3 per cent of the udder tests.

The percentage of shedders of *Brucella abortus* found among cows with blood titers of 1–100 or over, increased rapidly as successive tests of the cows were made over a period of time.

The diagnosis of shedder condition, based on several consecutive blood-serum agglutination tests, was possible and feasible in these 20 animals at any time throughout the period of the tests.

The agglutination titer of the whey is less dependable as an indicator of infection, and is more subject to fluctuations, than is the blood titer.

Brucella abortus was not recovered from any udder in which the milk from all individual quarters showed titers of less than 1–25.

In only 2 instances was *Brucella abortus* obtained from a quarter which had a negative whey titer, and in each case other quarters of the same udder had high titers and were excreting the organism.

Of 38 quarters proved to be infected with *Brucella abortus,* 23 eliminated the organism at every test throughout the period. The remaining 15 infected quarters failed to infect guinea pigs on more or less irregular occasions only.

The spread of infection from quarter to quarter, as indicated by the excretion of *Brucella abortus,* was very slow.

None but the "S" type of *Brucella abortus* were found in the milk of these naturally infected cows.

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APPENDIX

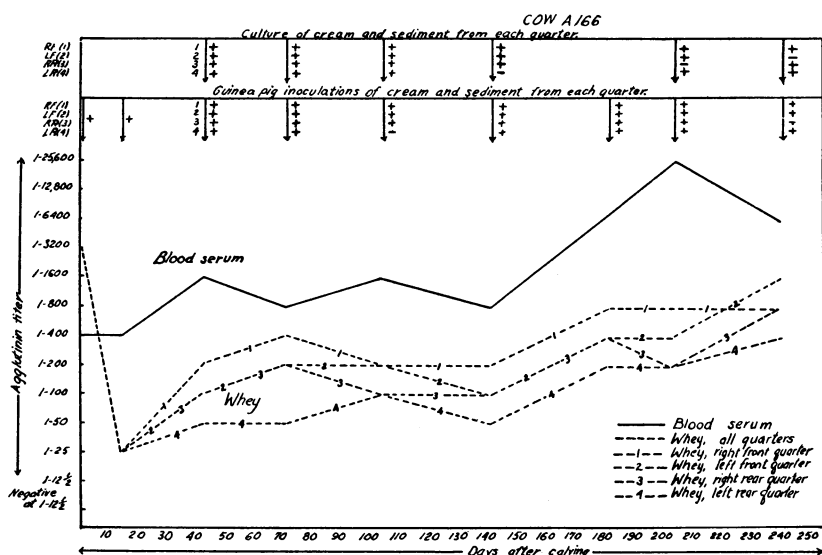## RECORDS OF TYPICAL INDIVIDUAL COWS

Fig. 3.—Cow A–166: calved June 18, 1930, dry February 20, 1931; eight-year-old Jersey; positive to the agglutination test for *Brucella abortus* for at least 18 months previous to the beginning of this study.

Note the sharp drop in whey titer on the fifteenth day after calving, which illustrates graphically the well-known phenomenon of antibody concentration in the colostrum. The gradual rise in titer at the end of the lactation period, as mentioned in the text (page 557), is shown here.

Counts of the number of *Brucella abortus* organisms per cc of milk from the four quarters of the udder, are as follows:

Days after calving	RF	LF	RR	LR	Days after calving	RF	LF	RR	LR
42	180	10	20	10	140	90	50	10	0
70	20	10	0	0	202	1,080	60	0	160
103	0	0	0	0	239	920	0	490	1,090

Tests made during the next lactation period indicated that the shedder condition persisted, and there was little change in the blood or whey titers.
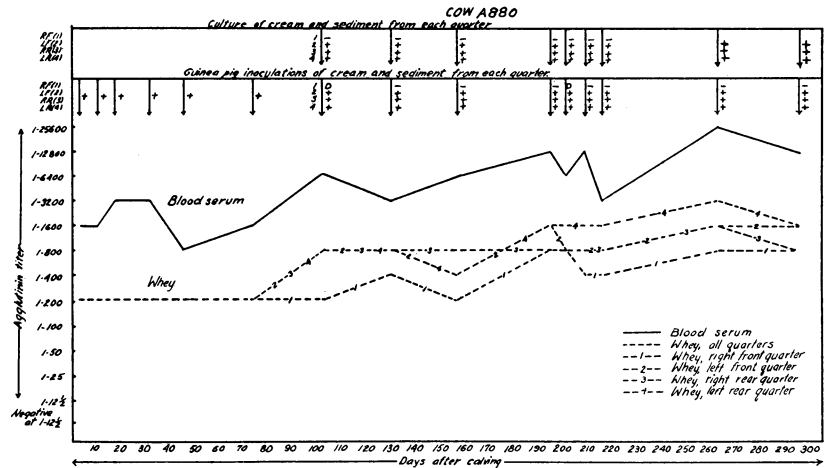
Fig. 4.—Cow A–880: calved April 20, 1930, dry February 12, 1931; nine-year-old Jersey; first became a reactor to the agglutination test for *Brucella abortus* 9 months previous to the beginning of the lactation period shown in the graph. At the end of 190 days of lactation, this cow's production fell below the minimum for profitable marketing, and the animal was brought to the laboratory and kept in lactation until 298 days after calving.

The numbers of *Brucella abortus* organisms per cc of milk from the various quarters were as follows:

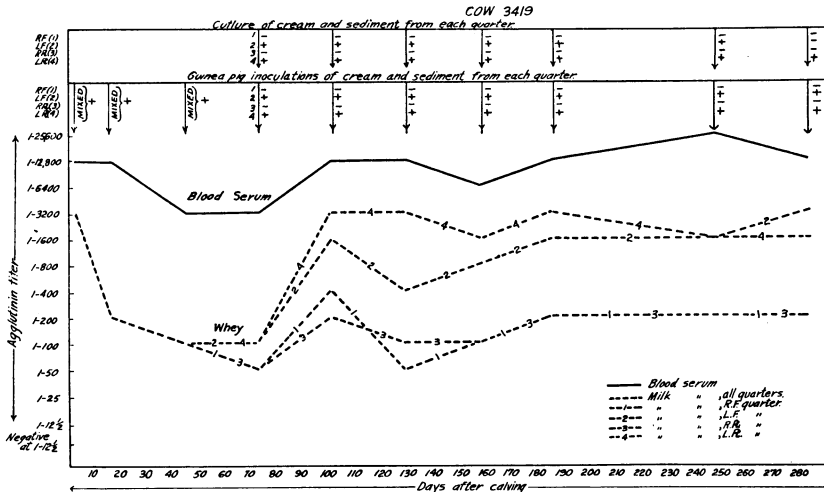| Days after calving	RF	LF	RR	LR	Days after calving	RF	LF	RR	LR
101	0	60	30	220	208	0	50	—	70
129	0	80	20	50	215	0	60	20	50
156	0	50	10	70	264	0	30	20	230
194	0	40	30	40	298	0	30	20	110
200	0	50	0	20					

Fig. 5.—Cow 3419: calved May 5, 1930, dry February 27, 1931; nine-year-old Holstein; positive to the agglutination test for *Brucella abortus* for at least 18

months previous to the first test shown on the graph. Previous calving was normal, with shedding condition during that lactation period.

Note the drop in the whey titer after calving, the relatively higher titer for the infected quarters, and the constancy of the shedder condition from the left half of the udder.

Numbers of *Brucella abortus* organisms per cc of milk from infected quarters during the lactation period shown in the graph were as follows:

Days after calving	LF	LR	Days after calving	LF	LR
100	90	360	184	90	1,200
128	630	1,360	246	1,580	1,070
157	80	970	283	0	170

Subsequently calved May 23, 1931, and three monthly tests of blood and milk after this calving showed little change from the previous lactation findings.
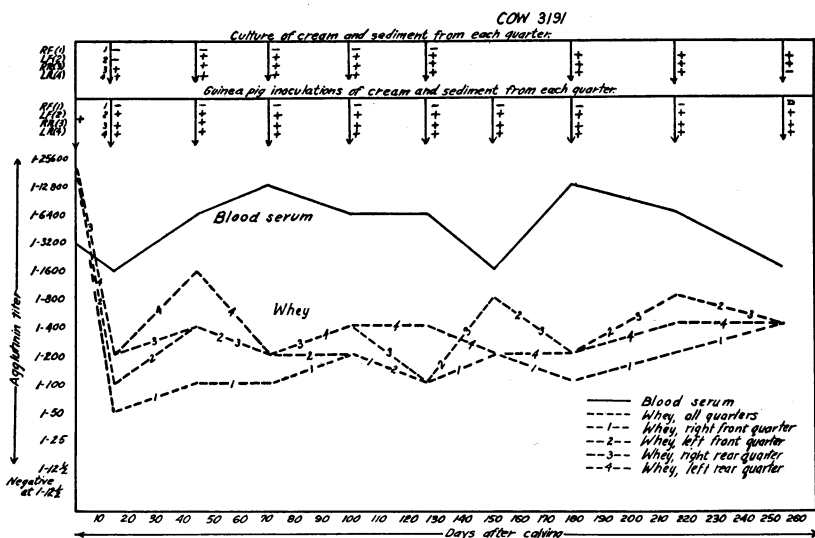

Fig. 6.—Cow 3191: calved July 2, 1930, dry April, 1931; eight-year-old Holstein; positive to the agglutination test for *Brucella abortus* for at least 15 months previous to tests shown on the graph.

Note the drop in whey titers soon after calving.

The numbers of *Brucella abortus* organisms per cc of milk from the infected quarters are as follows:

Days after calving	LF	RR	LR	Days after calving	LF	RR	LR
42	0	80	60	178	480	200	10
70	50	260	210	215	140	100	0
99	190	190	210	254	160	40	0
126	220	100	20				

A single test, made one month after the next calving, gave results comparable with those shown in the graph.
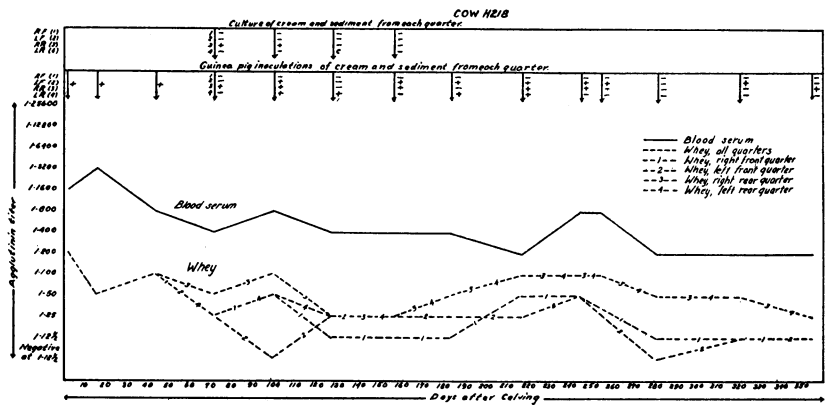
Fig. 7.—Cow H–218: aborted an eight-months fetus on May 5, 1930, dry August 31, 1931; four-year-old Jersey; became infected with *Brucella abortus* between seven and twelve months previous to the records shown in the chart.

Although three quarters of the udder of this cow produced milk containing *Brucella abortus* from time to time, the number of organisms was never sufficiently great in any sample to yield *Br. abortus* colonies when 0.1 cc of milk was plated.

Because of lack of space, only the data collected during the first 357 days of lactation are given in figure 7. The results of tests continued until the 451st day of lactation were similar to those of the earlier tests shown in the chart.
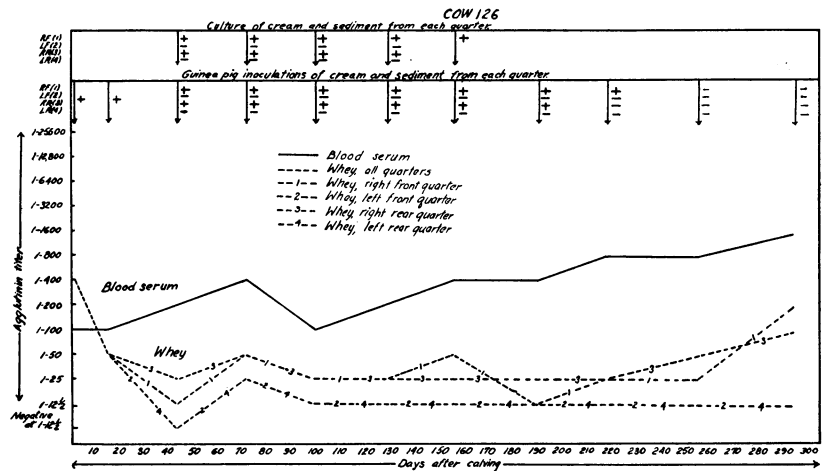

Fig. 8.—Cow 126: calved June 3, 1930, dry March 25, 1931; eight-year-old Holstein. Negative to the agglutination test for *Brucella abortus* 19 months previously, but positive to the test 8 months before the beginning of the lactation period shown in the chart.

The number of *Brucella abortus* organisms per cc of milk from the infected quarters of the udder of this animal was very small, and cultures were obtained from noncentrifuged milk on only three occasions, although guinea pigs were infected in all but the last two tests.

Days after calving	RF	RR	Days after calving	RF	RR
71	20	0	217	0	0
99	0	0	254	0	0
128	40	10	293	0	0
155	30	0			

Guinea-pig inoculations made one month after the next normal calving, failed to show *Brucella abortus* in the milk of any quarter. The blood agglutination titer was 1–200.
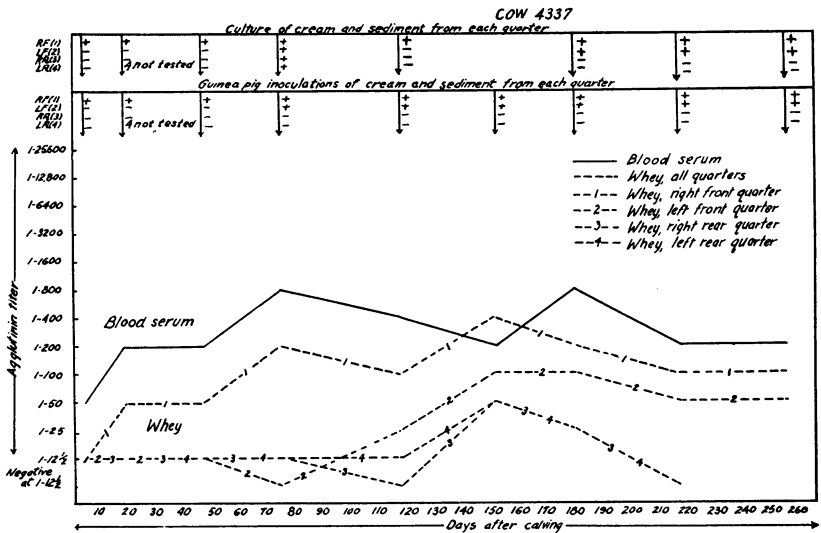

Fig. 9.—Cow 4337: calved July 12, 1930, dry April, 1931; three-year-old Holstein; negative to the agglutination test at 18 months before the records shown in the graph, but had a titer of 1–50 for at least 6 months before the beginning of the lactation period recorded.

Note the rise in the titer of the left front quarter after the development of a shedder condition.

The numbers of *Brucella abortus* organisms per cc of milk, were found to be as follows:

Days after calving	RF	LF	Days after calving	RF	LF
4	+	0	117	250	0
18	+	0	179	310	50
46	+	0	216	40	20
74	150	0	255	160	10

The "plus" signs in the above tabulation indicate that cultures were obtained from centrifuged milk, but since the noncentrifuged milk produced no colonies, it was not possible to estimate the number per cc.
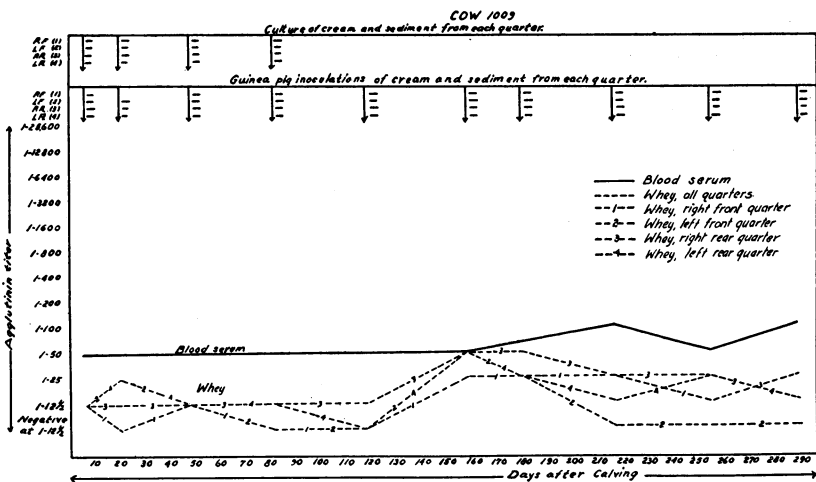
Fig. 10.—Cow 1009: calved July 10, 1930, dry May, 1931; seven-year-old Jersey. Agglutination tests for the preceding 16 months were all similar to those shown in the chart. The continuous low titers would seem to indicate that this animal had recovered from infection with *Brucella abortus*, and that the blood reaction noted was caused by residual agglutinins due to this infection.

The organisms were not recovered from the milk or cream of this animal at any time.
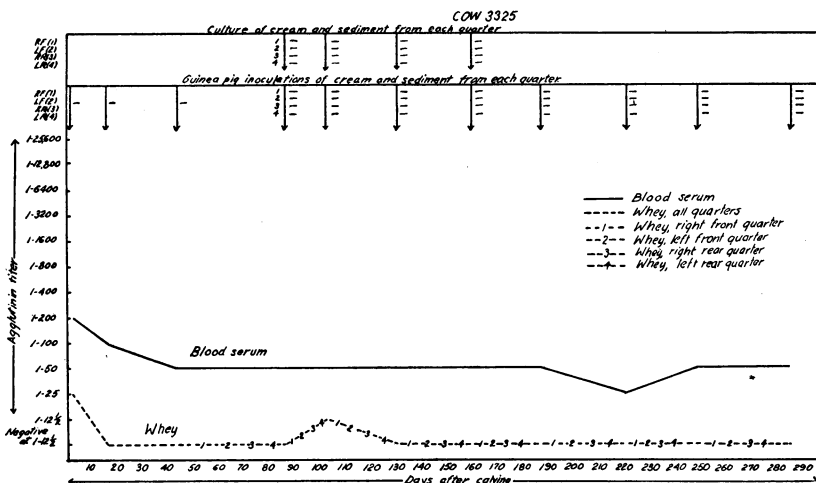

Fig. 11.—Cow 3325: calved May 5, 1930, dry February, 1931; eight-year-old Guernsey. Previous to this lactation, the agglutination tests for *Brucella abortus* were as follows: 16 and 14 months previous, negative; 12, 10, and 4 months previous, titers were 1–200; 23 days previous, titer was 1–50.

Brucella abortus was not recovered from the milk or cream during this lactation period, nor in three tests made after a subsequent parturition.