BRUCELLA ABORTUS IN MILK.

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As a result of the extensive investigations in America and on the Continent into the relationship of *Br. abortus* to undulant fever in man, attention in this country has been directed to cases of fever due to obscure causes, with the result that an increasing number of cases of undulant fever are being reported. So far, however, the cases in Great Britain appear to be less numerous than those in the United States and in Denmark where every effort has been directed to tracing sources of infection and where the conclusion has been reached that, although a certain number are infected by contact with the secretions and carcasses of infected animals, many infections occur through ingesting milk containing *Br. abortus*.

In this country, Wilson and McNutt (1926) at Manchester examined 488 samples of cows' milk, and found that 5·7 per cent. of single milks and 8·8 per cent. of mixed milks were infected with *Br. abortus*. In America, King (1928) reported that 50 per cent. of the dairy herds in Indiana were infected with this organism; Carpenter and Boak (1928) found that 24·4 per cent. of 120 samples of raw milk showed evidence of infection as shown by injection of the cream into guinea-pigs; while Hasley (1930), by the plating method, has shown that *Br. abortus* was present in certified milk in 10 out of 230 samples examined. In Denmark, similar evidence of extensive infection of the milk supply has been reported by Andersen and Thomsen (1930), since these workers found 11, or 30 per cent., of 36 samples to be infected.

The question of the thermal death-point of *Br. abortus* has also been considered by various workers. In the report by Dalrymple-Champneys (1929) it is stated that Priesz (1903) found *Br. abortus* to be killed by heating at 55° C. for 3 min., that Dalton and Eyre (1904) found *Br. melitensis* to be killed by heating in a water bath for 10 min. at 57·5° C., and that Fabyan (1913) found that various strains differed in their susceptibility to heat, but that 10 min. at 59° C. was generally sufficient. Similarly, Park (1928) found that various cultures of *Br. abortus* and *Br. melitensis* were killed by exposure to heat at 60° C. for 10 min.

Again, Carpenter and Boak (1928) found that seven strains of *Br. abortus* were killed by heating at 60° C. for 15 min., while one porcine strain resisted this temperature for 20 min., and they concluded that temperatures of 142° F. (61° C.) to 145° F. (63° C.) for 20–30 min. were satisfactory for pasteurising milk and freeing it from living forms of *Br. abortus*. Similar results have been obtained by Zeller and his co-workers (1928). Arnold (1930),

however, states that 40 min. exposure to a temperature of 140° F. was necessary for effective pasteurisation, while Hardy, Jordan, Borts, and Hardy (1931) have not found living organisms following exposures to temperatures of 144–145° F. for 30 min.

That a characteristic disease could be produced in guinea-pigs by inoculation with a culture of *Br. abortus* was first pointed out by Smith and Fabyan (1912), while a more detailed study was presented by Fabyan (1912). Work on guinea-pig infections was extended by Smillie (1918), who found that the number of living bacteria in the body of the guinea-pig began to decline at about the fourth week after infection, and this decline in numbers slowly continued until the tenth or twelfth week, whereupon a rapid decline occurred. Hagan (1922), however, showed that immunity in guinea-pigs was relative, and attempted to determine the number of organisms necessary to produce infection. In numerous experiments he found that subcutaneous inoculation with 10,000 organisms always produced infection and that in some cases less than 100 were sufficient, while in one experiment he found that 1750 organisms failed to produce infection in one animal and 87 organisms produced infection in another.

METHODS.

The milk samples were collected on a basis arranged by the Department of Health for Scotland for an investigation into the incidence of tuberculous infection. The samples were divided into three categories, A samples—raw milks received from the producers, B samples—milk subjected to any form of pasteurisation, and C samples—milk ready for retail distribution. Sterile 100 c.c. bottles were supplied to the City Veterinary Inspector who, using every precaution to avoid any possible contamination, collected the samples. The bottles were completely filled in each case, and on receipt at the Laboratory the whole was centrifuged—50 c.c. quantities being treated at 3000 R.P.M. for half an hour. The cream was discarded and the deposit was resuspended in 6 c.c. of sterile normal saline, 3 c.c. of this suspension being inoculated into the left groins of each of two tuberculin-negative guinea-pigs. The first animal of each pair was killed after 28 days and the second animal after 56 days. The animals were etherised, the large neck blood vessels cut through, and the blood collected from each animal in sterile centrifuge tubes. Later a post-mortem examination was carried out, and the spleen and a piece of the liver were removed aseptically and placed in a sterile Petri dish. Then 3 c.c. of sterile saline were added, and with sterile forceps the tissues were broken up and emulsified. Large drops of this emulsion were placed on the surface of two plates containing Huddleson's liver agar medium and distributed over the plates with a sterile spreader. One series of plates was incubated in the normal incubator atmosphere, and the other series in a jar containing 10 per cent. carbon dioxide, the percentage of CO_2 being adjusted by a method already described (Smith, 1932). After an incubation period of 3 days at 37° C.,

the plates were examined for colonies of *Br. abortus*, the golden sheen of the transparent colony, as seen by transmitted light, making recognition easy. Colonies were selected and inoculated into liver agar slopes, and incubated for a further period of 3 days under the same conditions in which they originally appeared. After a further incubation period of 3 days the profuse growth thus obtained was, after subculture, suspended in normal saline and an agglutination test was carried out. Volumes of various dilutions of *Br. abortus* agglutinating serum (1/100 to 1/6400) were placed in Dreyer agglutination tubes, and one volume of diluted suspension was then added to each tube. The mixtures were incubated in the water bath at 52° C. for 4 hours. If the strains agglutinated, an absorption test was carried out by making a subculture on a liver agar plate, and after the 3-day incubation period the growth was washed off in 1·5 c.c. saline and added to a tube containing 0·03 c.c. of agglutinating serum. The suspension and serum were left in contact overnight, then centrifuged, and an agglutination test set up with the supernatant fluid; 0·5 c.c. volumes were used and the dilutions ranged from 1/50 to 1/1600, so that when a volume of the standard homologous formalinised suspension was added the dilution of the serum ranged from 1/100 to 1/6400. The tests were incubated along with the necessary controls in the water bath at 52° C. for 4 hours.

The blood from each animal was collected, and with the sera agglutination tests were set up using 0·5 c.c. volumes containing dilutions ranging from 1/12½ to 1/800. In this series of tests, in order that uniformity might be obtained, the Oxford standard suspension of *Br. abortus* was employed throughout, and when 0·5 c.c. of this suspension was added to each tube the serum dilutions ranged from 1/25 to 1/1600 in the first instance and were continued if necessary, the mixtures being incubated as already described.

Agglutinating sera for *Br. abortus* were readily produced by giving rabbits an intravenous dose of 50 million living organisms of a recently isolated strain. The progressive infection in the rabbit caused agglutinins to reach a titre of about 1/6400 in 10 days' time.

A post-mortem examination was made on all animals, and in those animals which showed signs of a tuberculous infection the usual staining methods were employed to demonstrate the presence of tubercle bacilli.

Results.

Samples of raw milk.

Samples of raw milk were collected from 279 different sources. The daily delivery from the various dairy farms varied from a few to over 100 gallons. By guinea-pig inoculation, 79 of these samples or 28·3 per cent. were found to contain *Br. abortus*, while 29 or 10·3 per cent. were found to contain tubercle bacilli. The various supplies were divided up according to the amount of milk delivered daily, and the incidence of infection in relation to amount, and in consequence in relation to the actual size of the dairy herd, is given

in Table I. This table shows that, in general, the greater the bulk of the original sample the higher was the incidence of infection with *Br. abortus*. In a general way also, the lowest incidence of infection with tubercle bacilli occurred in supplies from the smaller dairy farms, while a much greater incidence was obtained in the larger supplies.

Table I. *Samples of raw milk.*

Total amount of daily supply in gallons	Total no. of different samples	Incidence of *Br. abortus*			Incidence of *B. tuberculosis*		
		No. +	No. −	% +	No. +	No. −	% +
1–10	41	9	32	21·9	1	40	2·4
11–20	57	15	42	26·3	6	51	10·5
21–30	56	11	45	19·8	5	51	8·9
31–40	46	15	31	32·6	5	41	10·8
41–50	34	12	22	35·2	4	30	11·7
51–60	17	7	10	41·1	2	15	11·7
61–70	10	3	7	30·0	2	8	20·0
71–80	4	0	4	0·0	0	4	0·0
81–90	9	5	4	55·5	1	8	11·1
91–100	3	1	2	33·3	2	1	66·6
101–110	2	1	1	50·0	1	1	50·0
Totals	279	79	200	28·3	29	250	10·3

Pasteurised milk samples.

Milk samples were collected from eight pasteurising plants, 246 samples being collected directly after pasteurisation at the source and 133 samples as retail samples in bottles ready for issue to the consumers. The distribution of the samples in relation to the dairy and in relation to the presence of *Br. abortus* and tubercle bacilli is given in Table II. Thus, out of a total of 379 samples, 36 or 9·4 per cent. were found to contain *Br. abortus* and 23 or 6·0 per cent. tubercle bacilli.

Again, 187 of the 379 samples were collected from three pasteurising plants in which the "holding" method was employed, and none of these showed the presence of *Br. abortus* and only 4 or 2·1 per cent. showed tubercle bacilli. This is in marked contrast to the results obtained with the milk samples from dairies using the "flash-point" method. From these, 192 samples were obtained, and 36 or 18·7 per cent. contained *Br. abortus* while 19 or 9·8 per cent. contained tubercle bacilli.

At this point it should be stated that the term *flash-point pasteurisation* used in subsequent pages only applies to the method employed in heating the milk, and bears no relationship to any specified temperature. Apparently in Aberdeen certain commercial interests do not approve of heating the milk to 80° C. (176° F.), and are satisfied with totally inadequate temperatures of 140–145° F.

Our results will now be discussed in relation to each dairy.

Dairy No. 1 has a modern pasteurising plant using the "holding" method, and pasteurising the milk at 145° F. for 30 min. This plant deals with some-

thing like 6000 gallons per day. Raw milk samples were collected from 66 supplies, representing a daily delivery of 1981 gallons to this dairy. Nineteen samples or 28·7 per cent. were found to contain *Br. abortus* and 5 or 7·5 per cent. tubercle bacilli. Seventy-eight samples of the pasteurised milk were obtained on 9 different days, but animal inoculation failed to show the presence of *Br. abortus* or tubercle bacilli in any of these specimens.

Dairy No. 2. This dairy also uses the retarding process for pasteurising, but it differs in type from No. 1. Raw milk specimens from 42 supplies, representing a total of 1566 gallons, were obtained; 12 specimens or 28·5 per cent. were found to contain *Br. abortus* and 7 or 16·6 per cent. tubercle bacilli. Samples of pasteurised milk were collected on 29 different days, making a total of 78. Not a single pasteurised sample was found to contain *Br. abortus*, but 3 or 3·8 per cent. contained tubercle bacilli, the three positive results being obtained from samples taken on 3 different days.

Table II. *Pasteurised milk samples.*

Pasteurising plant no.	Type	Pasteurisation at ° F.	No. of B samples taken	No. of C samples taken	Total	No. + for *Br. abortus*	No. + for *B. tuberculosis*
1	Holder	145	78	Nil	78	0	0
2	,,	145	57	21	78	0	3
3	,,	145	31	Nil	31	0	1
4	Flash	145	70	15	85	27	9
5	,,	145	2	22	24	2	3
6	,,	140	4	30	34	3	1
7	,,	145	1	28	29	0	2
8	,,	140	3	17	20	4	4
		Totals	246	133	379	36	23

Dairy No. 3 has a pasteurising plant similar to No. 2, and theoretically retards the milks for 30 min. at 145° F. From 74 raw milk supplies, representing a daily amount of 1867 gallons, 20 samples or 27 per cent. containing *Br. abortus* and 7 or 9·4 per cent. tubercle bacilli. Thirty-one milk samples were obtained after pasteurisation on six occasions, but none was found to show the presence of *Br. abortus* and only 1 or 3·2 per cent. tubercle bacilli.

Dairy No. 4. The plant consists of a single "flash-point" pasteuriser which brings the milk up momentarily to a temperature of 145° F. Samples of raw milk were obtained from 66 supplies, representing a daily amount of 2594 gallons; 21 specimens or 31·8 per cent. contained *Br. abortus* and 4 or 6 per cent. tubercle bacilli. Eighty-five pasteurised samples were collected on 24 days, 27 or 31·7 per cent. being found to contain *Br. abortus* and 9 or 10 per cent. tubercle bacilli. On one occasion, from 12 samples collected during one morning's run, 5 were found to contain *Br. abortus*; on another morning 10 contained *Br. abortus*; and on a third, 7 out of 12 contained *Br. abortus* and no less than 6 tubercle bacilli. The only apparent effect that this pasteurising plant has is to mix the infected milk with the uninfected.

Dairy No. 5. The plant consists of a small "flash-point" pasteuriser

working at a temperature of 145° F. None of the raw milk supplies was sampled at this small dairy, but a total of 24 specimens of the pasteurised milk was obtained on 12 days. Two samples or 8·3 per cent. contained *Br. abortus* and 3 or 12·5 per cent. contained tubercle bacilli.

Dairy No. 6. This dairy carries out "flash-point" pasteurisation at 140° F. No specimens were obtained of the raw supplies, but 33 samples of the treated milk were obtained on 17 days; 3 samples or 9 per cent. were found to contain *Br. abortus* and 1 or 3·3 per cent. tubercle bacilli.

Dairy No. 7. "Flash-point" pasteurisation here was carried out at 145° F. Specimens of raw milk from 12 supplies, representing a daily delivery of 287 gallons, were obtained. One sample or 8·5 per cent. contained *Br. abortus* and 2 or 17 per cent. tubercle bacilli. Thirty samples of the pasteurised milk were obtained over 15 different days, but none was found to contain *Br. abortus* and only 2 or 6·6 per cent. tubercle bacilli.

Dairy No. 8. This dairy conducts its pasteurisation by the "flash-point" method at 140° F. Raw milk samples from 10 supplies, representing a daily delivery of 469 gallons, were obtained; 3 samples or 33·3 per cent. contained *Br. abortus* and a similar number contained tubercle bacilli. Twenty specimens of the pasteurised milk were obtained on 10 days; 4 or 20 per cent. contained *Br. abortus* and a similar number tubercle bacilli.

Retailed samples.

A total of 189 samples were collected of milk ready to be retailed, and 18 or 9·5 per cent. contained *Br. abortus* and 16 or 8·4 per cent. tubercle bacilli. Of these samples 133 had been treated by heat, 21 being pasteurised by the "holding" method and 112 by the "flash-point" method. None of those treated by the "holding" method showed *Br. abortus* but 3 showed tubercle bacilli, while, as already shown, 10 samples treated by the "flash-point" method contained *Br. abortus* and 10 tubercle bacilli. Twenty-nine samples from untreated milk showed 4 positive for *Br. abortus* and 2 for tubercle bacilli, while from 27 retailed samples, where the previous treatment was unknown, 4 positive abortus and 1 positive tubercle results were obtained.

THE RESULTS OF THE ANALYSIS OF GUINEA-PIG INFECTIONS.

The results obtained, using the guinea-pig as the test animal for the presence of *Br. abortus* in milk, have now been set forth, and in view of the paucity of the literature on the actual susceptibility of this animal to the infecting dose of *Br. abortus* in milk the infections in the guinea-pig will now be analysed. It has been demonstrated that an injection of very few tubercle bacilli can produce a progressive disease in guinea-pigs, but the work of Hagan (1922) showed that several thousands of abortus bacilli may be required to be injected before a Brucella infection is established. Furthermore, if the guinea-pig killed after being inoculated for a period of 28 days shows evidence of a tuberculous infection, the second guinea-pig inoculated with part of the same specimen

will undoubtedly show more advanced lesions when killed 56 days after inoculation. The variation of individual susceptibility in guinea-pigs does not permit the same conditions to apply to Brucella infections, as it has been frequently found that the animal killed after the 28-day inoculation period showed evidence of infection, whereas the animal killed after being inoculated for 56 days with part of the same sample showed no evidence of infection.

Furthermore, as certain of the positive results are based on the results of positive agglutination tests only, it is necessary to examine the serological findings in normal or non-infected guinea-pigs and the serum reactions of animals inoculated with milk which must have contained killed *Br. abortus*, otherwise it might be suggested that the agglutinin response was due to normal agglutinins or to agglutinins produced as a result of a response to dead organisms.

For the purpose of studying the presence or absence of normal agglutinins in guinea-pigs, specimens of sera collected from animals killed for complement for the Wassermann Reaction and from animals killed after inoculation with pus, urine, and sputum from human sources for the presence of tubercle bacilli were tested. In all, sera from ninety-eight animals were tested in dilutions ranging from 1/12½ to 1/800, giving a final dilution of 1/25 to 1/1600 when the volume of standard Oxford suspension of *Br. abortus* was added. In not one single instance was agglutination obtained in any dilution. This agrees with the work of others who believe that normally no agglutinins are present in the sera of these animals even when used in 1/10 dilutions.

For the purpose of controlling the response of the animals to killed Brucella organisms, the samples collected from the three pasteurising plants using the "holding" method were utilised. From these three plants raw samples showed, Dairy No. 1, 66 raw samples with 19 or 28·7 per cent. positive abortus samples, Dairy No. 2, 44 raw samples with 12 or 28·5 per cent. positive abortus infections, and Dairy No. 3, 74 raw milk samples with 20 or 27 per cent. positive abortus infections, giving a total of 184 raw samples with 51 or 28 per cent. positive. From these plants 187 pasteurised samples were inoculated into 374 guinea-pigs, and not a single animal showed agglutinins even in the lowest dilution of the serum—1/25, 187 animals being killed after 28 days and 187 after an inoculation period of 56 days. It seems unlikely, therefore, that killed abortus bacilli, as found in milk, are capable of stimulating the production of agglutinins when injected subcutaneously. It is believed, therefore, that when agglutinins have been present in the serum of a guinea-pig an actual infection with living organisms has occurred, and thus the course of the infection in the guinea-pig will depend on the size of the infecting dose and the relative susceptibility of the animals infected.

GUINEA-PIG INFECTIONS.

The results of the guinea-pig infections are set forth in Table III, and are divided into groups depending on the results of the cultural and agglutination tests in the animals killed after inoculation for 28 days and for 56 days. Thus, out of a total of 123 positive infections, only nineteen pairs of animals showed both positive cultural and agglutination tests; in ten pairs the first animals killed showed both a positive culture and positive agglutination, while the second animals gave negative cultural and agglutination findings; in four

Table III. *Analysis of guinea-pig infections with* Br. abortus.

Guinea-pig killed after inoculation period of 28 days		Guinea-pig killed after inoculation period of 56 days		A, B, and C samples: no. of pairs in each category
Culture of spleen and liver	Serum agglut.	Culture of spleen and liver	Serum agglut.	
+	+	+	+	19
+	+	−	−	10
+	+	+	−	0
+	+	−	+	4
+	−	+	+	1
+	−	−	−	2
+	−	+	−	0
+	−	−	+	0
−	+	+	+	13
−	+	−	−	8
−	+	+	−	0
−	+	−	+	0
−	−	+	+	53
−	−	+	−	2
−	−	−	+	11
			Totals	123

+ = positive culture or positive serum agglutination.
− = negative culture or negative serum agglutination.

instances the first animals showed positive cultural and agglutination tests, while the second animals showed only the presence of agglutinins; in one pair the first guinea-pigs gave a positive culture only, while the second gave both a positive culture and a positive agglutination test; in two instances the first animals only gave a positive culture without showing the presence of agglutinins, while the second animals showed neither a positive culture nor agglutinins; similarly in two instances the second animals gave a positive culture, while neither the first nor the second showed agglutinins in the serum in the lowest dilution tested—1/25; in thirteen instances the first animal gave a negative culture, while the second gave both a positive culture and a positive agglutination test; in eight instances the first guinea-pigs gave only a positive agglutination test, while in eleven pairs the second animals gave only a positive agglutination test; in no less than fifty-three instances the first animals failed to give a positive culture and positive agglutination test, while the second gave both a positive agglutination test and a positive culture. Finally, 104

positive cultural results were obtained from 123 pairs of guinea-pigs, giving a percentage of 84·5, while 19 or 15·5 per cent. of results were based on positive agglutination results only.

Agglutination reactions with sera of infected guinea-pigs.

The titres associated with infection in animals killed are given in Table IV. It will be seen that fifty-four animals gave positive agglutination tests after the 28-day inoculation period, while the sera from 101 animals reacted positively after an inoculation period of 56 days. The highest titre occurring in the first group was 1/1600, while the majority of reactions ranged from 1/100 to 1/400. In the second group the sera from three animals showed a maximum titre of 1/6400, while altogether a greater percentage showed higher titres than those in the first group.

Table IV. *Agglutination reactions with serum from guinea-pigs.*

	Titre of sera and number of positive reactions									
	1/25	1/50	1/100	1/200	1/400	1/800	1/1600	1/3200	1/6400	Total
Animals killed after inoculation period of 28 days	1	7	11	15	10	5	5	0	0	54
Percentage +	1·8	12·9	20·3	27·7	18·5	9·2	9·2	0	0	
Animals killed after inoculation period of 56 days	3	2	10	17	20	10	26	10	3	101
Percentage +	2·9	1·9	9·9	16·8	19·0	9·9	25·9	9·9	2·9	

Cultural and serological characteristics of strains of *Br. abortus*.

The cultural characteristics of *Br. abortus* have not shown any particular deviation from the normal types. All strains, with the exception of two, grew only in the presence of 10 per cent. carbon dioxide, and these two grew in the normal atmosphere of the incubator. Strain A13/2 grew on liver agar plates after an incubation period of 3 days, the growth, however, on the plates incubated in 10 per cent. CO_2 was more profuse than that obtained in the normal atmosphere, while strain A66/2 however grew better on the plates in the normal atmosphere than on those incubated in 10 per cent. CO_2. Inquiry at the farms from which the original milk samples were derived showed that there had been repeated outbreaks of infective abortion and that as a result the farmers had had their cows inoculated with a living abortus vaccine. The cultural characteristics of the two strains may, therefore, have been due to the fact that the attenuated living strains used for immunisation had developed the faculty of growing in the normal atmosphere.

As regards the serological characteristics of the strains seven agglutinating sera were prepared from various strains, and cross agglutination and cross absorption tests with these various strains and their sera showed that each serum agglutinated all strains to practically full titre, and, furthermore, the

cross absorption tests showed that all strains could completely remove the agglutinins from the various sera. This showed that there was no serological difference in any of these freshly isolated strains. Accordingly, in all, 104 strains as isolated were tested against the serum prepared for strain A16/2; all were agglutinated to practically full titre (1/6400) and all absorbed from the serum the agglutinins for the homologous strain. Serologically, therefore, only one race of *Br. abortus* has been encountered in this investigation.

POST-MORTEM FINDINGS IN GUINEA-PIGS.

The post-mortem pathological findings in guinea-pigs infected with tubercle bacilli are so very characteristic that they require no discussion here, but the gross macroscopic pathological findings associated with infections due to *Br. abortus* have not been so definite. The guinea-pigs were inoculated in the left groin and, considering the fact that the milk deposit was untreated, remarkably few animals died from immediate infection. In animals found to be infected with *Br. abortus*, no local abscess formation occurred; occasionally there was slight enlargement of a left abdominal lymph node, but this gland contained no pus. The most characteristic feature appeared to be enlargement of the spleen, without the formation of any tubercle-like nodules. From the enlargement of the spleen the presence of an abortus infection could be foretold before the cultural and agglutination results were available. The other organs failed to show any characteristic signs of Brucella infections.

DISCUSSION.

There is no necessity to discuss in detail the incidence of tuberculous infection of milk, the results of which will, in due course, be dealt with by the Department of Health. The raw milk supplies showed a very marked infection with tubercle bacilli and a still more gross infection with *Br. abortus*. As already stated, the "holding" method of pasteurising the milk at 145° F. for 30 min. has been found to be effective in destroying *Br. abortus* but has not been so successful in dealing with tubercle bacilli, although the incidence of infection would appear to be greatly reduced. One "holding" pasteurising plant was entirely successful but another gave 3 positive samples out of a total of 78, while from a third 1 positive sample from 31 was obtained. The two pasteurising plants showing the positive tubercle results were of the same type structurally, and the results may be due to either mechanical defects or to laxity on the part of the staff in conducting the pasteurisation. The so-called "flash-point" method of pasteurisation at a temperature of 140–145° F. has not been successful. Admittedly this method is employed by dairymen, mainly with the object of maintaining the keeping qualities of the milk, but as the method has little or no lethal effect on *Br. abortus* and tubercle bacilli, it seems unlikely that it will destroy other organisms with an optimum growth temperature of 37° C. On the other hand, McIntosh and

Whitby (1931) have shown that pasteurisation by the "holding" method fails in regard to killing of thermophilic and thermoduric bacteria, whereas "flash-point" pasteurisation using a temperature of 160–163° F. is effective. The results suggest the need for efficient pasteurisation of all ordinary milk samples as produced at present.

In another paper, Smith (1932), it has been shown that only ten cases of undulant fever have been encountered over a period of two years, and these were practically all diagnosed in the first instance on the basis of a positive agglutination test with the patient's blood serum. Thus, out of 273 specimens of blood sent for the Widal reaction, in 10 or 3·6 per cent. of patients the illness was due to a *Br. abortus* infection. Considering the large number of people who must constantly be ingesting living abortus bacilli, the strains must be relatively avirulent for human beings or human beings must have, on the whole, a very high immunity for this organism.

The analysis of the guinea-pig infections suggests the possibility that the actual number of milk supplies infected with *Br. abortus* is underestimated by this method, and that milk supplies with comparatively few organisms present do not always set up infection when inoculated. Furthermore, in those guinea-pigs in which a positive agglutination test but no positive cultural result was obtained, the infecting dose had probably been very small and the guinea-pig was rapidly able to deal with the infection. In a recent paper by Boak and Carpenter (1931) it was shown that when 1 c.c. quantities of milk containing 110 to 135 millions of killed *Br. abortus* were injected subcutaneously the guinea-pigs failed to produce any agglutinins, even in a 1/10 dilution of the sera.

The problem of establishing dairy herds free from abortus infection seems to be as great, if not greater, than establishing tubercle free herds. In America the need for such an effort has already been envisaged. Meyer (1931), in an extensive paper dealing with the eradication of cows infected with *Br. abortus* in dairy herds producing certified milk, showed that an initial survey of 3701 animals in five dairy herds in 1927 revealed 38·2 per cent. reactors. A system of segregation and replacement was introduced, and in 1929 the same herds showed only 1·7 per cent. reactors. The difficulties involved in disposing of infected cows and of purchasing fresh animals free from infection are duly emphasised, also the immense amount of laboratory investigation necessary. When, for instance, there are isolated herds free from Bang's disease surrounded by infected herds, Meyer contends that it would be necessary to conduct serum agglutination tests every thirty days to prevent the possibility of catastrophe in the non-infected herd. Meyer further admits that the experimental stage in this preventive measure has not been passed, and it is within the bounds of possibility that it will be eventually necessary to advocate the pasteurisation of even certified milk in order to free it from living forms of *Br. abortus*.

Further investigation will doubtless, in due course, show the extent to

which cases of undulant fever are occurring in this country, and should the prevalence be similar to that found in Denmark and the United States, then the dairying industry has a very extensive problem to solve if milk is to be produced and made safe for human consumption without using pasteurisation. Not only so but the possibility of milk-borne outbreaks of Salmonella, streptococcal, and diptheria infections due to actual cow infections, and the possibility of contamination of milk supplies by the workers makes one wonder whether after all it would not be better to establish effective pasteurising methods rather than to aim at creating and trying to maintain abortus and tubercle free herds. Furthermore, the arguments that have been produced to show that pasteurisation has a deleterious action on the vitamins and chemical constituents have not been particularly conclusive, and so far appear to be offset by experiments showing that the heat as used in pasteurisation has no injurious effect.

CONCLUSIONS.

1. An investigation into the incidence of *Br. abortus* and tubercle bacilli in milk has shown *Br. abortus* to be present in 79 or 28·3 per cent. of 279 different specimens of raw milk, and *B. tuberculosis* in 29 or 10·3 per cent. of the same samples.

2. In a total of 379 samples collected from eight pasteurising plants, 36 or 9·4 per cent. were found to contain *Br. abortus* and 23 or 6·0 per cent. tubercle bacilli.

3. From 187 samples treated by "holding" pasteurisation at 145° F. for 30 min., *Br. abortus* was not obtained, but 4 or 2·1 per cent. of samples showed the presence of tubercle bacilli.

4. From 192 samples treated by "flash" pasteurisation, 36 or 18·7 per cent. were found to contain *Br. abortus* while 19 or 9·8 per cent. contained tubercle bacilli.

5. No normal agglutinins for *Br. abortus* were found in the sera of 98 non-infected guinea-pigs when tested in dilutions ranging from 1/25 to 1/1600.

6. The guinea-pig infections with *Br. abortus* have been analysed, the serum agglutination and cultural tests being compared and the results suggest that the actual total incidence of *Br. abortus* in milk is underestimated by the guinea-pig inoculation method.

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