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INQUIRIES INTO THE PATHOGENIC EFFECTS PRODUCED BY *BRUCELLA ABORTUS* IN THE UDDER AND CERTAIN OTHER ORGANS OF THE COW

BY

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ILLUSTRATED BY TWENTY-SIX PLATES WITH FIFTY FIGURES AND ONE SCHEME IN THE TEXT

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A. Introduction [1]).

The discovery of *Brucella abortus* as the originator of bovine infectious abortion by B. Bang and Stribolt in 1896, in Denmark, indicates the beginning of a new era in the investigation of the most significant cattle disease of the present age — "Bang's abortion disease" or cattle brucellosis. That in many cases bovine abortion was contagious and an artificial infection possible, was already known before [Cruzel (1832) (37), Johne (1872) (86), Bräuer (1873) (17), Franck (1876) (50), Lehnert (1878) (108), Nocard (1886) (131), Trinchera (1882) (178), *et al.*]. But with the discovery of *Brucella abortus* and the explanation of its aetiological significance by B. Bang and Stribolt, the inquiries into the disease caused by the above-mentioned microbe were placed on a reliable basis and could be grounded on scientific evidence.

In the first decade of the present century, cattle brucellosis was found in various parts of Europe and America, and at present its occurrence throughout the world has been proved by numerous researches.

Later researches show that the pathogenic effect of *Brucella abortus* does not confine itself to cattle, but that the microbe infects also men, swine, goats, in rarer cases sheep and horses, in several cases dogs [Saceghem (149), Planz and Huddleson (135)], cats [Koegel (100), Makkawejsky and Karkadinowskaja (119)], and certain birds, *viz.* hens and turkeys [Huddleson and Emmel (80), Fabyan (44), Gilman and Burnett (55), Koegel (100)]. All the animals mentioned above have also been infected with *Brucella abortus* by means of artificial inoculation, and in addition to the usual object of experiments — the guinea-pig — also apes, rabbits, mice, rats, pheasants, geese, ducks and pigeons.

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1) The studies here reported were carried out within the years 1930—1933, and were submitted to the Veterinary Faculty as a thesis in 1933. On account of its having been abbreviated and the delay of its translation into English, I was not able to have it printed earlier.

There is no doubt that brucellosis is the most momentous of all diseases affecting cattle, owing to its frequent occurrence all over the world and the great economical damage caused by it.

This appears also from the large number of inquiries made into the character of this disease for the purpose of elucidating and fighting it. The direct damages caused by brucellosis in herds are as follows: 1) the waste of calves, 2) the damage effected by indisposition of the genitals leading to sterility, and 3) the decrease of milk production. Hitherto but slight attention has been paid to the decrease of milk production due to *Br. abortus*, and the causes of this fact have been little studied. Usually the diminution of milk production in cases of abortion is explained as an indirect result of the latter, and very few authors have ever tried to connect the decrease of milk production with brucellous infection of the udder. Evidently these problems have not been sufficiently studied hitherto and fully explained, because brucellosis as a very widespread infection was not known to be the chief cause of the pathological-histological changes in cattle, and specially in the udder.

The economic damage to stock-farming caused by brucellosis in many countries has been estimated in money. Thus the amount of the annual damage from brucellosis in the United States of America, in 1916, was about 20 million dollars [Giltner, Hallmann, and Cooledge (58)], in 1925, it was more than 100 millions, and in 1930, it amounted to 200—300 million dollars [Barnes (10)]. The damage caused by brucellosis in Germany, in 1925, was estimated at 100 millions [Klimmer (93)], in 1926 — at 200 millions (according to the statistics of the hygienic exhibition at Düsseldorf), in 1930 — at 250 millions [Zwick (191)], and in 1931 — at 200 millions RM every year [Zeller (188)]. The annual damage in Estonia, in 1932, is estimated at 2 million Est. crowns [Laja (105)].

In addition to the direct damage caused by brucellosis, the expense connected with the researches into this disease ought to be specially mentioned. According to the data of Williams (182), in USA, for the investigation of no cattle disease has there been spent so much money as for the purpose of elucidating brucellosis.

In the years following the discovery of *Br. abortus*, cattle brucellosis was considered to have its seat only in the pregnant uterus and in the genitals of bulls, without including hereditary brucellosis in new-born calves. This view, however, could be held

plausible only until 1912, because up to that time *Br. abortus* had not been found either in the excretions and secretions, or in any organ besides the genitals. But already in the inquiries dating from the years 1912 and 1913, Smith and Fabyan (165), Melvin (124), Schroeder and Cotton (152), Mohler and Traum (128) proved for the first time the presence of *Br. abortus* in milk by means of experiments on guinea-pigs. A little later, Zwick and Krage (192) reared a culture of *Br. abortus* eliminated with milk. These investigations were followed by numerous additional experiments. It became evident that a high percentage of naturally infected cows, both pregnant and non-pregnant, and cows that had aborted and that had not, eliminate *Br. abortus* with their milk [Robinson (144), Cotton (35), Schroeder and Cotton (157), Cooledge (34), Winkler (183), Bogenschneider (15), Hart and Traum (70), Buck and Creech (18), Carpenter (24), Prillwitz (138), Pfenniger and Krupski (134), Bang and Bendixen (79), Steck (170), Mitchell and Humphreys (127), Pröscholdt (140), Lerche (111), *etc.*]. Besides infecting the udder, the microbe was discovered before long also in the lymph nodes of the udder and the pelvis [Schroeder and Cotton (155), Robinson (144), Prillwitz (138), *etc.*]. Now, from these inquiries into the matter it was clear that the infection affects not only the genitals but also the udder and the lymph nodes belonging to it. But the prevailing opinion was that brucellosis as such was not a general infectious disease in the bovine organism, but was located only in the organs already mentioned. More observations and inquiries ensued which showed that in cases of natural infection *Br. abortus* established itself in the organism of the cattle in various organs and parts of the body and produced its pathogenic effects. Thus *Br. abortus* has been detected in the articulations and the tendon sheaths in cases of extensive changes of the articulations and inflammations of the tendon sheaths [Buck and Creech (19), Bang and Bendixen (8)], and comparatively frequently in the hygromata of the knees [Boyd, Delez and Fitch (16), Panisset and Comptois (133), Magnusson (177), van der Hoeden (77), Roots and Ridala (145)]. Studying cattle sputum in connection with tuberculosis, Pröscholdt ascertained [1926 (139)] the presence of *Br. abortus* by means of experiments on guinea-pigs in 2·3 per cent. of the sputum-samples. Considering these results,

the author was of the opinion that *Br. abortus* is eliminated also with bronchial mucus.

The microbe is supposed to be carried to its habitats in the haematogenic or lymphogenic way, but no one has as yet tried to ascertain the actual possibility of the blood-infection in the case of a natural infection. Only in 1931, Götze and Müller (64) declared that they succeeded in infecting a cow artificially, transfusing the blood of a brucellous cow into an undiseased one. Relying on this result, Götze and Müller presume that in cases of bovine brucellosis a general bacteriemia is possible for at least a while. Krüger [1932 (102)] detected *Br. abortus* by guinea-pig inoculation in the diaphragm of 3 slaughtered cows.

Roots and Ridala [1932 (145)] demonstrated in their work for the first time by a number of experiments cases of natural brucellous infection, and proved the presence of *Br. abortus* and the considerable histological changes due to it, besides the other organs of cattle, also in the spleen and the thyroid gland. Thus the inquiries of late years greatly enhance our knowledge of brucellosis in the bovine organism. It has been ascertained that besides its usual habitat (the genitals and the udder), *Br. abortus* is present in many parts of the body.

Bovine abortion is a comparatively frequent symptom of the pathogenic effect produced by *Br. abortus*. Brucellosis attracted notice through abortion already in former years, and that is just why inquiries into the disease began. It is natural that the disease was then named "abortus infectiosus". The disease bore this name down to our own times, and it is still called so by some authors. But, since later examinations showed that abortion was only one of the pathological symptoms and did not occur in a large percentage of brucellous cases, the denomination "abortus infectiosus" was no longer suitable. More general names had to be found which were independent of the symptoms of the disease. Now "Bang's infection", and in most recent times "abortus brucellosis" (opposite to melitensis-brucellosis) or simply "brucellosis" are used. In the present work the denomination "brucellosis" is made use of, as melitensis-brucellosis does not occur with us. The morbific agent has likewise had different names, e. g. "*Bacterium abortus infectiosi Bang*", "*Alcaligenes abortus*", "*Brucella abortus (Bang)*" etc. "*Brucella abortus (Bang)*" is used throughout

this work as corresponding best to the rules of the systematic nomenclature of bacteria.

The pathological changes in the fetal membranes *(placentitis et cotyledonitis necrotica)* and endometritis in cattle, caused by brucellosis, have already been known since the discovery of *Br. abortus*. Further, a comparatively frequent inflammation of the testes of bulls and the testicles, in connection with coagulation necrosis and abscesses caused by *Br. abortus*, was ascertained [Schroeder and Cotton (156), Buck, Creech and Ladson (20), Marcis (120), Schlegel (150), Ohlson (132), Bluhm (14), Christiansen (27), Ehrlich (40), Magnusson (116), Mirri (126), Lerche (110), Cominotti (28), Richter (142), Witte (184), *etc.*]. But an inflammation of the seminal vesicle and prostata and the nodes on the mucous membrane of the penis have been found less frequently [Bürki (22), Bluhm (14), Buck, Creech and Ladson (20), Gilman and Hopper (56), Magnusson (116), *etc.*]. The histological finding in the genitals of bulls is severally similar to the changes due to tuberculosis. In singular brucellous cases, acute, subacute, and chronic inflammatory foci in the thyroid gland were observed [Roots and Ridala (145)], and an inflammation of sheaths of tendons and arthritis was noted by Buck and Creech (19), O. Bang and Bendixen (8), *etc.* *Br. abortus* has been found also in the hygromata of the knees, which were first of all considered to be caused by various mechanical injuries (stone-floors etc.), but when the damaged tissue proved to be propitious for *Br. abortus* to establish itself [Boyd, Delez and Fitch (16), Panisset and Comptois (133), Magnusson (117), van der Hoeden (77), Roots and Ridala (145)], the above-mentioned microbe would also produce its pathogenic effect there.

Inflammation due to brucellosis has been observed in the abomasus and intestines of the embryo and new-born calves [Zwick and Zeller (193), Klimmer (91), Witte (185), *etc.*], in the serous membranes [Zwick and Zeller (193), Haupt (72), *etc.*], in the lungs [Smith (164), *etc.*], and in the urinary bladder. Further, an edema of the umbilical cord, of the belly and the chest has been noticed.

According to the information we have, $12 \cdot 5 - 91 \cdot 8\%$ of cows naturally infected with *Br. abortus* eliminate the bacterium in question, mostly for quite a long period, in some cases for about

7 years. The problem that presents itself is whether *Br. abortus* is present in the udder as a loyal commensal, or whether it causes changes there in the same way as in the other parts and organs of the body. There is no doubt that the milk production of cows decreases in connection with brucellosis, but most contemporary authors ascribe this exclusively to the fact of abortion, which, as we know, leaves the udder unsatisfactorily prepared for lactation. Despite the importance of this factor, it is certain that it is not the only one to which the decrease of milk production is due. In this connection, the data of Simms and Miller (162) are of special importance, showing, as they do, that the diminished milk production in cases of brucellosis may last for years, even though the number of abortions among cattle should steadily decrease. Similarly, Laja (104, 106) notes that three herds of brucellous cows that had not aborted, though continuing to yield milk, produced about 25 per cent. less milk than those that were unaffected. This shows that over and above abortion there must be other agents causing the decrease of milk production in infected cows. Of these agents, those that have to be examined in the first place, are the direct functional disturbances in the udder due to *Br. abortus*.

The few statements to be found in literature show that but little attention has been paid to inquiries into the pathogenic effects produced by *Br. abortus* in the udder. Certain authors [Cooledge (33), Tweed (180), Runnels and Huddleson (147), O. Bang and Bendixen (9)] have noted a high cellular count in the milk in cases of *brucellosis*, but in a number of cases no attention was paid to other possible infections of the udder that may likewise bring about a high cell count of the milk, without causing any clinical symptoms in the udder. Furthermore, it has been observed that the quantity of milk sugar and fat in the milk decreased, whereas there was an increase in catalase, chlorine and sediments [O. Bang (5)]. Only Schroeder and Cotton (154) have noted macroscopically small foci of induration in cases of brucellosis in the udders of cows. In the study of brucellous udders histologically [Runnels and Huddleson (147), Smith, Orcutt and Little (167)], there have been noticed small acute, subacute, and chronic inflammatory foci, whereas other possible infections have not always been taken into consideration. No one has so far ascertained with any accuracy the nature of the changes

observed, nor have the possible variations or the extent of these changes been studied with any care. It is not clear whether there was any *Br. abortus* in the parts thus changed, or whether this microbe could be found in the histological sections. Scientists are of different opinions as to the changes in the udder, and some of them have failed altogether to notice any pathological changes there [Friedemann (52)].

Hence it appears that our knowledge concerning the pathogenic effects produced by *Br. abortus* in the udder of the cow is even now insufficient. It is therefore most urgent that, because of the aetiological significance of brucellosis and for the purpose of fighting the disease, the following questions should be elucidated:

1) Do any pathological changes take place in the udder if *Br. abortus* is eliminated with the milk?

2) In what parts of the udder and to what extent do the changes take place?

3) What is the form and duration of these changes?

4) Is there any *Br. abortus* in the parts thus altered?

5) Are the amount and composition of the milk affected by these processes?

In the present study I have set myself the immediate task of solving these problems. I have specially studied the pathological changes in the bovine udder and in the adjoining lymph nodes, as well as the presence of *Br. abortus* in the changed parts. Nor have I omitted any other possible agents. In addition I have examined the same animals, as to finding possible excitants of diseases and pathological changes in the various other organs and parts of the body.

B. Literature concerning the Question.

a. The Presence of *Brucella Abortus* in the Milk of Cows.

Smith and Fabyan (165), Melvin (124), Schroeder and Cotton (152), Mohler and Traum (128) were the first to find *Br. abortus* in the milk of cows, studying it by means of experiments on guinea-pigs for *Mycobacterium tuberculosis*. The internal organs of the injected guinea-pigs showed changes which might have easily been taken to be the changes observed in cases of tuberculosis, but, as they proved to be negative for *Mycobacterium tuberculosis*, the inquiries had to be continued. The above-mentioned

scientists succeeded indeed in isolating then a culture of *Br. abortus* from the organs thus changed.

Zwick and Krage (192) cultivated *Br. abortus* (1913) from the milk sediment of cows that had aborted 3—14 days before and even 6 and 13 months before. There was no evidence of any macroscopical changes in the milk of these cows nor any clinical symptoms in the udder.

Klimmer and Winkler (95) observed that after aborting, cows eliminate *Br. abortus* with the milk for quite a long time. The authors did not find any macroscopical changes in the milk, in the udder, nor the adjoining lymph nodes.

Robinson (144) ascertained that there was brucellous infection in the udders as well as in the lymph nodes of the udders and pelvis of several non-pregnant cows.

Schroeder and Cotton (157) found out that 60 per cent. of naturally infected cows eliminate *Br. abortus* with their milk. The elimination may last for about one week or for several months, and in some cases even for about 6 to 7 years. They made sure that *Br. abortus* was eliminated with the milk of cows that had not aborted at all, and of those about to abort in a month's time.

Cooledge (34) studied the milk of 118 cows from 7 farms, and found that 27 per cent. of the cows examined eliminated *Br. abortus* with their milk. But he did not find the bacteria always present when the milk contained agglutinins of *Br. abortus*. The milk taken in the middle of milking was most abundant in agglutinins. In determining the immune corpuscles of *Br. abortus* in the milk, the complement fixation and the agglutination tests were equivalent.

Thomsen (173) takes it for granted that *Br. abortus* is eliminated with the milk of every infected cow.

Steck (170) detected *Br. abortus* in the milk of several cows that had not aborted. In one case he found the microbe present in the milk several months previous to the time of aborting. The author of this study is of the opinion that *Br. abortus* may occur also in the udder of perfectly healthy cows.

Winkler (183) studied the milk of 32 infected cows; 13 (41 per cent.) of the cows eliminated *Br. abortus* with their milk after they had aborted, but not continuously.

Bogenschneider (15) found that 2 (12·5 per cent.) out of 16 naturally infected cows had *Br. abortus* in their milk.

Zeller (187) states that in cases of brucellosis, cows often eliminate with their milk the microbe in question. Further, he declares that the general methods — the cultivation methods and guinea-pig inoculation are not absolutely reliable methods for determining the presence of *Br. abortus* in milk.

Smith, Orcutt, and Little (166) injected a living and a killed culture of *Br. abortus* into one quarter of the udder. Further, an injection of the cultures was given subcutaneously and intravenously. The milk of the injected quarter showed in the first case many more agglutinins than in the latter cases. Taking into consideration the results of the experiments, the authors are of the opinion that the anti-bodies of *Br. abortus* also originate in the udder.

Schroeder and Cotton (153) studied repeatedly by means of the guinea-pig experiment the milk of 56 cows, taking the milk samples from each quarter of the udder individually. They noted that none of the cows whose blood serum agglutinated *Br. abortus* in dilutions 1 : 100 or less, eliminated *Br. abortus* with their milk, whereas 25 (83·3 per cent.) out of 30 cows eliminated the microbe, the aggl. titer of their blood serum being 1 : 200 and above. Hence, based upon this finding, the authors draw the conceivable conclusion that only those cows eliminate *Br. abortus* with their milk whose blood serum titer is above 1 : 100.

Hart and Traum (70) noted that *Br. abortus* was eliminated with the milk of cows whose blood serum did not agglutinate the above-mentioned microbe, and with that of vaccinated cows.

Buck and Creech (18) found that 17 (56·7 per cent.) out of 30 brucellous cows eliminated the microbe with their milk. They did not succeed in exterminating the disease from the udder through vaccination.

Carpenter (24) observed *Br. abortus* also in the milk of non-pregnant cows. The author examined two herds by comparing them. He vaccinated one herd, being uncertain of the extent of the infection in it, and found that the agglutination of *Br. abortus* was positive in 86 per cent. of the cows, and 38 per cent. of them eliminated the microbe with their milk. The second herd was not given an injection of *Br. abortus* vaccine; it appeared that the agglutination titer of the blood serum of 72 per cent. of the cows was positive, and 66 per cent. of the cows eliminated *Br. abortus* with their milk. Thus, in the second herd the microbe was eliminated

by 91·8 per cent. of the cows that had reacted positively to the blood test. Later on Carpenter serologically examined one more herd where no abortion had occurred. Two cows in this herd proved brucellous, one of them eliminating the microbe with her milk. The author was of the opinion that the subsequent epidemic in the herd was due to the lastmentioned cow.

Prillwitz (138) serologically studied 160 cows assigned for slaughter, and found that 11 of them were infected with *Br. abortus*. The microbe was to be found in the milk of 4 cows and in the lymph nodes of the udder of one cow.

Carpenter and Boak (26) found the presence of *Br. abortus* by means of the guinea-pig experiment in the cream samples of 23 (6·08 per cent.) cows, where the sum total of the cream samples for the purpose of the study was 378, taken from 378 cows individually.

Bang and Bendixen (7) examined the milk of cows belonging to 7 herds. The results were as follows: in the first herd 4 (33·3 per cent.) out of 12 cows eliminated *Br. abortus* with their milk; in the second herd 1 (5·5 per cent.) out of 18 (7 samples taken mostly from cows that had aborted were lost), in the third herd 2 (66·7 per cent.) out of 3 cows eliminated the microbe; in the fourth herd 7 (43·1 per cent.) out of 16, in the fifth herd 4 (44·4 per cent.) out of 9, in the sixth herd 1 (25 per cent.) out of 4, and in the seventh herd 3 (33·3 per cent.) out of 9 cows eliminated the microbe. Thus, 35·9 per cent. of all the cows belonging to the 7 herds eliminated *Br. abortus* with their milk. The authors think that the guinea-pig experiment is not an absolutely reliable method of ascertaining the presence of *Br. abortus* in milk. Brucellous infection is to be noticed more frequently in the rear quarters of the udder.

King and Caldwell (89) did not find *Br. abortus* by guinea-pig inoculation in the milk samples of those cows that had an agglutination titer of their blood serum 1:60 or less (in 151 cases). But 23 out of 56 cows, the agglutination titer of whose blood serum was 1:120 or above, eliminated the microbe with their milk. *Br. abortus* was detected twice in the milk of a cow, but there was no evidence of agglutinins in her blood.

Van der Hoeden (78) found *Br. abortus* present in the milk of cows from a farm where there had not been any cases of abortion for several years. Furthermore, he observed a cow that had not

aborted but whose milk, nevertheless, contained *Br. abortus*, though the agglutinins of the microbe had never been found in her blood.

Drescher and Hopfengärtner (39) ascertained the presence of *Br. abortus* in the milk of 21 per cent. to 83 per cent. of cows that were infected with *Br. abortus*.

Graham and Throp (59) studied the milk of 78 cows whose blood reaction with *Br. abortus* was positive, and found in the milk of 45 (57·7 per cent.) of these cows the agglutinins of *Br. abortus*.

Traum (175) states that, according to the agglutination reaction, 20 per cent. of the milk cows in the U. S. A. are affected with *Br. abortus*, and 30 per cent. to 50 per cent. of them eliminate the microbe causing the disease.

Jensen (84) thinks that at least 10 per cent. to 20 per cent. of brucellous cows eliminate *Br. abortus* with their milk. Further, he is of the opinion that about 20 per cent. to 30 per cent. of market-milk contains *Br. abortus*.

Gilman (53) studied the milk of 34 cows, taken from each quarter of the udder individually, to find out whether it showed any agglutinins and bacteria present or not. He never noticed any *Br. abortus* when the agglutination titer of the milk serum was less than 1:80, nor when the titer of the blood serum was under 1:320. But the above-mentioned microbe was observed in 53·7 per cent. of the milk from the quarters of those udders that had an agglutination titer of the milk serum 1:80 and above.

Schumann and Lerche (159) found brucellous infection in 278 (22 per cent.) milk samples taken from 1265 herds. The blood test for *Br. abortus* was positive also in farms where cows had never aborted, and some of the cows eliminated the bacterium in question. *Br. abortus* was found in the milk of 49 per cent. of the 51 cows that had reacted positively to the serological test; in another case, 52·9 per cent. of 19 cows that reacted positively but had never aborted, had *Br. abortus* in their milk. According to the opinion of the authors, based on the results of these experiments, half the number of brucellous cows eliminate the excitant of the disease with their milk.

Lerche's (111, 112) data show that the occurrence of brucellosis in cattle is very frequent indeed; 20 per cent. is the average amount of infection. The author notes that *Br. abortus* was eliminated with the milk of cows that had aborted and that

had not aborted. 54 (52·3 per cent.) out of 104 cows whose blood serum was positive to *Br. abortus* agglutination, contained the microbe in their milk. Among these 104 cows examined, there were 23 that had calved normally, and 14 (60·9 per cent.) of them eliminated *Br. abortus*. In another farm, 38 (45·2 per cent.) out of 84 cows that had aborted eliminated *Br. abortus*. Hence it follows that *Br. abortus* may be present even more frequently in the milk of those cows that have calved normally than of those that have aborted. According to the same author, *Br. abortus* was present in the milk of 18 cows (58 per cent.) out of 31 belonging to one herd. The elimination of the above-mentioned microbe with milk is not always regular; sometimes the presence of the bacteria cannot be ascertained in milk, though the udder be infected. *Br. abortus* is not always eliminated with the milk from all the quarters of the udder, sometimes only one or two of the quarters eliminate the bacterium in question. The elimination of *Br. abortus* with milk may last for months and even for years. *Br. abortus* was found by means of the animal experiment in 18 milk samples, but by means of the cultivation method it was found only in 4 milk samples out of 18. Lerche ascertained the germ count of *Br. abortus* eliminated with the milk of two cows: in 1 ccm milk there were about 60—320 bacteria. The author succeeded in determining *Br. abortus* by means of the cultivation method in 11 cases in the milk taken at the end of milking from 14 cows (that eliminated the microbe with their milk); in the milk samples taken at the beginning and in the middle of the milking it was present only in four cases. Considering these results, Lerche is of the opinion that *Br. abortus* is located in the parenchyma of the udder, whence it is driven into the milk at the end of milking by the natural massage of the udder.

Makkawejsky, Karkadinowsky, Michejeff, Gawriloff, and Dawydowsky (118) studied the milk of 68 cows that had aborted, and found that 14·7 per cent. of them eliminated *Br. abortus*.

Mitchell and Humphreys (127) detected *Br. abortus* in the milk of 12 (75 per cent.) cows out of 16 that had reacted. Two cows yielded milk that was infected in one quarter, three cows had it in two quarters, five cows in three quarters, and two in all four quarters of the udder.

Gwatkin (63) examined the milk of 31 cows that had

calved normally. It followed that 2 cows the agglutination titer of whose blood serum was 1:50, did not eliminate the germs of the disease with their milk, 30 per cent. of 10 cows with an aggl. titer of 1:100 eliminated *Br. abortus*, 100 per cent. of 3 cows with an aggl. titer of 1:250 also eliminated *Br. abortus*, 75 per cent. of 4 cows with an aggl. titer of 1:500, and 50 per cent. of 12 cows with an aggl. titer of 1:1000 eliminated the above-mentioned microbe with their milk. On the whole, 15 (48·4 per cent.) out of the 31 examined cows eliminated *Br. abortus* with their milk.

Cotton and Buck (36) found that only few of the cows whose aggl. titer of the blood serum was 1:100 or less, eliminated *Br. abortus* with their milk; they were either lately infected or their aggl. titer was constantly rising. The above-ementioned microbe was eliminated by 50 per cent. of the cows whose aggl. titer was 1:200, and almost all of the cows whose aggl. titer was 1:1000 eliminated *Br. abortus* with their milk.

Tullberg (179) could seldom make sure the presence of *Br. abortus* by means of experiments on the guinea-pig in the milk of cows, the aggl. titer of whose blood serum was 1:10 to 1:30; he found it present in 19 per cent. of cases with an aggl. titer of 1:70, and in 50 per cent. of cases where the aggl. titer was 1:100 or above.

Pröscholdt (140) has made extensive inquiries into the occurrence of *Br. abortus* and its agglutinins in bovine milk. Table I presents the results of these studies.

Hence it appears that 203 (53 per cent.) out of the 383 cows examined (with an aggl. titer of the blood serum 1:100 to 1:64 000) eliminated *Br. abortus* with their milk. Further, he found that only 3 per cent. of cows, the aggl. titer of whose blood serum was 1:100 and less, eliminated the bacterium in question. 94 per cent. to 96 per cent. of cows eliminating *Br. abortus* had an aggl. titer of the blood serum 1:100 and above, and about 81 per cent. to 86·6 per cent. of the cows had likewise agglutinins of *Br. abortus* in their milk. Among 208 cows eliminating *Br. abortus*, there were 145 cows that had aborted and 63 cows that had calved normally. The aggl. titer of the milk serum remained in all cases one and the same, whether the milk for the tests was taken at the beginning, in the middle, or at the end of milking. The specific agglutinins of *Br. abortus* were not always present in all the quarters of the udder. The author is of the opinion that the agglutinins may

originate also in the udder. Pröscholdt studied by means of experiments on the guinea-pig the sediment of 989 milk and as many cream samples, in order to determine which of them contained more *Br. abortus*. 267 (31 per cent.) out of the 859 samples taken into account, were infected with *Br. abortus*. The microbe was found in the cream in 169 (63·26 per cent.) cases, and in the sediment in 198 (74·15 per cent.) cases. In making the corresponding experiments, it became evident that for determining *Br. abortus*, guinea-pig inoculation was not an absolutely reliable means, although injections of cream, sediment, and a small quantity of centrifuged milk were made. The author therefore always recommends two guinea-pigs to be injected simultaneously for the purpose. *Br. abortus* has been detected in several cases in the secretion of non-functional udders in the dry period.

Table 1.

(Composed according to Pröscholdt's Data.)

Number of infected Cows	Blood Sera Positive to Agglut. Test	Number of Cows eliminating *Br. Abortus* with Milk		*Br. Abortus*' Agglutinins in the Milk of Cows eliminating the Bacteria		The Bacteria were not eliminated by		*Br. Abortus*' Agglutinins in the Milk of Cows not eliminating the Bacteria	
		Number	%	Number	%	Number	%	Number	%
94	1 : 100	17	18	15	88	77	82	7	9
83	1 : 200	31	37·35	24	77·4	52	62·65	14	27
56	1 : 400	35	62·5	31	88·6	21	37·5	9	24
32	1 : 800	23	72	19	82·6	9	28	5	55·5
20	1 : 1000	14	70	12	85·7	6	30	6	100
47	1 : 2000	36	74·5	34	94·4	11	25·5	11	100
26	1 : 4000	24	92·3	21	87·5	2	7 7	1	50
25	1 : 8000—1 : 64000	23	96	23	100	2	4	1	50
383	1 : 100—1 : 64000	203	53	179	88·2	180	47	54	30

Klimmer (95) made examinations of 187 cows and found that 83 (47 per cent.) of them had a positive aggl. titer of the blood serum, and in 64 (36 per cent.) cases the reaction of the milk serum was positive. *Br. abortus* was eliminated in both cases: in the first case it was eliminated from the milk of 42 (51 per cent.) cows, and in the second case from 42 (65·6 per cent.) cows. Further it was observed that the agglutination of the blood serum of one cow that eliminated *Br. abortus* with her milk, was positive, and

that of her milk serum, negative, but that the reactions of both the blood and the milk sera of another cow eliminating *Br. abortus* with her milk, were negative.

Klimmer thinks the aggl. titer of the blood serum to be remarkably congruent with the elimination of *Br. abortus* with the milk. He found the highest aggl. titer of the blood serum *viz.* 1:100 in 75 per cent. of the cows that were infected but did not eliminate the microbe; the blood serum of 83 per cent. of the cows eliminating *Br. abortus* with their milk titered at least 1:500 to the agglutination test. An aggl. titer (of the blood serum) of 1:200 was observed in cows that did not eliminate *Br. abortus* (25 per cent.), as well as in those cows that eliminated the bacterium in question (17 per cent.). Further, Klimmer informs us of the studies made in a herd where some cows were injected with living cultures of *Br. abortus*. It appeared that the blood tests showed in some cases an aggl. titer of 1:3000, but the cows did not eliminate *Br. abortus* with their milk; the author ascribes this fact partly to the injections of living cultures of the bacterium. According to the summarized results of his inquiries, Klimmer states that about 98 per cent. of cows eliminating *Br. abortus* with their milk have an aggl. titer of the blood serum at least 1:100, about 95 per cent. have a titer of 1:200, and all cows whose blood serum titers at least 1:4000 to the agglutination test, eliminate *Br. abortus* with their milk. Naturally infected cows may eliminate *Br. abortus* when the aggl. titer of the milk serum is a minimum of 1:100; in cases when the aggl. titer of the milk serum is 1:800 or above, the microbe is always present in the milk. The aggl. titer of the milk serum of cows eliminating *Br. abortus* may decrease thanks to the injection of living cultures of the bacterium to about 1:25, and it may amount to about 1:400 when the microbe is not eliminated with the milk. It was observed that the guinea-pig experiment was not an absolutely reliable means for determining *Br. abortus*, but the cultivation method proved to be even less so for that purpose.

Summary. According to the literature enumerated, 12·5 per cent. to 91·8 per cent. of brucellous cows eliminate the bacterium in question. Most of the investigators have noticed the elimination of *Br. abortus* with the milk by 50 per cent. of cows or more. The elimination of the microbe may last for quite a long period, in some cases even for about 7 years. *Br. abortus* is eliminated with

the milk of pregnant and non-pregnant cows, and also with the milk of cows that had aborted, and of those that had calved normally. Usually, when cows eliminate *Br. abortus*, the specific brucellous agglutinins are present in the blood and in the milk. In single cases, when there are no agglutinins to be found in the blood, in the milk, or in both simultaneously, the microbe is still eliminated with the milk. Those cows that have the specific agglutinins of *Br. abortus* in their blood, as well as those having the above-mentioned agglutinins in their milk, do not eliminate *Br. abortus* with their milk. The bacterium has been detected in several non-functional udders in the dry period. For the purpose of determining *Br. abortus*, the guinea-pig experiment has not proved absolutely trustworthy, but it is as yet the best of all the known means.

b. The Elimination of *Br. Abortus* with Milk ensuing from Vaccination with Living Cultures of *Br. Abortus*.

Zwick and Krage (192) were the first to ascertain that goats injected subcutaneously with living cultures of *Br. abortus* eliminate the above-mentioned microbe with their milk in 24—48 hours after the injection.

Buck and Creech (18) found that one (33·3 per cent.) out of three cows injected with living cultures of *Br. abortus* eliminated the bacterium in question.

Hart and Traum (70) ascertained that 10 (62·5 per cent.) out of 16 cows that were injected with living cultures of *Br. abortus* eliminated the microbe with their milk.

Birch (12) examined 11 cows injected with *Br. abortus* and detected the bacteria in the milk of 3 (27·3 per cent.) cows. The cows from another group treated in a like manner eliminated *Br. abortus* with their milk.

O. Bang (6) noted *Br. abortus* in the milk of 1 (20 per cent.) cow out of 5 vaccinated cows.

Carpenter (24) found that 38 per cent. of the cows injected with living culture eliminated *Br. abortus* with their milk.

Schumann and Lerche (159) fed a cow, which had been pregnant for about 147 days, and a heifer with the contents of the stomach of a fetus richly containing the germs of *Br. abortus*. Both animals were unaffected before the feeding. The first of them calved normally, but the microbe was detected in the uterine

exudate, in the fetal membranes, and in the milk 11 days after she had calved until the end of the experiments. The heifer was bred repeatedly but she never conceived. Further, Schumann and Lerche injected living cultures of *Br. abortus* subcutaneously into two pregnant cows and a heifer. One of the cows calved normally, but the other cow and the heifer aborted. *Br. abortus* was found in the fetal membranes of the two animals that had aborted, all three revealed the microbe in their uterine exudate, and it was present in the milk of the heifer.

Zeller and Beller (189) injected 10 pregnant cows belonging to herds that were not affected with brucellosis; the result was that 7 of them aborted. Further experiments made in a like manner caused 4 cases of abortion in a group of 5 animals. Some of the cows already mentioned (exact data are lacking) eliminated *Br. abortus* with their milk.

Karsten (87) made experiments by injecting cows with living cultures of *Br. abortus*. In 5 cases there followed sterility; 8 cows conceived but 3 of them aborted, and four cows that calved normally eliminated the above-mentioned microbe with their milk.

Cominotti (29) inoculated 20 heifers twice with living cultures of *Br. abortus*. Hence it followed that all the animals calved normally, but 50 per cent. to 66·5 per cent. of them eliminated *Br. abortus* with their milk during 15—18—19—24 months. The biological qualities (especially the aerobic growth) of the bacteria found in the milk were similar to those found in the injected culture. For the purpose of investigating milk for *Br. abortus*, the author of this work recommends an injection of one and the same substance into two guinea-pigs.

Elfr. Ridala (143) injected 5 non-pregnant and 2 pregnant thorougly healthy cows with living cultures of *Br. abortus*, the culture being prepared for vaccinating purposes in the Governmental Serum Institute. The animals under examination were stalled in separate sheds and stall-fed by different persons. It appeared that all these cows eliminated *Br. abortus* in 7—21 days after the injection. The microbe was eliminated by 5 cows for about 4—6 months and by 2 cows till the end of the experiments (1·5 years). All the cows but one conceived and calved normally.

Besides the above-mentioned authors, there are other scientists, *e. g.* Wyssman (186), Connaway, Durant and Newman (30), Graig (60), Fischer (46), who have noted that

Br. abortus is eliminated with milk after cows have been injected with living cultures of the microbe.

Zeller (187), Bogenschneider (15), Schermer and Ehrlich (151), Tize (147), Fitch, Boyd, and Lubbehusen (47) a. o. are of the opinion that the injection of living cultures of *Br. abortus* does not cause a subsequent elimination of just the same bacteria with the milk. A majority of these authors are of the opinion that the living cultures of *Br. abortus* die very soon after being injected into the organism of cows. This view, however, has not been proved scientifically. Later researches by Zeller (189) have shown the opposite.

Summary. The above-described statements of various scientists show that the elimination of *Br. abortus* with the milk of cows is very frequently (20 per cent. to 100 per cent.) due to the injection of living cultures of *Br. abortus*. Besides, frequent cases of abortion have been caused by injecting pregnant cows, the microbe always being present in the fetal membranes. Furthermore, a large percentage of non-pregnancy has been noticed among cows injected with living cultures of *Br. abortus*.

c. The Germ Count of *Br. Abortus* eliminated with Milk.

Alice Evans (41, 42) was the first (1915) investigator to rear a culture of *Br. abortus* directly from milk and determine the germ count by the plate-cultivation method. She studied 46 milk samples taken from two dairy-farms producing certified milk, and got a culture of *Br. abortus* from 14 (30·4 per cent.) samples. The germ count in 1 ccm of the milk was about 110 to 4300 as judged by the full-grown colonies of *Br. abortus*. One sample of the uncertified milk showed in 1 ccm about 50 000 germs of the microbe in question.

Carpenter and Barker (25) ascertained a rise and fall in the germ count of *Br. abortus* in the milk drawn from one and the same udder: the milk samples examined showed 20 to 440 germs in 1 ccm of the milk. For the purpose of determining *Br. abortus* in the milk, the authors consider cream to be better and more appropriate than sediments.

Huddleson, Hasley, and Torrey (79) state that the cream spontaneously risen to the surface of milk most easily yields the richest culture of *Br. abortus*. The authors are of the

opinion that the germs of *Br. abortus* rise to the surface together with the fat-globules of milk. They ascertained the presence of *Br. abortus* in the milk of some cows, the germ count of the bacteria being about 10000 in 1 ccm. There was no marked difference between the milk from the morning and that from the evening milking.

Hasley (71) studied certified but raw milk in Detroit. He took for the purpose of his experiments 230 samples from 5 dairies; it appeared that *Br. abortus* was present in 10 milk samples taken from 3 dairies, the number of the bacteria in 1 ccm of the milk being about 2, at the most 8.

Lerche (111) examined 18 milk samples by means of the guinea-pig experiment and the cultivation method simultaneously. *Br. abortus* was revealed through guinea-pig inoculation in all the 18 samples, but by means of the cultivation method the microbe was detected only in 4 samples. Further, Lerche studied by means of the cultivation method the milk of 7 cows eliminating *Br. abortus*, the milk being drawn from each quarter of the udder individually. He succeeded in cultivating *Br. abortus* in the milk from two quarters of one cow, and from one quarter of two cows. The author placed 0·2, 0·1 and 0·02 ccm of the milk of the two latter cows on serum agar plates for the presence of *Br. abortus* and the number of germs. The growth was then studied, and it showed a various germ count — about 60 to 320 — of *Br. abortus* in 1 ccm of the milk.

O. Bang and Bendixen (9) state that the number of the germs of *Br. abortus* in milk is variable. They think that it amounts sometimes to 30000 germs in 1 ccm of milk when cows are naturally infected. The presence of the above-mentioned bacterium in the udder does not cause any macroscopical changes in the milk. The elimination may last for one or more lactation periods. The authors found *Br. abortus* by means of the cultivation method in 112 (64 per cent.) out of 176 milk samples that were previously examined by means of the animal-experiment and had revealed the microbe in question.

Traum and Haring (176) examined milk samples by means of the cultivation method for the presence of *Br. abortus*; 25 per cent. to 30 per cent. of the cases yielded unauthentic results.

Stockmayer (172) noted *Br. abortus* in the secretion of the udder in the dry period. There could be determined

about 10000 germs of the above-mentioned bacterium in 1 ccm of milk, but the same amount of the secretion of a dry udder and the colostrum showed 50000 germs and above. The cream of centrifuged milk revealed only a few of the bacteria; there were more of them to be observed in the sediment. On the contrary, the bacteria proved present in much larger numbers in the cream that had risen to the surface by standing of milk than in the sediment.

Summary. From the above-described literature it follows that the germ count of *Br. abortus* varies indeed; beginning with single germs in 1 ccm of milk it amounts to about 50000 bacteria. Many authors [Lerche (111), Bang and Bendixen (9), *etc.*] think these data inexact, considering the difficult and inconsistent growth of *Br. abortus* on the nutrient media for the culture of bacteria. Several authors [Lerche (111), Traum and Haring (176)] have not succeeded in finding *Br. abortus* by means of the cultivation method in a large percentage of milk samples, though the microbe was proved present in the same samples through guinea-pig inoculation.

d. Changes in the Quantity and the Compound of Milk due to *Br. Abortus* in Bovine Udders.

Barnes (10) found that the annual milk production of brucellous cows in a farm was about 2685 quarts (1 quart = about 1 litre), while unaffected cows yielded about 5248 quarts. Hence it follows that the milk production of the brucellous cows was about 2563 quarts (48·84 per cent.) less than that of the undiseased animals.

Hart (68) gives information about a herd of 1000 head, mostly infected with *Br. abortus*. Out of this herd a new herd was bred by raising 213 unaffected cows at a distance of several kilometres. Though the functional capacity of the new herd at the time of its examination had not fully developed (cows had calved only once or twice), yet the cows of this herd are said to have produced more milk in comparison with the old ones (data in figures are lacking).

Simms and Miller (162) noticed that brucellous cows yield about 35 per cent. less milk a year than undiseased animals. The authors made constant examinations for a considerable period in a herd where no abortions had occurred in the years 1913 and 1914. Four pregnant cows were added to the herd in 1915; three

of them proved brucellous and aborted. After that the infection spread rapidly among the cows in the herd, and the number of abortions reached its climax in the years 1919—1922.

Table 2.

Comparative Data concerning the Milk Production of Cows Infected and Unaffected by *Br. Abortus*.

(Composed according to Data by Simms and Miller.)

Year	Number of Cows	Of them		Abortions	Normal Parturitions	Average Annual Milk Product. of a Cow		Difference
		reacted	did not react			reacted	did not react	
1919	54	41	13	14	41	4965	6693	1728
1920	52	36	16	12	35	4506	5704	1198
1921	64	40	24	12	44	5617	7977	2360
1922	79	48	31	10	50	4710	8542	3832
1923	59	26	33	5	50	4544	7343	2799
1924	70	24	46	6	59	3262	6291	3029

Cows unaffected by *Br. abortus* were then, in 1923/24, isolated from the diseased animals and removed to a distance of one mile. The unaffected cows were stall-fed by persons who had no access to the brucellous herd. The owner of the cattle sold all his brucellous animals after he had understood the considerable difference, about 35 per cent., between the milk production of both herds. Thus, brucellosis was exterminated from the herd, while a partial isolation and a disinfection had not yielded the desired results. According to table 2, the decrease of milk production remains a fact constantly to be noticed in brucellous cows, even though the number of abortions among the cattle had steadily decreased.

Laja (104, 106), considering the milk production of three herds in two years, noted that the annual milk production of the regularly[1]) milking brucellous cows was about 25 per cent. less than that of the unaffected animals. But the percentage of the decreasing milk production amounted to about 28, when, in addition

1) Regularly milking cows are those that have not aborted during the year under examination, that are more than once in milk, that have not been acquired later by purchase nor been barren, those with the interval between two calvings of no more than 12 months, those that have not been afflicted with any clinical disease, *etc.*

to the former, there were considered those cows that did not yield milk regularly; cows that had aborted were not taken into account.

Hardenbergh (67) observed that the milk production of unaffected cows was 1 quart more per day than that of the brucellous ones.

Rich (141) found in control-data that, in general, the milk production of cows in the lactation period following abortion was smaller than after normal calving by about 22 per cent. of the milk and 19·5 per cent. of the cream. Cows whose blood serum reacted to the aggl. test for *Br. abortus* and, despite this fact, had calved normally, yielded about 6·9 per cent. less butterfat than cows unaffected by brucellosis.

O. Bang and Bendixen (9) made a profound study of the milk from 15 brucellous udders. The daily milk production of each quarter of one cow that had conceived for the first time and aborted on October 8, 1928, was weighed 29 times within 6 months (July 17, 29 — January 28, 30). The daily milk production of the quarters during that space of time was on an average:

right front	quarter	1579 g
right rear	„	615 „
left front	„	1308 „
left rear	„	335 „

Bacteriological investigations on May 27, 29 revealed *Br. abortus* in the milk from both the rear quarters; besides, in the milk from the left rear quarter micrococci *(Microc. aureus)* proved present, whereas the milk of both the front quarters did not show any bacteria. According to the examination made on October 29, 29, *Br. abortus* was present in the milk from three quarters (except the right front quarter), and, in addition to that, the milk of the left rear quarter contained micrococci. The cow calved normally on July 21, 30. Further bacteriological studies, made on July 22, 30, showed *Br. abortus* in the milk of three quarters of the udder (except the right front quarter) but to no considerable amount -- according to the authors' opinion, because the microbe could not be ascertained by means of the cultivation method. Besides, some micrococci were observed in the milk of the left rear quarter. According to the following examinations (September 20, 30, January 3, 31, and May 4, 31), *Br. abortus* proved present likewise in three quarters (except the right front quarter), but no other bacteria were found in any of the quarters.

The microbe was found on September 3, 31 in the milk of only the two rear quarters. The milk production of each quarter of the udder was controlled from September 27, 30 till May 23, 31 and weighed 5 times during that period of time. It appeared that the daily average was:

right front	quarter	2540 g
right rear	„	1400 „
left front	„	2230 „
left rear	„	1364 „

Despite the fact that the undiseased quarters of the udder were not equal in producing milk, and though, in the present case, the left quarter was infected with micrococci, the finding of O. Bang and Bendixen concerning the milk production of the right rear quarter (infected with *Br. abortus* for a short period) and of the left front quarter (a constant infection with *Br. abortus*), speaks for the fact that milk production is most disadvantageously affected when the above-mentioned microbe is to be found in the udder. In examining other cows Bang and Bendixen detected very frequent infections of the udder with micrococci and streptococci. It was therefore impossible to determine the decrease of milk production as caused by brucellosis only.

Cooledge (33) made studies into the amount of cellular elements in the milk of brucellous cows and noted that the cell count of the milk in cases of natural infection was over 5 times as high as that of the milk of cows that did not eliminate the bacterium in question. Cooledge was of the opinion that such a high cell count in the milk of apparently normal udders was indicative of their being infected with *Br. abortus*. Further examinations, however, revealed high cell-count milk in which *Br. abortus* was not present. The author injected living cultures of *Br. abortus* into the milk cistern of unaffected cows, which caused a rapid increase in the cell count of the milk.

Tweed (180) examined 96 cows, out of which 17 eliminated *Br. abortus* from one or more quarters of the udder. He observed that the cell count of the brucellous milk was over twice as high as that of the normal milk. The milk drawn at the end of milking showed a much larger number of cells than that drawn at the beginning, whereas the amount of the specific antibodies of *Br. abortus* remained unaltered. In making studies of the smears of the milk sediment and also of the histological sections, he found

that the milk contained mostly polynuclear cells, epithelial cells being absent. The author thought the cell count too small for the purpose of determining any catarrhal or suppurative inflammation.

Similarly, Runnels and Huddleson (147), O. Bang and Bendixen (9), *etc.* found a marked increase in the cellular elements of the milk when brucellosis became established in the udder.

O. Bang (5) noted that the changes in the milk compound caused by a brucellous infection of the udders were similar to those to be noticed in cases of a slight inflammation of the udder: the quantity of the milk sugar decreased, whereas there was an increase in chlorine. The quantity of chlorine and milk sugar in the milk of a brucellous cow drawn from each quarter of the udder individually was as follows:

left front quarter	0 bacteria	in	1	ccm of milk,	lactose	4·7	per cent.,	Cl	0·11	per cent.
right „ „	„	„	„	„	„	4·9	„ „	Cl	0·11	„ „
left rear „	8000 germs of *Br. abortus* and 4800 micrococci	„	„	„	„	3·9	„ „	Cl	0·18	„ „
right „ „	4800 germs of *Br. abortus*	„	„	„	„	3·9	„ „	Cl	0·17	„ „

O. Bang and Bendixen (9) studied the milk of 15 cows afflicted with a spontaneous and latent brucellous infection in the udders. The quarters showed also latent streptococcic and micrococcic infections and the presence of corynebacteria; therefore, the researches made into the pathogenic effects of *Br. abortus* have not been extensive. But the udders of several young cows were infected only with *Br. abortus*; the changes to be noticed in the milk compound of these cows were similar to those observed by Steck, Koestler, and Radosawlewitsch in cases of other latent infections in udders. They found that in different lactation periods the quantity of milk sugar decreased to about 3·06 per cent., and the percentage of the fat to 1 per cent. On the contrary, the amount of catalase increased (in 15 ccm of milk) to about 12·5 ccm of oxygen, the amount of sediments, according to Trommsdorff, to about $5^0/_{00}$, of Cl — to about 0·21 per cent., the chlorine-sugar number to about 6·99, and the cell count to about 2 580 000 in 1 ccm of milk. pH varied from 6·58 to 7·02. *Br. abortus* was determined by means of the cultivation method in 1 ccm of the milk, comprising the number of bacteria from some single ones to

about 30 400. The authors had never noted an inflammation o the udder clinically in cases of a brucellous infection of the latter.

Summary. From the above-described literature it follows that in cases of brucellosis, the milk production decreases to about 35 per cent. But, in general, the question has been little dealt with, and no great importance has ever been attached to the matter. In cases of the elimination of *Br. abortus* with milk, O. Bang (6), O. Bang and Bendixen (9), Lerche (111), Klimmer (95), *etc* have not found macroscopically any changes in the milk, but they noted some changes in the compound of the milk which were similar to those already ascertained in cases of other latent infections of the udder. It has been observed that the quantity of fat and milk sugar decreased, whereas there was an increase in chlorine, in the number of catalase, and the cell count. Attention should also be paid to the fact whether studies are made at the beginning, in the middle, or at the end of lactation. The great difference to be noticed in the compound of the milk of each quarter shows that there are some changes which could be ascribed to brucellosis.

e. Pathological Changes due to *Br. Abortus* in the Bovine Udder.

Schroeder and Cotton (154) found macroscopically some small induration foci in the udders of cows in cases of brucellosis. Histological studies were not made.

Friedemann (52) made histological studies of the tissue of one naturally infected udder, but did not find any changes there.

Smith, Orcutt, and Little (167), in studying the source of agglutinins in the milk of cows, injected living cultures of *Br. abortus* through the teat canal of individual quarters, and studied the results clinically, bacteriologically, and histologically. The animal was slaughtered 23 hours after she was injected; it appeared that the secretory alveoli of one quarter were filled up with polynuclear leucocytes. The same kind of cells were found in the epithelium and the interstitial connective tissue. This animal was a tuberculin reactor and also showed haemolytic streptococci in the milk from all quarters.

The same authors introduced a living culture of *Br. abortus*

into three quarters of another udder. A histological examination of the three treated quarters as well as of the one untreated showed some alveoli singly or in large groups, filled with polymorphonuclear cells and some acidophiles. Each cell of the secreting epithelium in these foci had one large vacuole (fat). The interstitial connective tissue was infiltrated with lymphoid and plasma cells. In one section there was found a focus occupying $^2/_3$ of the lobule, in which the alveoli were compressed by endothelial cell accumulations, and the surrounding epithelium showed some vacuoles. The described focus resembled lesions noted in the guinea-pig due to *Br. abortus*. This animal was also a tuberculin reactor and showed haemolytic streptococci in all quarters of the udder.

Smith and his co-workers assert that the invasion of the udder by *Br. abortus* means the presence of the above-mentioned microbe and its multiplication in the milk, in the alveoli and lactiferous ducts, but not in the parenchyma. In other words, the residual milk in the alveoli and ducts appears to be the seat of multiplication.

Runnels and Huddleson (147) think it impossible to state definitely that the changes found by Smith, Orcutt, and Little in the udders of two injected cows were caused by *Br. abortus* only, because in all of these quarters haemolytic streptococci were found, and none of the injected quarters showed the presence of *Br. abortus*.

Runnels and Huddleson (147) made bacteriological, serological, and histo-pathological studies of the udders of three cows and a heifer. One of the cows had been naturally infected (from associating with brucellous animals), but two cows and the heifer had been inoculated (by injecting and feeding with living cultures of *Br. abortus*). The milk and the udders of all the animals were examined bacteriologically according to the plating method and the guinea-pig experiment. In making experiments, no other types of bacteria were found but *Br. abortus* which was detected in the udders and the milk of all the four cows, except that of one injected cow. The authors examined the presence of the specific agglutinins for *Br. abortus* in the milk by means of the aggl. test, experimenting directly with the milk; in doing that they did not find it necessary to separate the serum from the whole milk. For the purpose of histological studies, bits of tissue were taken from three regions (from the upper, the central and the lower region) of the quarters

of the udder. The material was fixed in Zenker's fluid and then embedded in paraffin. The sections were stained according to van Gieson's, Mallory's eosin-methylene blue and Goodpasture-Weigert's combined methods. The two latter methods were used for the purpose of staining the bacteria.

The essential data concerning the material and the results of the investigations are as follows: a naturally infected cow (case 2) had calved for the first time on July 15, 1920, the calving was normal; then she aborted on May 9, 1921; the third time she calved normally on March 26, 1922 and was slaughtered on Dec. 18, 1922, containing a 5-months' fetus. *Br. abortus* was detected by means of the guinea-pig experiment only in both the rear quarters. Histologically it was ascertained that the connective tissue of about one half of the alveoli of the front quarters had moderately increased. In this portion the alveoli were irregular in shape, their lumina were comparatively empty, and the fat globules were almost totally absent. Small foci of lymphocytes were detected in the interalveolar and interlobular connective tissue of the left quarter especially. Only a slight proliferation of the interalveolar connective tissue was noticed, or it was quite absent, and most of the epithelial cells contained fat globules. In the lumina of most of the alveoli there was found a pale-pink granular mass which contained polynuclear and desquamated epithelial cells, chromatin granules and clear vacuoles.

The histological changes in both the rear quarters of the udder were most marked in the basal and the middle parts of the quarter, while in the portion near to the teats no considerable alterations were noticed. Almost all of the tissue from the basal and middle portions of the sections was damaged. The changes were of an acute, subacute, or chronic type, the latter being predominating. In cases when the lobules showed more acute changes, the interalveolar connective tissue remained unaltered, or it was but very slightly changed. The changes in the glandular epithelium varied from fatty degeneration to necrosis and disintegration. The exudate in the alveoli consisted in varying amounts of a pink granular or stringy material, in which polymorphonuclear cells were present, either normally or in all stages of disintegration; further it consisted of occasional deep-pink staining hyaline masses and large masses of chromatin granules enclosed in mononuclear cells. In the lumina of the alveoli a few

corpora amylacea were observed. In some areas the changes were even more marked than the above-described ones. There was a partial or complete degeneration and disintegration of the epithelium. The alveoli were filled up with exudate, and frequently the structure of three or four adjacent alveoli was completely lost. Because of chromatolysis, these areas took only the eosin stain in the sections stained according to Mallory's method. There was much interalveolar congestion and inflammatory edema adjacent to the last-mentioned foci. Also a considerable subepithelial lymphocytic infiltration of the larger lactiferous ducts was observed in several places. In places where the changes were of a subacute or chronic type, the proliferation of the interalveolar elements increased extremely in amount. Proliferating fibroblasts were found, and among them numerous leucocytes. The latter consisted of lymphocytes, polymorphs, eosinophiles, plasma cells, and polyblasts, appearing in numbers in the order named. In places there was to be noticed a massy accumulation of lymphocytes. With the proliferation of the interalveolar connective tissue there was a corresponding atrophy or a complete disappearance of the alveoli. The lumina of the changed alveoli were either empty or they contained only a moderate amount of pink-staining granular material, in which were to be noticed disintegrated leucocytes, desquamated and disintegrating epithelial cells, and chromatin granules.

The most important change in the supramammary lymph nodes was the increase in the thickness of the trabeculae in the cortical substance; furthermore, one or two dense foci of the hyaline masses were found in the central part of the germinating centres. In the cortical substance of the left lymph node occurred a few haemorrhages. In the medullary substance, the reticular connective tissue was replaced by fibrous connective tissue, and the lymphoid tissue had considerably decreased, only a few islands had remained. There were also diffuse haemorrhages in this area.

The changes found in the udders of one artificially infected cow and a heifer that was slaughtered 9 months after she had been injected, resembled those noticed in the rear quarters of naturally infected cows. Furthermore, there was observed an increase in the number of the capillaries, in a few places some purulent exudate of the alveoli, a subepithelial hyaline degeneration of the larger lactiferous ducts, and seldom a perivascular infiltration of the lymphocytes. The supramammary lymph nodes of the heifer

presented a picture of chronic lymphadenitis, whereas no changes were observed in the same lymph nodes of the cow.

The udder of one injected cow (case I) that had no *Br. abortus* in her milk and died through *peritonitis* due to *perforatio uteri*, was used for the researches as normal material.

Runnels and Huddleson failed to find *Br. abortus* in the histological sections of the udders examined and the lymph nodes belonging to them.

Hart (68) did not make any histological studies of udders, but still noted a great difference between the milk production of infected and unaffected cows, and was sure that the decrease of milk production is generally caused by changes due to brucellosis in the udder.

Hallmann, Scholl, and Delez (65), in publishing the results of their investigations and observations of the pathogenic effect produced by *Br. abortus* in the bovine organism, positively declare that in cases of brucellosis, first of all an accumulation of fibroblasts and of small round cells is to be noticed in the affected organs. The necrosis may occur, but need not always occur. Subacute and chronic inflammations of the udder due to brucellous infection are to be noticed together with a cellular exudation, degeneration, and desquamation of the parenchyma.

Sholl and Torrey (161) have made histological studies of the udders of 41 cows that had reacted positively to the serological test for brucellosis. They ascertained the presence of *Br. abortus* in the milk and the tissue of the udder by means of the cultivation method. Examinations with regard to other possible agents were made superficially and most insufficiently: for the purpose of their isolation, the authors used only liver agar plates and gentian violet liver agar plates that were incubated in 10 per cent. of CO_2 concentration, for the purpose of determining the presence of *Br. abortus*. The bacteria that were found were not fully differentiated, except *Br. abortus*. The presence of *Mycobacterium tuberculosis* was totally left out of consideration. Nevertheless, the udders of the examined cows frequently showed the presence of streptococci and staphylococci. The investigators observed most frequently interstitial mastitis, whereas exudative mastitis, suppurative mastitis, and fibrosis were seldom found. There is a complete confusion as to whether the pathological changes were caused by

Br. abortus or by some other infection. Therefore one has to be most careful in estimating the results of these researches.

Summary. According to the literature concerning the question of brucellosis, the number of udders eliminating *Br. abortus* that have been studied histologically with any care is not large. The only studies that yield authentic results are those made of one naturally infected cow [Runnels and Huddleson (147)]. No conclusions can be drawn from the results of the investigations of other authors [Friedemann (52), Scholl and Torrey (161)], because of the small number of cases examined or the insufficiency of the researches made.

For the purpose of thoroughly elucidating the pathogenic effects produced by *Br. abortus* in the udder of the cow, it is of the utmost necessity to continue the researches already begun, and to carry them out thoroughly on a large scale.

It is already known that in cases of a natural and artificial infection with *Br. abortus* in the udder, there is to be noticed an acute, subacute, and chronic inflammation. The nature of the changes and their variableness has not been as yet exactly determined. It is not known either to what extent the changes take place in the infected quarter, whether the changes are present in foci all over the quarter, or whether they are localized only in certain quarters of the udder. Further, it has not been ascertained whether *Br. abortus* is present in the parts thus changed, and whether it could be found also in the histological sections. It has not been as yet elucidated whether the changes in the udders of brucellous cows that have calved normally are the only reason for the decrease in milk production.

C. Personal Inquiries.

a. Prospect of Inquiries.

A detailed bacteriological and histological examination of udders eliminating *Br. abortus* as well as of the lymph nodes belonging to them, seemed to be the most adequate and the easiest way of solving the problems the present study has set itself, and particularly of elucidating the changes in the udders infected with *Br. abortus*. One had naturally to make sure that the material used was free from all other germs causing diseases. In collecting the material, due attention had to be paid to the age of the

cow, her previous calvings, the duration of the infection, her milk production, and other facts that might be of value or of use for estimating the results of the inquiry.

b. Course of Inquiries and Methods.

aa. Bacteriological and Serological Examinations.

The cows fit for the purpose of examinations had to be chosen from larger farms in the neighbourhood of Tartu. The preliminary data concerning the cows that had reacted to the agglutination test for *Br. abortus* in the above-mentioned farms were obtained by me from the Bacteriological Station of the University (Director: Prof. Dr. F. Laja). In making use of these data for my purposes, I chose cows that had a considerably high agglutination titer from 4 farms, about 10 animals from each. In collecting milk samples from the cows already mentioned, I also took blood samples from most of the animals. Before drawing the milk, I cleaned the teats and the udder with a clean wet cloth, then I wiped the teats and partly the udder surrounding the teats with a piece of bandage moistened with 70 per cent. alcohol. In cases where the cow yielded plenty of milk, I took in the middle of the milking about 300 ccm of milk from all the quarters into sterilized bottles, but in cases where the milk production was not great, I took the samples after five streams had been milked out. Filling up 15 ccm test tubes with the collected milk, two tubes from each sample, I centrifuged them for half an hour at a speed of about 2500—3000 revolutions a minute. After recording the outward qualities of the centrifuged samples, I plated the sediment from one of the tubes on bromcresol-purple-saccharose-alkalialbuminate agar plates and on modified Brown blood agar plates (pH 7·8). Though these nutrient media are in the first place used for ascertaining streptococci and *Bact. pyogenes* in the milk, they are also appropriate for rearing all other microbes (*Escher. coli*, micrococci etc.) causing udder infections, except *Mycobacterium tuberculosis* and *Br. abortus*. After the plates had stayed about 24 and 48 hours in the thermostat (37° C), each time I made thorough examinations of the sown plates with regard to the growth, and if the plates proved sterile, I controlled them once more 96 hours later. I prepared 4 smears of the remnant of the sediment in the test tubes. The first smear I stained by Ziehl-Neelsen for *Mycobacterium tuberculosis*, the second by

Gram for gram-positive bacteria *(Strept. agalactiae, Bact. pyogenes, Staphyloc. pyogenes)*, the third I stained by Pappenheim, to ascertain the cell count, and the fourth of the preparations was laid in store.

The presence of *Br. abortus* and *Mycobacterium tuberculosis* was ascertained by means of the guinea-pig experiment. I added to the sediment in the second test tube about $^1/_4$ of cream of the same milk and about 1 ccm of skimmed milk, stirred the mixture and injected guinea-pigs subcutaneously in two legs. The beginning of the brucella-infection in the injected guinea-pigs was ascertained first of all by the appearance of the specific agglutinins. For the purpose of ascertaining that, I took at least two blood samples from each guinea-pig, usually 5 and 8 weeks after they had been injected, previous to their slaughter. When the blood samples showed negative results for the agglutination test for *Br. abortus*, new samples of blood were collected once or twice afterwards, and also at the slaughter. I took the blood samples from the ear of the guinea-pig, and at the slaughter from the heart. The collecting of blood from the ear of a guinea-pig is wearisome but without any danger to the life of the animal; this is the reason why I availed myself of this mode. In the agglutination test I always used one and the same strain of *Br. abortus* as an antigen. The suspension was prepared in a physiological solution of NaCl (0·85 per cent.), and was preserved with phenol (0·5 per cent.). The blood serum of the guinea-pigs was diluted from 1 : 10 to 1 : 10240, and the dilutions of the blood serum of cows were made from 1 : 10 to 1 : 40 960. The injected guinea-pigs whose blood serum agglutinated *Br. abortus* in 5 to 8 weeks, were put under chloroform 8 weeks after they were injected, but the guinea-pigs that did not react positively to the agglutination test were chloroformed 10 weeks after the injection. In making a post-mortem examination of these guinea-pigs, I examined all organs for macroscopical changes. Further, I always made cultures of the spleen of the guinea-pigs on serum agar, and very frequently also of the superficial and deep inguinal lymph nodes; from the spleen I put a part into at least 3 test tubes, and from the lymph nodes into 1 to 2 test glasses. After substituting 10 per cent. of lighting gas for the air in the test tubes, I closed them with paraffin and placed them in the thermostat (37° C), leaving them there until colonies of *Br. abortus* appeared, which usually happened after 3 to 9 days; or the tubes

were left there even for 14 days if the case proved to be negative. Grown colonies that resembled the colonies of *Br. abortus* I transferred to serum agar, and made a final study of each culture by means of the agglutination test, to determine whether or not they belonged to the genus of *Br. abortus*. Further, from the spleen and the superficial and deep inguinal lymph nodes of each guinea-pig I made smears which I stained according to the Ziehl-Neelsen method for *Mycobacterium tuberculosis*.

According to the preliminary examinations, 90 per cent. of the examined cows eliminated *Br. abortus* with their milk. Several cows from three farms were fit for investigations, because there were no other types of bacteria present in their udders besides *Br. abortus*. It was not an easy matter to induce the farmers to slaughter the animals, though I explained to them the importance of the inquiries to stock-farming. In some farms I was advised to buy the cows myself, the price of the cows being raised about 50 per cent. higher than the average market-price. I could not afford to purchase the cows, and I had to wait till some of them were sold for slaughter because of their very markedly decreasing milk production. Thus, some material appropriate for the present study could be obtained from six cows only.

I took some more milk samples from the milk of four of these cows (cows I, II, III, and IV) — a few minutes previous to slaughter, but this time from each quarter individually.

Two cows (cows V and VI) had ceased to yield milk some days previous to slaughter, therefore no milk samples could be obtained. But in one case (cow VI) there could be drawn about 25 ccm of a serum-like secretion of the udder from each quarter individually, and in another case (cow V) from all quarters about 40 ccm of a similar secretion. The samples of the milk or of the secretion I collected and examined in a like manner to the samples of the milk for the preliminary investigation, except in two cases (cows III and IV), when the chloride content and the presence of brucella-agglutinins in the milk of single quarters was determined. I determined the chloride content by Koranyi's method as modified by Zaribnicky and Münchberg (129) in the following manner: about 2 ccm of concentrated halogenless nitrogen (spec. grav. 1·40) have to be put into a 100 ccm Erlenmeyer flask, then 1 ccm of milk has to be added and 1 ccm n/10 of a solution of nitrate of silver; the mixture has to be carefully shaken, then heated

on an asbestos net to boiling point; in continuing the heating, the organic substances are oxidized with a saturated solution of potassium permanganate (consum. nearly 4 ccm); for refrigeratory purposes there have to be added 30 ccm of distilled water, and, as the indicator, 0·5 ccm of a saturated solution of the sulphate of iron and ammonia; the superfluous quantity of the nitrate of silver is combined by titrating with n/50 solution of potassium sulphocyanide; the calculation of the consumed quantity of the nitrate of silver for the combination with chlorine is based on the former result, and from that follows the percentage of chlorine in the examined milk.

By coagulating the milk with rennet, I obtained the milk serum necessary for the agglutination test. In doing that, I added the solution of the rennet to the samples and left them for a few hours in the thermostat (37° C). The serum was filtrated, if necessary, and was investigated by means of the agglutination test in a like manner as the blood sera; the dilutions were made from 1 : 10 to 1 : 20 460.

One more sample of blood was taken from each cow under examination at the time of slaughter.

After the cows were slaughtered, I removed, for the purposes of my studies, the following organs and parts of the body: in two cases (cows I and II) the udder, the supramammary and deep inguinal lymph nodes, the ovaries and uterus, in one case (cow III) all the above-mentioned organs and in addition to them also the mesenteric lymph nodes. In two cases (cows V and VI) I took the udder, the supramammary and deep inguinal lymph nodes, the spleen, the mesenteric lymph nodes, the marrow (from metacarpus dex.), the thyroid gland, the uterus and ovaries. In one case (cow IV) all the organs mentioned in the two latter cases were removed, except the marrow, and besides those, I took the right superficial cervical lymph node, the lungs, the liver, the kidneys, and the suprarenal glands.

I took all organs of the animals I used for my inquiries myself, by cutting them with a knife that was cleaned properly with alcohol, after removing each organ from the organism of the cow. The smaller organs and parts of the body I placed immediately after removal in large sterile Petri's dishes, and covered the dishes with sterile paper, whereas the bigger organs were wrapped up in sterile papers and covered with clean ironed towels; then I placed them

separately in tin boxes. The material thus arranged was carried away from the town abattoir of Tartu to the Veterinary and Milk-hygienic Institute of the University. Arriving at the Institute, I registered the outward qualities of the organs, and, taking at once the material for the examinations, I made further arrangements for the bacteriological studies. The material from all the above-mentioned organs and parts was examined bacteriologically, except four udders (cows I, II, III and IV) that had not shown the presence or the absence of any germs after repeated study of the milk samples.

Before taking the material for making cultures, I seared the surface of the smaller organs and parts of the body several times with a glowing hot knife; then making cautious incisions with a sterile knife into the scorched places as deep as 1·5 cm, I obtained some juice from the tissue and pieces of the substance with the help of sterile instruments (such as knives, scissors, nippers). The greater organs I seared with a gas flame. The histological material for the purposes of my studies I took from a great many parts about 3 cm deep. Thus, some material was taken from various regions of each quarter of the udders (cows V and VI); further, I obtained some mucus and a part of the epithelium of the cervix and the cornua uteri; through the longitudinal incision I had made with a sterile knife at the seared places of the uteri, I drew the material with a sharp sterile spoon from the cervix and the cornua uteri. The material from each organ and part of the body was placed first in sterile Petri's dishes where it was refined and stirred according to requirement. I plated the material on the same nutrient media that were used for the previous studies of the milk samples as described above. Leaving the plates in the thermostat (37° C) for about 5 days, I controlled them every day. The remnant of each material was suspended in about 1 ccm of 0·85 per cent. solution of NaCl and injected subcutaneously into two legs of guinea-pigs. I made studies of the injected guinea-pigs in the same manner and order as applied at the examinations of the milk samples.

In order to study the physiological changes (involution) that took place in the udder during lactation, and the changes due to aging, I examined the udders, supramammary and deep inguinal lymph nodes of 9 undiseased cows of different ages and in varying stages of lactation or in the dry period. A few minutes previous to the slaughter of one cow (case VII), I took milk samples from

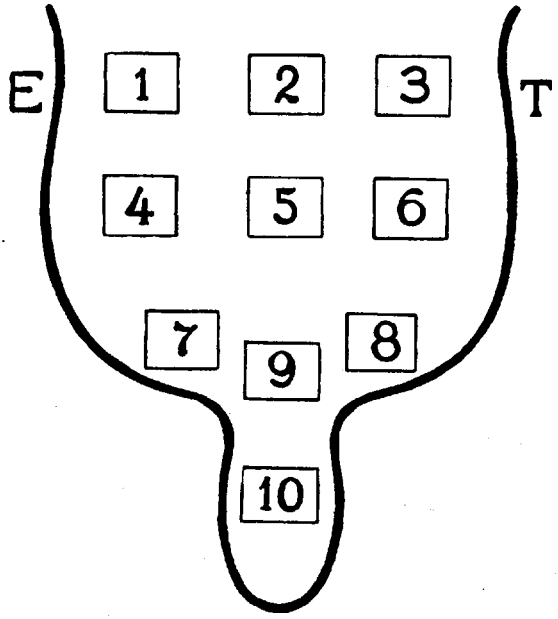
each quarter of the udder individually, and a composite milk sample from the milk of all the quarters of the udders of 5 cows (cases VIII, IX, X, XI and XII); further blood samples were taken from all the cows at the time of slaughter. I studied the samples of the milk and of the blood in the same way as in the cases of two brucellous cows (cases III and IV). The bacteriological study of the udders of three cows (cases XIII, XIV, XV) in their dry period was made in the same manner as that of the udders of brucellous cows (cases V and VI) in their dry period, as well as a study of the supramammary and the deep inguinal lymph nodes of all the cows.

bb. Histopathological Studies.

After the bacteriological work was finished, I carried the organs and parts of the body into the Institute for Pathological Anatomy of the Veterinary Faculty of the University. The material for the histological studies was taken in about 2—5 hours after the animals were slaughtered, and placed in the fluids for fixation. Besides the external qualities, such as the size and the weight registered before taking the bacteriological material, I took down the evidences of the macroscopical qualities of all the organs and parts of the body. The material for the histological studies was removed from 10 regions of each quarter in the order demonstrated in scheme 1, and besides this, from all places exhibiting macroscopical changes. The material from the uterus was removed only from those places that were not injured during cutting for the bacteriological examination. The histological material from the larger organs was taken from a great many parts. For my histological studies I took the whole of the lymph nodes, ovaries, and thyroid glands, with the exception of those parts that were damaged during removal for the bacteriological examination. From each region that was meant for the histological studies I cut off at least 6 blocks of tissue as big as $1{\cdot}4 \times 1 \times 0{\cdot}3$ cm. From each udder I took about 360 pieces of tissue, including those portions of the quarters that are not denoted in the scheme. The blocks cut one from the other were tied up in pieces of bandage by twos, by threes, and even by fours when more than 6 blocks were taken; the parcels were provided with labels with exact descriptions of the origin of the blocks and with various necessary notes. Thus, three parcels were made up from each portion; one of them was

fixed in alcohol (98°—99·9°), the second in 10 per cent. formalin, and the third in Orth fluid. For the purpose of a thorough fixation of the material, I changed the fixative 3 times during the first 48 hours. After making the general arrangements necessary in the technique of histological inquiries, I embedded the material from

Scheme 1.



Scheme 1 represents the outer side-view of a quarter of the udder.
E — the front part of the quarter. T — the back part of the quarter.

each parcel as follows: one block of tissue in paraffin and the remaining blocks in celloidin. From the histological material, embedded both in paraffin and celloidin, I made plenty of serial sections, the paraffin sections 2 to 5 microns, and the celloidin sections 5 to 10 microns thick. I studied the structure of the tissue and the cells mostly by using the celloidin sections, staining them according to the five general methods, and in one case making the oxydasic reaction. The histological sections were stained as follows:

1) according to van Gieson;
2) with haematoxylin-eosin;
3) according to Pappenheim (though this method is used for the purpose of staining blood preparations, I adapted and

modified it for staining the histological sections in order to determine the cells);

4) according to Unna-Pappenheim;

5) according to Unna (the two latter methods are used for the purpose of determining the plasma cells);

6) for ascertaining the granules in the mast-cells I used Winkler's α-naphthol-dimethyl-p-phenylen-diamin oxydasic reaction as modified by W. Schultze and Fursenko (158) for the technique of pathological histology. In order to have a choice, I stained two sections, at least, according to all methods.

The histological material from the udders, the supramammary and deep inguinal lymph nodes of unaffected cows (cases VII, VIII, IX, X, XI, XII, XIII, XIV and XV) I removed and studied in the same manner as that of brucellous cows, the material being fixed in 10 per cent. formalin.

For staining the bacteria, both in the paraffin and celloidin sections, I fastened the sections to the slides according to the Henneguy method as modified by Jordan (158). I eliminated the celloidin from the sections by means of absolute alcohol and a mixture of absolute alcohol with ether; in case of need, I dipped the sections either in distilled water or in 70° alcohol before they were stained.

For the purpose of determining *Br. abortus* in the histological sections of the infected udders and the adjoining lymph nodes, many methods and possibilities had to be tried out, because no fixed method has as yet been adopted for the purpose of staining the above-mentioned microbe in the histological sections. I paid special attention to those methods that are used for the purpose of ascertaining *Br. abortus* in the smears, as well as to those used for the purpose of staining the gram-negative bacteria in the histological sections, and bacteria not easily susceptible to staining (e. g. *Bact. typhi abdominalis*, *Bact. leprae*, *Bact. mallei*, *etc.*). For the purpose of staining the bacteria in the histological sections, the following methods were employed:

1) Giemsa method with various modifications;

2) Ziehl's staining methods with diluted and undiluted carbolfuchsin;

3) Löffler's methylene blue methods;

4) Saathoff-Pappenheim's method with methyl green pyronine dilution used for the purpose of staining gram-negative bacteria in the histological sections;

5) Löffler's universal method for sections;

6) Nicolle's methylene blue tannin method for typhus, chicken cholera, and other bacteria;

7) Baumgarten's method for *Bact. leprae*;

8) Marzinowsky's method for *Bact. leprae*;

9) Kühne's carbol methylene blue stain for *Bact. mallei*;

10) Gram's stain as modified by Weigert for the purpose of staining histological sections;

11) Ziehl-Neelsen's method as modified by Schmorl for *Mycobacterium tuberculosis* in histological sections.

In staining *Br. abortus* in the histological sections, I used all the above-enumerated methods, always paying attention to other possible bacteria as well. For the purpose of staining *Mycobacterium tuberculosis*, I adopted Schmorl's (158) method as modified by Ziehl-Neelsen, because the changes found in the organs were similar to those to be noticed in cases of tuberculosis. I have employed this method for many other cases, *e. g.* very frequently for the purpose of staining sections of tuberculous material which has always given good results.

In ascertaining *Br. abortus*, I began with the sections where I had noted very considerable changes. For the purpose of staining, I adopted first of all the Giemsa method as modified by Andersen and Torbjörnsen (3) which is used for staining *Br. abortus* in smears. The method is as follows: the preparation is fixed in methyl alcohol (10 min.) and then stained for about 15 min. in Giemsa solution (1 ccm of distilled water: 3 drops of Giemsa solution); after staining it for a short while, it is decolorized in 1 per cent. acetic acid, after which it is washed in water, dried and thus made fit for study. This method, however, did not give the desired results, because 15 min. proved too short a time for staining the sections, and the 1 per cent. acetic acid for decolorizing was too strong. Meanwhile I used various combinations: I altered the staining solution, the concentration of the acetic acid, and the duration of staining. After a long period of experimenting, I attained the best results by using the Giemsa solution in combination with the following method: I fixed the paraffin sections with capillary attraction to slides and cover-glasses thoroughly cleaned in alcohol and alcohol-ether, and dried them in the thermostat (at 37° C) for about 24 hours; I removed the paraffin from the sections as usual, by holding the sections in two xylols for

3 min. in each; further, I drew the preparations through four alcohols, diminishing the concentration (99·8°—50°), and, at last, through two distilled waters; I pasted the celloidin sections to the slides and cover-glasses and eliminated the celloidin in the above-described manner; I drew the celloidin sections likewise through four alcohols and two distilled waters. Then the sections were stained according to Giemsa in an azure-eosin-methylene-blue solution (the proportion being 1 ccm of double distilled water: 2 drops of stain) for 4 to 24 hours at about 30° C; during the staining I renewed the solution at least 4 times. The sections were stained in thoroughly cleaned cylinder-glasses provided with lids. After staining I rinsed the preparations for a short while in distilled water, and I decolorized them in 0·25 per cent. acetic acid for 5 to 30 minutes (the decolorizing was done according to the thickness of the sections and the duration of staining, but usually until the preparation lost its dark blue colour and acquired a reddish hue on its blue ground colour). Further, the preparation was rinsed in several distilled waters for 4 minutes, and at last it was drawn through one 70° alcohol and two absolute alcohols (1 to 2 minutes in all); this was followed by xylol (3 times renewed) and Canada balsam; the preparations on the slides were covered with cover-glasses and *vice versa*; then followed the investigation of the preparations. The whole procedure is very time-consuming indeed, and yields good results only when everything is performed punctually and regularly, beginning with the acquisition of the material and ending in the microscopy of the preparations. One cannot get preparations that are good enough to be photographed in a short time; it takes a comparatively long time till the technique and experience are finally acquired.

After attaining positive results by the above-described method, I made further experiments with Löffler's methylene blue solution. I prepared the sections for staining in the above-described manner, while the staining itself was made in the usual solution or in Löffler's 1:4 diluted methylene blue; in the first case I stained the sections for 15 to 30 minutes and in the second — for about 3 hours, I decolorized them in 0·5 per cent. acetic acid for 20 min.; the further treatment of the preparations was carried on in just the same way as in staining with Giemsa solution. Staining with Löffler's diluted methylene blue solution for a longer while gives better results than staining for a shorter time in a stronger

solution. The preparations stained in Löffler's methylene blue solution and suitably decolorized proved good enough for the bacteria to be photographed in the sections. For determining *Br. abortus* in the sections, staining by Löffler's method was not so suitable as staining with the Giemsa solution because of the monotony of its colour.

I made many experiments with Ziehl's carbolfuchsin, diluting it to 1:75; the staining lasted for about 24 hours at about 30° C, and the decolorizing in 0·25 per cent. to 0·5 per cent. acetic acid — according to the intensity of colour — for about 30 min. The rest of the staining procedure I carried out in the same way as with the Giemsa solution. This staining method likewise gives good results so that the bacteria can be photographed in the sections. For the purpose of searching and determining the bacteria, especially when they are present in small quantities in the sections, Ziehl's method is not so suitable as the Giemsa method.

I made less use of all the other staining methods enumerated. Although I succeeded in staining *Br. abortus* in the sections by these methods, they did not show any advantage, and the contrast in the colouring was often insufficient. For the purpose of acquiring technique and controlling the results, I injected a dense suspension of cultures of *Br. abortus* into normal and unaffected lymph nodes, and dipped the blocks of tissue after their injection into the suspension. These pieces of lymph nodes I fixed and embedded just in the same way as the whole examined material; further, I made sections and stained them according to the same methods I had used for the purpose of determining *Br. abortus* in the sections containing changes. The microbe was found with no difficulty on the uneven surface of the pieces of the lymph nodes.

I stained *Br. abortus* in the histological sections of the fetal membranes of 4 cows that had aborted (where the infection with *Br. abortus* had been previously ascertained by means of the guinea-pig experiment), and of the spleens and the lymph nodes of 5 brucellous guinea-pigs.

The sum total of the histological sections I have stained for the purpose of all my studies exceeds 8000.

I began with the present studies towards the end of the year 1930, and by working continuously, finished them in two years and a half.

I made the bacteriological studies at the Veterinary and Milk-hygienic Institute of the University of Tartu (Director: *Prof. Dr. E. Roots*), and the histopathological studies at the Institute for Pathology of the University of Tartu (Director: *Prof. Dr. A. Valdes*).

I made all the preliminary arrangements and the studies in connection with the present work personally, except in those cases where the work could not be carried out regularly and in due time by one person. My wife (Senior Assistant at the Veterinary and Milk-hygienic Institute of the University of Tartu) gave her technical assistance in studying the material bacteriologically and in making cultures, injecting guinea-pigs, and placing the material for fixation.

c. Results of Inquiries.

Cow I — "Koidu".

5 years of age (born in 1926), a cross-bred animal of the Estonian red breed ("angler"), bought from the J. estate by which she had been bought at the Helme fair in 1928. Her weight was about 425 kg. The cow was sold on January 27, 1931 because of her diminished milk production and non-pregnancy, and was slaughtered on the same day in the town abattoir at Tartu.

aa. Preliminary Data.

"Koidu" had calved normally at the J. estate on December 11, 28 and on February 17, 30. After that she had been bred repeatedly on May 17, 30, July 6, 30, July 26, 30, September 4, 30, October 15, 30, October 23, 30, November 23, 30, and January 6, 31, but had not conceived.

According to the serological examinations made on January 2, 28 at the Bacteriological Station of the University of Tartu (Director: *Prof. Dr. F. Laja*), "Koidu's" blood serum was positive in a dilution of 1 : 40 to the aggl. test for *Br. abortus*; later, on September 16, 29, the aggl. titer was 1 : 320+, on March 14, 30 — 1 : 320+, and on October 16, 30 — 1 : 1280+.

"Koidu" had not been given either living or killed cultures of *Br. abortus*.

According to table 3, *Br. abortus* and its agglutinins were not present in her milk on April 14, 30, but beginning with May 28, 30, *Br. abortus* and brucella-agglutinins were found time after time

constantly in her milk. The aggl. titer of her milk serum increased all the while, and on the date of slaughter (January 27, 31) it was 1 : 320 + (data obtained from *Elfriide Ridala*).

Table 3.

Dates of Examinations of Milk	Agglut. of *Br. Abortus* with Milk Serum	Agglut. of *Br. Abortus* with Blood Serum of Guinea-pigs injected with Sediment + Cream	Culture of *Br. Abortus* from the Spleen of Guinea-pigs
14. IV 30	negative (1 : 5±)	negative	negative
28. V 30	positive (1 : 80)	positive (1 : 80)	positive
3. VII 30	„ (1 : 160)	„ (1 : 80)	„
11. VIII 30	„ (1 : 160)	„ (1 : 500)	„
3. X 30	„ (1 : 160)	„ (1 : 2560)	„
17. XI 30	„ (1 : 320)	„ (1 : 2560)	„
7. I 31	„ (1 : 320)	„ (1 : 640)	„

Table 4 presents data concerning "Koidu's" milk production; no special conclusions, however, can be drawn from these data.

Table 4.

Month	1928		1929		1930		1931		Notes
	Milk kg	Butterfat kg	Milk kg	Butterfat kg	Milk kg	Butterfat kg	Milk kg	Butterfat kg	
January	—	—	383	13·79	139	5·14	120	—	On the days previous to slaughter "Koidu" yielded about 2 kg of milk a day.
February	—	—	245	6·89	103	3·19	—	—	
March	—	—	243	7·29	379	14·40	—	—	
April	—	—	259	7·35	372	13·76	—	—	
May	—	—	256	7·42	356	11·75	—	—	
June	—	—	252	8·57	304	9·42	—	—	
July	—	—	240	7·68	222	7·33	—	—	
August	—	—	238	7·85	240	7·92	—	—	
September	—	—	221	6·63	229	7·33	—	—	
October	—	—	157	5·97	204	6·93	—	—	
November	—	—	167	5·51	190	6·08	—	—	
December	230	7·82	188	6·02	177	6·19	—	—	
Amount	230	7·82	2849	90·97	2915	99·39	120	—	

bb. Results of Inquiries concerning Milk and Blood Sera.

In making use of the above data, I examined "Koidu's" milk on January 13, and January 27, 31, to find out which of the quarters was infected with *Br. abortus*, and whether there were other bacteria causing diseases or not. Table 5 presents the data of the examinations.

Table 5.

Milk examined January 13, 31 and January 27, 31 *	Aspect of Milk	Quantity of Sediment	Microscopical Finding in Sediment			Bacteriological Finding in Sediment		Agglut. of *Br. Abortus* with Blood Serum of Guinea-pigs injected with Milk Sediment and Cream	Culture of *Br. Abortus* from the Spleen of Injected Guinea-pigs	Examinations of Injected Guinea-pigs for Tuberculosis
			Cellular Elements	Microbes		Saccharose Plates	Brown's Plates			
				Gram's Staining	Ziehl-Neelsen's Staining					
Right Front Quarter	Normal	Normal	Abundant	Negative	Negative	Negative	Negative	Positive (1 : 640)	Positive	Negative
Right Rear Quarter	"	"	"	"	"	"	"	" (1 : 320)	"	"
Left Front Quarter	"	"	"	"	"	"	"	Negative	Negative	"
Left Rear Quarter	"	"	"	"	"	"	"	Positive (1 : 1280)	Positive	"

Table 5 indicates brucellous infection in three quarters of "Koidu's" udder; the milk from the left front quarter did not show the above-mentioned microbe to be present. No other bacteria were detected by repeated bacteriological examinations in any of the quarters. The sediment of the milk from each quarter showed a good many cellular elements; the polynuclear leucocytes were most abundant, whereas the number of epithelial cells and lymphocytes was smaller, also single plasma cells were present.

On the date of slaughter on January 27, 31, her blood serum titered 1 : 5120 to the aggl. test for *Br. abortus*.

cc. Macroscopical Finding at the Time of Slaughter.

General Condition.

The cow "Koidu" was in a wellnourished condition. Her fleshy body suggested rather that of fattened cattle than of a milch cow.

Udder.

The whole udder weighed 5560 g, being of normal shape, of an even and unusually dense and fleshy consistency.

The right front quarter weighed 1510 g, the sections showed a variegated colour; on the surface of the sections there

* All examinations listed in the table were made on January 27, 31, except the infection of the guinea-pigs.

were to be noticed alternate yellowish-grey, reddish-yellowish-grey, and reddish-grey spots of varying size. In the whole quarter there were to be found lobules which contained certain spots and specks that were partly of a lighter, partly of a darker shade than the ground colour, and they were rather irregular in outline. The consistency of the present quarter was denser than the usual tissue of the udder that was noticeable especially on the border portions of the quarter, but partly also in the middle of the quarter. The usual porous structure of the lobules in those places was absent, and the lobules had an even lardaceous appearance. There was an intense proliferation of the interstitial connective tissue which was thoroughly mixed with lobules in all directions, and particularly in the above-described places. The tissue of some lobules was substituted by connective tissue. In the border portions of the quarter there were to be noticed in places some haemorrhages which extended over all the lobules. In the smaller lactiferous ducts there were in places somewhat spongy, oblong and roundish, dull, slightly uneven formations, rising about 1·5 mm above the surface of the ducts. In the border portions of the quarter there were in places observable cystiform formations, of about 4 mm in diameter, that were surrounded with capsules of a dense connective tissue; their lumina contained partly a glassy, and partly a brittle, doughy mass of yellowish-grey colour.

The right rear quarter weighed 1710 g and was in general of the same qualities as the right front quarter, but it showed differences in that haemorrhages were to be observed only on the outside of the quarter, and the proliferation of the connective tissue was slighter.

The left front quarter weighed 1130 g. It resembled the right rear quarter, but haemorrhages and cystiform formations were absent.

The left rear quarter weighed 1210 g. It resembled the left front quarter, but there were also to be found cystiform formations.

Lymph Nodes.

There were two right supramammary inguinal lymph nodes *(Lln. inguinales superficiales dextri)*, one of them very much resembling a kidney; it weighed 70 g and was $11 \times 6 \times 2{\cdot}5$ cm large; the other, having a round shape, was $3{\cdot}1 \times 2{\cdot}4 \times 1{\cdot}9$ cm large and weighed 10·2 g. The above-mentioned

lymph nodes were not joined by lymphatic vessels; they were dilated (swollen); besides the usual unevenness on their surface there were astrictions drawn inwards. The cortical substance of the lymph nodes showed in sections a pale-grey colour, and the medullary substance a brownish-dark-grey colour. Both the substances, particularly the cortical substance, showed an observable reticular connective tissue that was partly formed into firm bundles of 6 mm in diameter. The surface of the sections was abundantly covered with succus that resembled a liquid, farinaceous porridge. The sections did not cover each other when restored to their previous position, the border parts keeping apart from each other.

There were two left supramammary inguinal lymph nodes *(Lln. inguinales superficiales sinistri)*: one of them weighed 55 g, and its size was 7·5 × 4·7 × 2·2 cm; the other weighed 11·5 g, and its size was 3·4 × 3·1 × 2·8 cm. All the other qualities of these lymph nodes were similar to those in the right supramammary inguinal lymph nodes.

The right deep inguinal lymph node *(Ln. inguinalis profundus dexter)* weighed 47·2 g, and its size was 9·5 × 5·6 × 2·1 cm. Its other qualities resembled those of the supramammary inguinal lymph nodes, only the colour in the sections was somewhat darker.

The left deep inguinal lymph node *(Ln. inguinalis profundus sinister)* weighed 43·1 g, and its size was 9 × 6·4 × 2·2 cm. The other qualities of this lymph node were similar to those in the right deep inguinal lymph node.

Uterus.

The uterus resembled that of a normal, non-pregnant cow. The wall of the uterus was 2·2 cm thick. The mucous membrane was very considerably corrugated, of a reddish-grey colour, covered with quite an abundant quantity of a half-muddy, glassy mucous layer.

Ovaries.

The size of the right ovary was 3·8 × 3 × 2 cm. The surface was rather coarse and rough. Its consistency was softer than that of the tendon. Its intersection showed a dense connective tissue with single follicles in it. There was one corpus luteum in the ovary which was 2 × 1·6 × 1·4 cm large.

The size of the left ovary was 3·5 × 3·1 × 1·8 cm.

All its other qualities resembled those of the right ovary, only there was no corpus luteum in it.

No macroscopical changes were noted in the other organs.

dd. Bacteriological Findings and Results of Injections, in Animals used for Experiments.

For the purpose of determining *Br. abortus* and *Mycobacterium tuberculosis*, I used the guinea-pig experiment. In pursuing other conceivable germs of diseases or some occasional microbes in the material under examination, I made cultures on bromcresol-purple-saccharose-alkalialbuminate agar and on Brown's modified blood agar. The results of these studies are illustrated in table 6.

Table 6.

Material	Cultures		*Br. Abortus*, Agglut. with the Blood Serum of Injected Guinea-pigs	Culture of *Br. Abortus* from the Spleen of Injected Guinea-pigs	Examination of Injected Guinea-pigs for Tuberculosis	Culture of *Br. Abortus* from the Examined Material
	Saccharose Plates	Brown's Plates				
Lln. inguinales sup. dext.	sterile	sterile	positive (1:5120)	positive	negative	—
Lln. inguinales sup. sin.	„	„	„ (1:5120)	„	„	—
Ln. inguinalis prof. dext.	„	„	„ (1: 320)	„	„	—
Ln. inguinalis prof. sin.	„	„	„ (1: 160)	„	„	—
Uterus	„	„	—	—	—	positive

From table 6 it follows that besides the three quarters of the udder of the cow "Koidu", the supramammary and deep inguinal lymph nodes and uterus were infected with *Br. abortus*. No other types of bacteria were present.

ee. Histopathological Finding.

Udder. Right Front Quarter.

a) In regions 1, 5, and 9 (see scheme 1, page 41) there are plenty of foci which vary in size, and some of which reach the size of lobules; single foci, however, are to be found in regions 2, 4 and 8. In the centre of these foci, in the finely granulated and netlike reddish-brown (by van Gieson) ground substance, there

are cells which are not close-set, being partly swollen and set irregularly. These cells resemble the epithelioid cells which appear in tuberculous tubercles. Among the above-mentioned cells, in some foci there appear parts of already decayed and of still disintegrating cells. In regions 1, 6, and 7, in one of the foci, there is one giant-cell of the Langhans' type, and in one focus of region 5 there are two giant-cells of the same type side by side. In the superficial portions of the foci, plasma cells and lymphocytes appear very close to each other, polynuclear leucocytes and fibroblasts being less numerous, with only a few mast cells. Between the cells mentioned there are numerous capillaries abundantly filled with blood, and fibres of collagenic connective tissue singly and in groups. In the boundary portions of the foci, single well preserved alveoli are to be observed; the epithelium of some of the alveoli is swollen, proliferated and desquamated, so that the lumina of the alveoli are filled with epithelial cells; some of these cells are pyknotic and they are disintegrating, or they have already partly decayed; single lymphocytes, plasma cells, and polynuclear leucocytes are to be found between the epithelial cells. The epithelium of a part of the alveoli is much less proliferated, but set irregularly, and here also some of the epithelial cells are swollen, others are pyknotic, and still others thoroughly decayed. Between the epithelial cells, similar cells appear as in the other alveoli mentioned already. In the lumina of the last-mentioned alveoli there are moderately desquamated epithelial cells (some of these are much swollen and some are polynuclear), single polynuclear leucocytes, plasma cells, parts of decayed cells, and in small quantities a netlike mass of a brownish-pale-red colour (by van Gieson). In region 2, in the preserved alveoli of the boundary portions of the foci in question a great majority of the epithelial cells contain fat globules; in the lumina of the alveoli there are to be found numerous polynuclear leucocytes, desquamated epithelial cells, fat globules, and a moderate quantity of a netty mass of brownish-pale-red colour. The tissue between the alveoli is dilated, showing numerous lymphocytes and plasma cells closely accumulated in places, with single polynuclear leucocytes among them.

In the histological sections of the above-described foci I found *Br. abortus* by means of the Giemsa and Löffler's methylene blue staining as modified by me.

b) In regions 1 and 6 there are numerous foci similar

to the foci a in the quarter in question, but which differ from these in that the finding in their centre, though containing occasional epithelial cells, is otherwise similar to the finding in the boundary portions of the foci.

c) In regions 1 and 9, the epithelium of most of the lactiferous ducts is proliferated, flattened and partly swollen, some of the cells having several nuclei — especially those that are against the lumen of the duct. Between the epithelial cells and especially in the nearest surroundings of the duct, we find a very dense infiltration of the plasma cells and some lymphocytes, but the infiltration of the polynuclear leucocytes is less in density. In the whole area, between these infiltration cells, there appear numerous fibres of collagenic tissue, singly and fasciculated, fibroblasts, and some mast cells. In the lumina of the ducts, the brownish-pale-red (by van Gieson) mass of various density shows desquamated epithelial cells in various quantities (in places some of them are much swollen and polynuclear), lymphocytes, polynuclear leucocytes, and parts of decayed cells, and in places single plasma cells. Beside other changes in one lactiferous duct of region 9, the epithelium is proliferated, flattened and stratified, and in the superficial portion partly cornified. In the border portion of the horny layer there appear bruised, flat, pyknotic and decayed nuclei, as is usual in the case of a cornification of the flat epithelium. Similar alterations are to be found in the lactiferous ducts of regions 5 and 6, as in regions 1 and 9, only in smaller quantities and in a slighter degree, not taking into account the cornification of the epithelium. Besides, in region 5, in the epithelium of one lactiferous duct which shows alterations, there is also a focus of epithelioid cells among the rest, with one giant-cell of the Langhans' type in it. No alterations are to be observed in the lactiferous ducts of the areas in the other quarters in question.

d) In the neighbourhood of other changes, the interlobular connective tissue in regions 1 and 9 shows in places an acute proliferation; in this tissue, an infiltration mostly of the plasma cells and lymphocytes appears in varying density and partly in groups, and in places there are also less numerous polynuclear leucocytes. In regions 5, 6, and 7, the changes in the interlobular connective tissue are slighter than in regions 1 and 9. In places a considerable amount of the fat tissue is observable in the interlobular connective tissue of region 9.

e) In all the regions, in the lumina of the alveoli of the lobules, which are normally preserved, there is to be found a brownish-pale-red reticular mass, partly in small, partly in moderate quantities; amid this mass there are some fat globules and very rarely occasional polynuclear leucocytes and desquamated epithelial cells; the epithelium of the alveoli is more often cubical in shape, but also cylindrical, though the latter occurs more rarely. In the lumina of some alveoli, in region 8, polynuclear leucocytes appear moderately in places, and between the alveoli, here and there, there are small groups of lymphocytes and plasma cells. In region 2, occasional lobules are present which are in a state of inaction.

Amyloid corpuscles appear abundantly in the whole quarter; these are in places more numerous in the normal part of the quarter.

There is no alteration to be observed in regions 3 and 10 (teat).

The approximate percentage of the changes that have taken place in the tissue of the examined quarters in question is shown in table 7.

Right Rear Quarter.

In regions 2, 3, 5, 6, and 9 there are numerous foci of varying size and density of the cells which are similar to those in the histological finding in the right front quarter, as described in a and b (fig. 3, 4, 6). Among the epithelioid cells of several foci in regions 5 and 9, usually a giant-cell of the Langhans' type is present, but in one focus of region 9 there appear three giant-cells of the same type side by side. Besides, in some of the foci in area 6, a very dense infiltration has occurred, which extends over the whole focus and consists of lymphocytes, plasma cells, and less numerous polynuclear leucocytes. Furthermore, in one altered lobule of region 6 and in several changed lobules of region 9, besides other alterations there are to be observed numerous capillaries densely filled with blood. In two foci, however, which otherwise are similar to the other foci, there appears an area of necrosis (fig. 12), extending over several alveoli, where there are parts of not very dense decayed cells, and disintegrating cells in the reticular ground substance of a pale-reddish-brown colour; the transition from the necrotic part into the part free of necrosis is slow. In one of the lobules the epithelium of the alveoli is considerably atrophied and mostly pyknotic, and the lumina of the alveoli are empty.

In regions 4 and 8 some foci are similar to those in the right front quarter, as described in a. Besides, in region 8 there are two foci of the size of a lobule, in the centre of which there do not appear any epithelioid cells, but mostly close-set plasma cells and lymphocytes. Among these only here and there disintegrating alveoli are to be found. Similar to the border portions of the foci, the epithelial cells of the alveoli have fat globules, and they are partly swollen, partly polynuclear, and in some alveoli mostly desquamated. In the lumina of the alveoli there are to be found numerous desquamated epithelial cells, polynuclear leucocytes, fat globules, and in smaller numbers lymphocytes; among the infiltration cells, outside the preserved alveoli, there appear likewise fat globules which evidently originate from the decayed alveoli.

In region 5 there are to be found some foci of various sizes, where the alveoli are decayed and replaced by a collagenic connective tissue with many capillaries and a dense round-celled infiltration (fig. 17).

In the alterations in regions 2 and 5 I found *Br. abortus* in the histological sections.

In regions 2, 3, 4, 5, 6, and 9, several lactiferous ducts show similar changes to those in the corresponding lactiferous ducts of the right front quarter (fig. 27). Similar to the epithelium of one lactiferous duct in region 9 of the right front quarter, two lactiferous ducts in region 2 (fig. 24), and one in region 3 of the quarter just under examination, show a proliferation, flattening and cornification in the superficial portion of the epithelium.

In regions 2, 3, 5, 6, 8, and 9, changes of the interlobular connective tissue in a varying amount are observable, and they are similar to those in the corresponding connective tissue of the right front quarter.

No changes are observable in regions 1, 7, and 10.

Also in the normal tissue of the udder, in general, the finding is the same as in the corresponding part of the right front quarter.

Left Front Quarter.

No pathological changes have taken place in the left front quarter; the finding in the parenchyma of the udder is alternately similar to the normal state in the lobules of the right front quarter. The interlobular connective tissue has moderately proliferated here and there, especially between the lobules that are in a state of

inaction, but there is no infiltration in it. Only the interalveolar connective tissue, considerably dilated in the lobules which are in a state of inaction, shows in places rare lymphocytes and occasional plasma cells.

Left Rear Quarter.

In regions 4, 5, 6, 7, and 9 there are numerous foci which resemble those in the histological sections of the right front quarter, as described in a (fig. 15) and b, whereas regions 2 and 3 show only a few of the above foci; in the corresponding foci of regions 7 and 9, the cellular infiltration is still more dense, and in places there are to be found single dilated capillaries. In regions 7 and 9, among the epithelioid cells of several foci, there appear giant-cells of the Langhans' type, and in one focus of region 4, there are two giant-cells of the above type. In region 5 there are some foci which also appear in region 8 of the right rear quarter.

In the corresponding foci of the changes I found *Br. abortus* in several places in the histological sections of regions 4 and 7 (fig. 47).

In regions 7 and 9, the lactiferous ducts are changed in their whole extent; the changes are in general similar to those in the lactiferous ducts of the right front quarter of the cow in question, only the degree of the changes is in places much higher: *e. g.* the lumina of the smaller lactiferous ducts are totally closed by the intense proliferation of the epithelium. The wall of the lactiferous ducts has considerably thickened, consisting chiefly of collagenic fibres and fibroblasts; among the latter there are numerous lymphocytes, plasma cells, and occasional polynuclear leucocytes. Six lactiferous ducts of region 7 and nine ducts of region 9 show in places a flattening and stratification of the epithelium, and a cornification of the superficial portions which lie towards the lumina. Besides other changes in the walls of the lactiferous ducts, in places there are to be observed also foci of the epithelioid cells, in some of which there are also some giant-cells of the Langhans' type. In regions 2, 4, 5, and 6 there are some alterations in the single lactiferous ducts, in general to a much slighter degree than in regions 7 and 9, but in region 5, the epithelium of a considerably changed lactiferous duct shows cornification. In the interlobular connective tissue, on the whole, changes of varying intensity are to be observed, which are similar to those in the corresponding tissue of the right front

quarter. Greater changes are to be found in the interlobular connective tissue of region 2, whereas the changes noticed in the same tissue in regions 3, 4, 5, 6, 7, and 9 are slighter, notwithstanding the very severe changes in the rest of the udder tissue in regions 7 and 9.

No changes are to be observed in regions 1, 8, and 10.

The finding in the unchanged tissue of the quarter under investigation is on the whole similar to that in the corresponding tissue of the right front quarter, but in general amyloid corpuscles are fewer in number.

The cystiform formations, as described in the macroscopical finding and observed in the right front, right rear, and left rear quarters, may be classified according to the histological sections into three different kinds:

a) The changes of the lactiferous ducts are very much like those of the ducts in the right front quarter, but they differ from these in that the ducts are dilated, round, and the changes around the ducts are extensive, the collagenic connective tissue is considerably proliferated, with a very dense cellular infiltration in it, which resembles the infiltration around the changed lactiferous ducts in the right front quarter. Besides, a cornification is often to be found in the superficial portions of the very intensely proliferated, swollen and flattened epithelium (fig. 25). In the lumina of the lactiferous ducts, there are numerous particles of decayed cells, disintegrating cells, and a mass of a reddish-yellowish-brown colour (according to van Gieson), amid which, particularly in the border portions, there appear numerous swollen (also polynuclear) and desquamated epithelial cells, polynuclear leucocytes, lymphocytes, and less numerous plasma cells. In these cystiform formations, particularly in the swollen epithelial cells and around them, I found *Br. abortus*.

b) The lactiferous ducts are dilated, and their epithelium is in places proliferated, partly atrophied and pyknotic. The lumina of the ducts are abundantly filled with a yellowish-reddish-brown mass (according to van Gieson), which is very finely granulated (almost homogeneous); in this mass, especially in the border portions of the lumina, there appear in places intensely swollen and desquamated epithelial cells, and in smaller numbers lymphocytes and polynuclear leucocytes. Between the epithelial cells, and particularly in the subepithelial tissue, there appear in places lymphocytes of various

density, plasma cells, polynuclear leucocytes in smaller numbers, numerous fibroblasts, and fibres of collagenic connective tissue.

c) Dilated and confluent alveoli with an intensely atrophied epithelium, and the contents of the lumina resemble those of the cystiform formation b.

In the right front and right rear quarter the macroscopically observable haemorrhages appear in lobules, which show no other intense changes besides an intense atrophy of the epithelium of the alveoli.

Table 7.

Extent of the Changes in the Quarters of the Udder according to Histological Studies.

Cow I.

Studied Regions	Approximate Percentage of the Changes of Tissue in the Studied Regions (Scheme 1, Page 41)										Approximate Average Percent. of the Changes of Tissue in the Whole Quarter [not considering Region 10 (Teat)]
	1	2	3	4	5	6	7	8	9	10	
Right front quarter	50	25	—	5	40	60	20	5	75	—	31·1
Right rear quarter	—	40	50	10	100	60	—	20	100	—	42·2
Left front quarter	—	—	—	—	—	—	—	—	—	—	—
Left rear quarter	—	25	10	40	33	40	100	—	100	—	38·7

The dash in table 7 shows that no changes occurred.

Lymph Nodes.

The right supramammary inguinal lymph nodes show intense changes of the usual structure of the lymph nodes. Only single germ-centres have survived in places. The connective tissue shows in places intense proliferation, particularly in the medullary substance, where only injured portions of the lymph nodes are preserved here and there (fig. 38). Beside other cells in the last-mentioned areas, plasma cells appear in great quantities diffusely and focally, and single mast cells. The lymph sinuses and lymph capillaries are in places dilated, and besides lymph

they contain various cells in great quantities (part of them on the point of disintegration), parts of decayed cells, and in places red corpuscles in small numbers. Over all the lymph nodes in several places there are foci of epithelioid cells, with rare giant-cells of the Langhans' type among them (fig. 37). The mentioned foci of the epithelioid cells differ from the foci of the epithelioid cells found in the udder insomuch that lymphocytes, plasma, and other cells have frequently forced their way between the epithelioid cells.

I found *Br. abortus* often in groups and in sparse sprinklings in the parenchyma of the lymph nodes also outside the foci of the epithelioid cells, and sometimes in the lymph sinuses of the histological sections.

In the left supramammary inguinal lymph nodes the finding resembles the one in the right inguinal lymph nodes; the only difference is that in one place, among the epithelioid cells, there even appear two giant-cells of the Langhans' type side by side.

The finding in the right deep inguinal lymph node is similar to that in the right supramammary inguinal lymph nodes, with the difference that in places the changes are less.

The finding in the left deep inguinal lymph node is similar to that in the right deep inguinal lymph node.

In all the above-mentioned lymph nodes I found *Br. abortus* in the histological sections.

Uterus.

The mucous membrane is of various thickness, in places being almost as thick as half of the wall of the uterus. The epithelium is in places desquamated, and a part of the preserved epithelial cells in these places prove to be atrophied and pyknotic; between the epithelial cells there are plasma cells, lymphocytes, and single polynuclear leucocytes; in the injured subepithelial tissue, the above-mentioned infiltration cells are to be found diffusely or they are in places densely focal, and there are numerous fibres of collagenic connective tissue and fibroblasts among them. The corrugated uterine mucous membrane has in places grown together. In the granulation tissue there appear numerous plasma cells, lymphocytes, and single polynuclear leucocytes; in places there are groups of epithelial

cells that have survived, and numerous capillaries moderately filled with blood.

The glands are mostly in a state of inaction; and around them, particularly around those adjacent to the epithelium, there is in places an infiltration chiefly of plasma cells and lymphocytes.

Ovaries.

In both ovaries there is to be found a great deal of connective tissue and very few follicles, and particularly small numbers of primary follicles; for the rest the finding is normal.

Cow II — "Loora".

7 years of age (born in 1924), a cross-bred animal of the Estonian red breed ("angler"), bought from the J. estate, by which she had been bought on March 12, 29. Her weight was 460 kg. The cow was sold on January 28, 31 because of her diminished milk production and non-pregnancy, and was slaughtered on the same day in the town abattoir of Tartu.

aa. Preliminary Data.

"Loora" had calved normally on March 17, 29 and on March 22, 30 at the J. estate. After that she had been bred repeatedly on June 2, 30, July 9, 30, September 4, 30, October 20, 30, November 2, 30, and November 28, 30, but had not conceived.

According to the serological examinations made on November 16, 29 at the Bacteriological Station of the University of Tartu, "Loora" proved to be infected with *Br. abortus* (aggl.-titer 1:6400+); later, on March 14, 30, the aggl.-titer was 1:1280+ and on October 16, 30 — 1:2560+.

On April 14, 30 "Loora" was vaccinated with 10 ccm of the living culture of *Br. abortus*, and on May 2, 30 she was injected with 20 ccm of the same culture.

According to table 8, the agglutinins of *Br. abortus* were found constantly in the milk of the above-mentioned cow, and the presence of *Br. abortus* was ascertained by means of the guinea-pig experiment (data got from *Elfriide Ridala*).

Table 8.

Dates of Examinations of Milk	Agglutination of *Br. Abortus* with Milk Serum	Agglut. of *Br. Abortus* with Blood Serum of Guinea-pigs injected with Sediment of Milk + Cream	Culture of *Br. Abortus* from the Spleen of Guinea-pigs
14. IV 30	positive (1 : 80)	positive (1 : 320)	positive
28. V 30	„ (1 : 160)	„ (1 : 160)	„
3. VII 30	„ (1 : 160)	guinea-pig died 20. VII 30	„
11. VIII 30	„ (1 : 160)	positive (1 : 1600)	„
3. X 30	„ (1 : 160)	„ (1 : 1280)	„
17. XI 30	„ (1 : 320)	„ (1 : 1600)	„
7. I 31	„ (1 : 160)	„ (1 : 320)	„

According to table 9, "Loora's" milk production was small all the time she was at the J. estate.

Table 9.

Month	1929		1930		1931		Notes
	Milk kg	Butterfat kg	Milk kg	Butterfat kg	Milk kg	Butterfat kg	
January	—	—	46	2·07	113	4·29	
February	—	—	dry		—	—	On the days
March	during 10 days 100	3·30	54	1·84	—	—	previous to slaughter
April	346	11·42	352	11·79	—	—	"Loora" yielded about 1,5 kg milk a day
May	286	10·01	367	11·05	—	—	
June	225	10·57	303	11·21	—	—	
July	202	7·67	245	9·80	—	—	
August	224	8·51	277	11·08	—	—	
September	161	6·28	233	8·62	—	—	
October	179	6·80	271	9·21	—	—	
November	129	5·16	205	7·17	—	—	
December	80	3·12	193	6·75	—	—	
	1932	72·84	2486	90·59	113	4·29	

For want of data it is impossible to ascertain whether the cow was infected with *Br. abortus* at the J. estate, or whether she had been infected before she was taken there.

bb. Results of Inquiries concerning Milk and Blood Sera.

In making use of the above data concerning "Loora", I examined her milk on January 13 and 27, 31 to find out which of the quarters were infected with *Br. abortus*, and to make sure whether there were not present other bacteria causing diseases. Table 10 presents the results of the inquiries.

Table 10.

Milk examined on 13. I 31 and 28. I 31 *	Aspect of Milk	Quantity of Sediment	Microscopical Finding in Sediment			Bakteriological Finding in Sediment		Agglut. of *Br. Abortus* with Blood Serum of Guinea-pigs injected with Sediment + Cream	Culture of *Br. Abortus* from the Spleen of Injected Guinea-pigs	Examination of Injected Guinea-pigs for Tuberculosis
			Cellular Elements	Microbes						
				Gram's Staining	Ziehl-Neelsen's Staining	Saccharose Plates	Brown's Plates			
Right front quarter	normal	normal	abundant	negative	negative	negative	negative	positive (1:640)	positive	negative
Right rear quarter	"	"	"	"	"	"	"	" (1:160)	"	"
Left front quarter	"	"	"	"	"	"	"	" (1:160)	"	"
Left rear quarter	"	"	"	"	"	"	"	" (1:320)	"	"

Table 10 indicates brucellous infection in all the quarters of Loora's udder. No other bacteria were detected by repeated bacteriological examinations in any of the quarters. The sediment of the milk from each quarter showed a good number of cellular elements; the number of polynuclear leucocytes was the highest, the epithelial cells and lymphocytes occurred in smaller numbers, and there were only single plasma cells.

On the date of her slaughter (January 28, 31) the agglutination titer of her blood serum was 1:5120+.

cc. Macroscopical Finding at the Time of Slaughter.

General Condition.

"Loora" was in a wellnourished condition, her fleshy body suggested rather that of fattened cattle than that of a milch cow.

Udder.

The whole udder weighed 4735 g, being of a normal shape; its consistency was evenly dense and fleshy.

The right front quarter weighed 1105 g; the section showed a variegated colour. On the surface of the section, in the yellowish-pale-reddish ground colour there were pale- and

* All examinations listed in the table were made on these dates, except the injection of the guinea-pigs.

dark-grey spots indistinct in outline. The colour of the individual lobules was also variegated in places; the lobules of a darker ground colour showed lighter spots, and in the lobules of a lighter ground colour there were darker spots which gradually disappeared in the surroundings. The lobules were mostly porous, but between them, especially in the basal and border parts, there were lobules singly or in groups which could not be perceived as porous. The last-mentioned lobules were of a dense consistency and lardaceous appearance, and protruded over the surface of the section. There was a moderate flow of milk of normal appearance on the surface of the section made in the middle portion of the udder, but on the border part it flowed out in small quantities. The surface of the sections in the whole quarter of the udder showed an even discharge of blood in small quantities from the blood-vessels. The interlobular connective tissue was clearly perceivable, in places it was proliferated into big cords, the diameter of which amounted to 3 mm between the groups of lobules; in some places it was quite possible precisely to separate the connective tissue from the lobules, but in places this was impossible, because the connective tissue had intruded into the lobules and disappeared there by degrees. The inner surface of the lactiferous ducts was generally even (except the opening of the smaller ducts where there were crater-shaped concavities), shining and of a silver-grey colour. But in some places, the walls of some of the bigger lactiferous ducts, and more frequently those of the smaller ducts, were about 1,5 mm thick. These thickened places in the ducts consisted of a grey, brittle mass which had a dull surface with small elevations.

The right rear quarter weighed 1285 g and had in general the same qualities as the right front quarter. It differed from the latter in that it had a redder ground colour, and the shades of the colour were more contrasting. Also the proliferation of the connective tissue was intenser, and the lardaceous lobules without pores were more frequent.

The left front quarter weighed 1100 g and was of the same qualities as the right rear quarter.

The left rear quarter weighed 1245 g and was of the same qualities as the right rear quarter.

Lymph Nodes.

The right supramammary lymph nodes (*lln. inguinales superficiales dextri*) consisted of one bigger and one

smaller node which were connected by lymphatic vessels. Both lymph nodes were slightly dilated. The bigger one weighed 45 g and was 7·5 × 6·5 × 1·7 cm large. The smaller node weighed 10·2 g; it was of a roundish shape and had a diameter of 3·2 cm. The further qualities of the above-mentioned nodes were as follows: the surface was even, except in several places that were covered with single astrictions. The cortical substance in the sections was of a pale-grey colour, and the medullary substance was brownish-dark-grey.

In both the substances and particularly in the cortical one there was found a clearly observable reticular connective tissue which had cords in places as thick as 0·7 mm. The surface of the sections was abundantly covered with liquid farinaceous succus. The sections did not cover each other when restored to their previous position, the border parts kept apart from each other.

There were three left supramammary inguinal lymph nodes (*lln. inguinales superficiales sinistri*). The biggest of them weighed 47 g and was 10 × 5·5 × 2·8 cm large. The next in size weighed 10 g, and it was 3·5 × 3 × 2·5 cm large. The smallest node weighed 4 g and was 1·6 × 1·5 × 1 cm large. The former two were connected by lymphatic vessels. The other qualities of these lymph nodes were similar to those in the right supramammary inguinal lymph nodes.

The right deep inguinal lymph node (*ln. inguinalis profundus dexter*) weighed 38·2 g, and its size was 6·8 × 3·4 × 1·2 cm. Its other qualities were similar to those in the supramammary inguinal lymph nodes.

The left deep inguinal lymph node (*ln. inguinalis profundus sinister*) weighed 40·1 g and was 7 × 3·5 × 1·4 cm large. The other qualities of this lymph node resembled those of the supramammary inguinal lymph nodes.

Uterus.

The uterus was outwardly quite the same in appearance as that of a normal non-pregnant cow. The wall of the uterus was about 2 cm thick. The mucous membrane was considerably corrugated, of a red colour, and was moderately covered with a half-muddy, glassy mucous layer.

Ovaries.

The right ovary was 3·5 × 3 × 1·9 cm large. Its surface was rough, and in comparison with the flesh it was of a more solid consistency. Sections showed single follicles which were surrounded by stout cords of connective tissue. There was one corpus luteum there which was 2·4 × 2 × 1·9 cm large.

The left ovary was 3·2 × 2·9 × 1·8 cm large. Its other qualities resembled those of the right ovary, except that the corpus luteum was absent.

The other organs did not show any macroscopical changes.

dd. Bacteriological Findings and Results of Injections, in Animals used for Experiments.

For the purpose of determining *Br. abortus* and *Mycobacterium tuberculosis*, I used the guinea-pig experiment. In pursuing other conceivable germs of diseases or any occasional microbes in the material under examination, I made cultures on brom-cresol-purple-saccharose-alkalialbuminate agar and on modified Brown's blood agar. The results of the above-mentioned studies are illustrated in table 11.

Table 11.

Material	Cultures		*Br. abortus*' Agglut. with Blood Serum of Injected Guinea-pigs	Culture of *Br. Abortus* from the Spleen of Injected Guinea-pigs	Examination of Injected Guinea-pigs for Tuberculosis	Culture of *Br. Abortus* from the Examined Material
	Saccharose Plates	Brown's Plates				
Lln. inguinales sup. dext.	sterile	sterile	positive (1 : 1280)	positive	negative	—
Lln. inguinales sup. sin.	„	„	„ (1 : 160)	„	„	—
Ln. inguinalis prof. dext.	„	„	„ (1 : 320)	„	„	—
Ln. inguinalis prof. sin.	„	„	„ (1 : 160)	„	„	—
Uterus	„	„	—	—	—	positive

From table 11 it follows that besides all the quarters of "Loora's" udder, also her uterus, supramammary and deep inguinal lymph nodes on both sides were infected with *Br. abortus*. The examined material did not show any other bacteria present.

ee. Histological Finding.

Udder. Right Front Quarter.

a) In regions 1, 6, and 7 (fig. 5) there are several foci of varying size to the extent of three lobules, whereas in region 2 these foci appear in great quantities. Instead of the usual alveolar structure of the udder, a dense cellular infiltration of these foci is observable, which abounds with lymphocytes and plasma cells, but there are also fibroblasts in smaller numbers and some rare polynuclear leucocytes and mast cells. Besides this cellular infiltration in the larger foci, particularly in region 1, there are to be found some compressed alveoli with the pyknotic epithelium, and in the centre of some foci epithelioid cells (fig. 16) and their groups are observable which are in some foci already partly decayed and partly decaying. Between the infiltration cells, particularly in the border portions of the foci, fibres of a collagenic connective tissue appear singly and in bundles, which are bound together with the interlobular connective tissue; a repeated proliferation of the latter has taken place here and there, and the above-mentioned cellular infiltration appears in it in varying density. In region 2, the changes in the foci in question are in general severer; a thorough destruction of half of the alveoli in these foci has taken place, and the majority of the remaining alveoli are in a state of disintegration; these latter are compressed, and their epithelium is in an irregular position; some of the epithelial cells are swollen and have other symptoms of degeneration, some of them are atrophied and pyknotic, and in these and in the less changed lumina of the alveoli there usually appear desquamated, and mostly beside the decaying epithelial cells, polynuclear leucocytes in great quantities, single lymphocytes, and rarely a finely reticulated mass of pale-reddish-brown colour (according to van Gieson). The above-mentioned alveoli often appear singly in the cellular mass, and their shape is oblong and angular.

In region 7 there is one focus of about half-lobular size; it is similar to the foci a described above, but in the centre of the foci, in the dense cellular mass there are free fat globules; in the lumina of the preserved alveoli in the border portion of the focus, among the rest, there appear numerous pieces (casein) of a dark-reddish-brown colour and of an indefinable appearance.

b) In region 7 there is one, in region 9 — there are two, and in region 2 (fig. 8, 10) numerous foci similar to those in the quarter in

question, as described in a, but these foci show necrotic places of varying size, particularly in their centre, even to the extent of half a lobule; in these necrotic places there appear disintegrated pieces of nuclei in great numbers, a netlike granular mass of yellowish-brown colour, occasional pus corpuscles, and various necrotic cells. In several directions of these necrotic places there are to be found epithelioid cells in groups and singly, some of which show symptoms of necrosis; the single cells appear dispersedly. Among the epithelioid cells, in one focus there is also a giant-cell of the Langhans' type. The necrotic places are surrounded by a dense wall of plasma cells, lymphocytes, fibroblasts, occasional polynuclear leucocytes, and mast cells; this wall is interwoven with a collagenic connective tissue, which slowly turns into tender funicles and disappears in the necrotic mass.

In the necrotic foci I discovered *Br. abortus* by means of staining the histological sections by the Giemsa method as modified by me, and by Löffler's methylene blue method.

In region 4 there appears one, and in regions 7, 8, and 9 there are several foci resembling those in the quarter in question, as described in b, with the difference that in the centre of the foci there are no necrotic places, and in region 9, the epithelial cells are denser, and among them in several foci there are giant-cells of the Langhans' type.

c) One lobule in regions 1 and 3, two lobules in region 6 contain alveoli over the whole extent of the lobules, and several lobules in regions 4 and 5 contain some alveoli the shape of which is more or less unchanged, but their epithelium has degenerated or atrophied in varying intensity, the nuclei being pyknotic, and some of the epithelial cells desquamated. The interalveolar tissue is often dilated to about five times its former extent, and in places even more; in this tissue there is in varying quantities mostly a focal infiltration of plasma cells, lymphocytes, fibroblasts, and to a smaller degree of polynuclear leucocytes and occasional mast cells. A comparatively intense dilatation of the capillaries has taken place in the quarters with the changes already mentioned, and they are richly filled with blood. In the lumina of the alveoli there appear desquamated epithelial cells in varying quantities, as well as polynuclear leucocytes (chiefly neutrophilic) and fat globules, and a pale-red, partly homogeneous, partly finely reticulated mass.

In the lumina of some alveoli the polynuclear leucocytes are in a state of fatty degeneration and disintegration.

d) In region 3, there is one focus as big as eight alveoli in one of the lobules, and it contains densely accumulated cells: aa) numerous polynuclear leucocytes, which are in denser agglomerations fatty-degenerated and contain decayed nuclei (pus corpuscles) (fig. 7); in places, however, where they are not so numerous, they are slightly or not changed at all; bb) lymphocytes and plasma cells are to be found particularly in the border portion of the focus. In the corresponding places of the udder tissue, single, as if dispersed, degenerated epithelial cells, and also the interalveolar connective tissue are preserved.

e) In one lobule of region 2 and in several lobules of region 6, the epithelium of the alveoli is atrophied to such a degree that even some of the alveoli prove to be confluent.

f) In region 7 in two lobules here and there, single compressed alveoli are still preserved among the densely accumulated plasma cells and lymphocytes; the epithelium of these alveoli is partly intensely atrophied and contains pyknotic nuclei. The majority of the above lobules are intergrown reticularly with the collagenic connective tissue, and the still free cavities show deposited fat (fig. 18, 19, 32).

g) A variously dense infiltration of lymphocytes, plasma cells, and polynuclear leucocytes is found to surround one lactiferous duct in region 5 and several larger ones in region 6. The epithelium of several lactiferous ducts in region 9 and of the majority of them in region 8, is flattened and has become many-layered. The epithelial cells are of varying size; a particularly large dilatation and a partial disintegration of the cells is to be found in the cells adjacent to the lumina of the ducts. There are numerous plasma cells and lymphocytes and in smaller quantities polynuclear leucocytes between the epithelial cells; the above cells are in places accompanied by a collagenic connective tissue. To a large extent in the surroundings of the ducts there are numerous accumulated lymphocytes and plasma cells, amid which there are also often to be found fibroblasts and polynuclear leucocytes, and occasional mast cells. In the lumina of the ducts there are to be found desquamated epithelial cells of varying density, further, lymphocytes, polynuclear leucocytes, occasional plasma cells, parts of entirely decayed cells, and a finely reticulated pale-brownish-red mass (according to van

Giesen). The desquamated epithelial cells in the lumina of the lactiferous ducts are mostly multiferously swollen, and their nuclei and the nuclei of other cells observable in the lumina, are often pyknotic.

In other regions, the lactiferous ducts of the quarter of the dder in question have not undergone any changes.

h) Between those lobules where changes are observable in regions 2, 6, and 8, the interlobular connective tissue is in places intensely proliferated; in this tissue there appears a diffuse infiltration of varying density, and more frequently a focal infiltration of lymphocytes and plasma cells. The interlobular connective tissue has undergone similar alterations in other regions too, but to a considerably slighter degree. In the still normal parts of the quarter in question, the epithelium of the alveoli is alternately cubical or cylindrical. In the lumina of the alveoli, a mass of pale-red colour is to be observed in varying quantities, in which occasionally some single polynuclear leucocytes are to be found in some of the alveoli. Varying numbers of amyloid corpuscles of different sizes appear both in the changed and in the normal parts of the whole quarter.

No changes are to be observed in region 10 (teat).

Right Rear Quarter.

On the whole, the changes resemble those in the right front quarter of the same udder (fig. 14, 33), not taking into account the changes d, e and f. Foci b appear only in region 1 (fig. 9, 11, 13), and of the changes described under c there is only one focus in region 5, and there are several foci in region 6.

The alterations in the lactiferous ducts are in places more considerable than those in the lactiferous ducts of the right front quarter, *e. g.* in regions 2, 3, 4, and 9; in some of the lactiferous ducts the epithelium is proliferated and flattened, and in places there is a cornification to be found (fig. 26); single smaller lactiferous ducts in regions 1, 2, 3, and 4 show an intense proliferation of the epithelium, which has caused an obstruction of the lumen (fig. 21, 22, 23, 29). In region 2, in one wall of an entirely changed lactiferous duct, besides other changes there appears also a focus of epithelioid cells with a giant-cell of the Langhans' type (fig. 28). In the altered interlobular connective tissue there are to be found polynuclear leucocytes (mostly neutrophilic and also single eosinophilic), and mast cells in smaller numbers, particularly in the surroundings of the blood-vessels.

Also the finding in the normal parts of the udder is on the whole similar to that in the corresponding parts of the right front quarter, but lobules in an inactive state are more frequent.

In general, amyloid corpuscles are rarer in the regions of the quarter of the udder in question than in the right front quarter.

In region 10 (teat) in two places, the teat canal almost as big as three alveoli shows subepithelial infiltration of lymphocytes and plasma cells.

Left Front Quarter.

The histological finding is on the whole similar to that in the right rear quarter, only the changes are fewer, not taking into account such foci as appear in the right front quarter b, for they appear more frequently just in regions 5 and 8. The changes in the lactiferous ducts are slighter, but in region 7, in the part towards the lumen of one entirely changed lactiferous duct, there is to be found a cornification of the epithelium which is proliferated into layers and flattened.

No changes are to be found in region 10 (teat).

Left Rear Quarter.

The finding is similar to that in the right rear quarter, only there are to be found more changes. Besides, in regions 7 and 9, there are several lactiferous ducts with a cystic dilatation; their preserved epithelium is composed of a single layer, being intensely atrophied and pyknotic. In the lumina of these ducts there is an abundant mass of brownish-pale-red colour, occasional desquamated epithelial cells, and in smaller numbers parts of decayed cells. Besides, in region 9, one more lactiferous duct, similarly to several other ducts, shows a cystic dilatation, but around this duct, particularly to the extent of half of its circumference, a very dense infiltration of mostly plasma cells and lymphocytes is widely observable, which reaches, though to a slighter degree, to the proliferated, swollen and flattened epithelium of the lactiferous duct, and in one part, together with the epithelium, it extends into the lumen of the duct, being of verrucous appearance; between the above-mentioned infiltration cells, fibres and bundles of a collagenic connective tissue are interwoven over its whole extent. In the epithelium of the duct there appears one giant-cell of the Langhans' type and other polynuclear giant-cells. In the lumen of the lactiferous duct, there

are numerous parts of decayed cells, further, a fine reticular and granular mass of brownish-pale-red colour, and numerous desquamated epithelial cells, polynuclear leucocytes and lymphocytes appearing in smaller numbers, and some plasma cells in the border portion of the lumen. In the lumen of the described lactiferous duct, I found *Br. abortus* between and in the epithelial cells adjacent to the lumen.

Further, in region 8, there is a lobule showing chiefly a somewhat flabby collagenic connective tissue, containing lymphocytes, plasma cells, fibroblasts, and polynuclear leucocytes in smaller numbers, and here and there numerous abundantly filled capillaries (fig. 20). Only a few atrophied and disintegrating alveoli are the remnants of the usual tissue of the udder.

In region 10 (teat) no changes are to be observed.

Table 12.

Extent of the Changes in the Quarters of the Udder according to Histological Studies.

Cow II.

Regions inquired	Approximate Percentage of the Changes of the Tissue in the Studied Regions (Scheme 1, Page 41)									Approximate Average Percentage of the Changes in the Whole Quarter [Region 10 (Teat) is not considered]
	1	2	3	4	5	6	7	8	9	
Right front quarter	14·3	60	5	20	6·6	20	20	80	40	29·5
Right rear quarter	75	50	20	25	10	14·3	12·5	33·3	8·3	27·6
Left front quarter	5	—	50	—	50	33·3	50	20	12·5	24·5
Left rear quarter	33·3	—	50	75	25	2	10	75	33·3	33·7

The dash in the table means that there were no changes.

Lymph Nodes.

The finding in the supramammary and deep inguinal lymph nodes is similar to that in the corresponding lymph nodes of cow I, also as regards the discovery of *Br. abortus* in the sections (fig. 48).

Uterus.

The finding in the uterus resembles that in the uterus of cow I. But besides this, in one place of the epithelium of the right oviduct and of the subepithelial tissue, the changes are similar to those in the epithelium and in the subepithelial tissue of the uterus.

Ovaries.

The ovaries show changes similar to those in the ovaries of cow I.

Cow III — "Tasane".

The cow "Tasane" was 3 years of age (born on December 20, 1928), a pure bred Estonian red "angler", bought from the J. estate. Her weight was 381 kg. The cow was sold on January 7, 32 because of her diminished milk production, and was slaughtered on the same day in the town abattoir of Tartu.

aa. Preliminary Data.

"Tasane" had never calved normally; she had aborted twice, on April 5, 30, and on May 10, 31; after that she had been bred repeatedly, and at the time of her slaughter she proved pregnant, containing a 4-months' fetus.

Table 13.

Month	Milk in kg	Butterfat in kg
January	—	—
February	—	—
March	—	—
April	128	4·22
May	191	6·11
June	210	7·14
July	201	7·64
August	135	5·13
September	51	2·05
October	dry	
November	"	
December	"	
Total	916	32·29

According to the serological examinations made at the Bacteriological Station of the University of Tartu, the agglutination titer of "Tasane's" blood serum was 1:40+ and 1:80±, later, on

March 14, 30 the agglutination titer was 1 : 640 +, on October 16, 30 it was 1 : 640 +, on March 9, 31 — 1 : 8000 +, and on November 14, 31 it showed 1 : 640 +.

The cow "Tasane" had never been given living or killed cultures of *Br. abortus*.

The milk production of the animal had been so inconsiderable that in 1931 she was not regarded as a milch cow at all. Table 13 presents data concerning her milk production in 1930.

bb. Results of Inquiries concerning Milk and Blood Sera.

In making use of the above data, I examined her milk on July 24, 31, to find out whether her udder was infected with *Br. abortus*, and to make sure whether there were not present other agents causing diseases. The results of the inquiries are found in table 14.

According to table 14, "Tasane's" udder was infected with *Br. abortus*. Further bacteriological examinations did not reveal any other bacteria in the milk. On the date of her slaughter, on January 7, 32, I took some milk for investigation, before the animal was slaughtered, from each quarter of the udder individually, in order to make sure which of the quarters were infected with *Br. abortus*, and if meanwhile there had not intruded other agents causing diseases. Table 15 presents the results of the inquiries.

Table 15 indicates brucellous infection in all the quarters of her udder; no other bacteria were detected by repeated bacteriological examinations in any of the quarters.

The sediment of the milk from each quarter showed a good number of cellular elements: polynuclear leucocytes were most numerous, epithelial cells and lymphocytes were less numerous, and plasma cells occurred seldom. After centrifuging the milk from the right front quarter, the right rear quarter, and the left rear quarter was watery and contained but little cream; and the milk from the left front quarter was very watery indeed, and there was very little cream in it. The quantity of sediment in the milk from the right front quarter was normal, but of a reddish-yellowish-grey colour. The reddish colour of the sediment was caused by red corpuscles occurring in the milk. The quantity of the sediment in the milk from the left rear quarter was a little above the ordinary quantity; in the right rear and the left front quarter it was a good

Table 14.

Milk examined on July 24, 31	Agglutin. of *Br. Abortus* with Milk Serum	Aspect of Milk	Quantity of Sediment	Microscopical Finding in Sediment			Bacteriological Finding in Sediment		Agglut. of *Br. Abortus* with Blood Serum of Guinea-pigs injected with Sediment + Cream	Culture of *Br. Abortus* from the Spleen of Injected Guinea-pigs	Examinations of Injected Guinea-pigs for Tuberculosis
				Cellular Elements	Microbes: Gram's Staining	Microbes: Ziehl-Neelsen's Staining	Saccharose Plates	Brown's Plates			
Composite Milk	positive (1 : 40)	normal	normal	abundant	negative	negative	negative	negative	positive (1 : 320)	positive	negative

Table 15.

Milk examined on January 7, 32	Aspect of Milk	Quantity of Sediment	Microscopical Finding in Sediment			Bacteriological Finding in Sediment		Agglut. of *Br. Abortus* with Blood Serum of Guinea-pigs injected with Sediment + Cream	Culture of *Br. abortus* from the Spleen of Injected Guinea-pigs	Examination of Injected Guinea-pigs for Tuberculosis	Quantity of Chlorine in Milk %	Agglut. of *Br. Abortus* with Milk Serum
			Cellular Elements	Microbes: Gram's Staining	Microbes: Ziehl-Neelsen's Staining	Saccharose Plates	Brown's Plates					
Right front quarter	milk watery with little cream	normal	abundant	negative	negative	sterile	sterile	positive (1 : 640)	positive	negative	0·129	positive (1 : 320)
Right rear quarter	"	abundantly above the standard	"	"	"	"	"	" (1 : 1280)	"	"	0·261	" (1 : 160)
Left front quarter	milk very watery with very little cream	"	"	"	"	"	"	" (1 : 1280)	"	"	0·301	" (1 : 320)
Left rear quarter	milk watery with little cream	a little above the standard	"	"	"	"	"	" (1 : 640)	"	"	0·135	" (1 : 160)

deal above the ordinary quantity. Also the chloride content in the milk of all the quarters was greater than the average standard, and it was most abundant in the milk of the left front quarter.

At the time of slaughter (on January 7, 32) the blood serum titered 1:2560+ to the agglutination test for *Br. abortus*.

cc. Macroscopical Finding at the Time of Slaughter.

General Condition.

The cow "Tasane" was in a wellnourished condition.

Udder.

The whole udder weighed 2940 g and was quite normal in shape. It was of an evenly dense and fleshy consistency.

The right front quarter weighed 710 g. In the intersection it was variegated, and there were alternate yellowish-grey, reddish-yellowish-grey, and reddish-grey spots of varying size and indistinct border-line on the surface of the sections. In places there were single lobules which were easily distinguished from the surroundings because of their lighter or darker shades. But the whole quarter showed lobules which contained whitish-grey specks that were lighter than the ground colour of the lobules and were rather irregular in outline. The interstitial connective tissue was considerably proliferated, very often it intruded into the lobules and substituted the usual tissue of the lobules. The lardaceous lobules in the udder described in the cases of former cows, were to be found mostly in the border portions of the quarter, whereas in the middle portion of the quarter they were fewer in number than the porous lobules. No macroscopical changes were found in the lactiferous ducts.

The right rear quarter weighed 900 g; it was similar to the right front quarter; in the border portions of the quarter there were cystiform formations (with about 0·8 cm in diameter) which were surrounded with capsules of a dense connective tissue, and the lumina contained in parts a somewhat translucent, in parts a porridge-like yellowish-grey mass.

The left front quarter weighed 600 g and was, in general, of the same qualities as the right front quarter.

The left rear quarter weighed 730 g; it was of the same qualities as the right front quarter, only its intersection was of an even reddish-yellowish-grey colour, speckled in places with darker spots.

Lymph Nodes.

There were two right supramammary lymph nodes, dilated (swollen) and roundish in shape. One of them weighed 70·2 g and was 10×8×2·5 cm large. The other weighed 9·8 g and was 2·9×2·6×2 cm large. Their other qualities resembled those of the right supramammary lymph nodes of "Koidu".

There were three left supramammary lymph nodes which were swollen, the largest of them resembling a pig's kidney, and the other two being roundish. The biggest node weighed 51 g and was 7·2×6×2·3 cm large. The next in size weighed 10 g and was 3×2·5×1·9 cm large. The smallest was 2·5×2×1·8 cm large and weighed 6·4 g. Their other qualities resembled those of the right supramammary inguinal lymph nodes.

The right deep inguinal lymph node had a roundish shape and weighed 47 g, being 8·9×6×2 cm in size. Its other qualities were similar to those of the same lymph nodes of "Koidu".

The left deep inguinal lymph node weighed 46·2 g and was 8·7×6·1×2 cm large. Its other qualities resembled those of the right deep inguinal lymph node.

Uterus.

The uterus contained a fetus about 4 months old. The uterus, fetus, and fetal membranes did not show any macroscopical changes.

Ovaries.

The right ovary was 4·2×2·9×2·6 cm large. Its outer surface was rough in places, but its consistency was normal. In the intersection there were to be seen follicles in moderate numbers between which the interstitial connective tissue was moderately proliferated. Besides, in the middle part of the ovary there was a corpus luteum 2·1×2×1·8 cm large.

The left ovary was 3·2×2·5×2 cm large. Its other qualities resembled those of the right ovary, only the interstitial connective tissue was more proliferated, and the corpus luteum was absent.

No macroscopical changes were noted in the other organs.

dd. Bacteriological Findings and Results of Injections, in Animals used for Experiments.

The investigations were made exactly in the same way as in examining the material taken from the cow "Koidu", except that more organs were examined. The results of the inquiries are presented in table 16.

Table 16.

Material	Cultures		*Br. abortus*' Agglutin. with Blood Serum of Injected Guinea-pigs	Culture of *Br. Abortus* from the Spleen of Injected Guinea-pigs	Examination of Injected Guinea-pigs for Tuberculosis
	Saccharose Plates	Brown's Plates			
Lln. ing. sup. dext.	negative	negative	positive (1 : 1280)	positive	negative
Lln. ing. sup. sin.	„	„	„ (1 : 80)	„	„
Ln. ing. prof. dext.	„	„	„ (1 : 320)	„	„
Ln. ing. prof. sin.	„	„	„ (1 : 320)	„	„
Lln. mesent.	„	„	negative	negative	„
Uterus	„	„	„	„	„
Ovar. dext.	„	„	„	„	„
Ovar. sin.	„	„	„	„	„

Table 16 shows that, besides all the quarters of her udder, also her supramammary and deep inguinal lymph nodes on both sides were infected with *Br. abortus.*

No other bacteria were present in the examined material.

ee. Histopathological Finding.

Udder. Right Front Quarter.

The finding is, in general, similar to the histological finding in the right front quarter of cow I, not taking into account the variety and intensity of the changes and the appearance of less numerous amyloid corpuscles.

Right Rear Quarter.

The histological finding is, in general, similar to the same finding in the right front quarter of cow I, not taking into account the intensity and variety of the changes in some regions. The main point of the changes is in regions 3, 4, 5, 6, and 7 (table 17). Besides, in region 7, the lumen of one lactiferous duct is thoroughly obstructed (fig. 30); in the centre of the duct appears a cornified mass, containing in places a deposit of lime and pieces of decayed nuclei, and in

the border portion compressed and pyknotic nuclei; the cornified mass is surrounded by the proliferated and flattened epithelium; the latter is followed by an intense infiltration of lymphocytes and plasma cells (in one direction to the extent of two lobules); amid this infiltration there are to be found numerous fibroblasts and occasional polynuclear leucocytes and mast cells; between the cells already mentioned there are to be found numerous fibres of a collagenic connective tissue, occurring singly and in groups. In this jumble of cells and fibres of connective tissue there are to be found foci in various places, and they are comparatively easy to discern; in these there appear epithelioid cells of varying density, but in general they are scarce in number; they show in places a comparatively intense tumefaction; some lymphocytes, polynuclear leucocytes, and plasma cells have penetrated in between the epithelioid cells.

Left Front Quarter.

The histological finding resembles the corresponding finding in the right front quarter of cow II, with the difference that the changes described in d, e, and f are not observable, and the foci described in b appear only occasionally in regions 5, 8, and 9; in general, however, other changes are to be found to a larger extent in the quarter of the udder under examination. Besides, there appear numerous foci that resemble those in the histopathological finding in the right front quarter of the udder of cow I, as described in a, and a cornification of completely changed lactiferous ducts in region 3. Further, the changes observed are of varying intensity and extent in all the regions, in comparison with the corresponding regions in the right front quarter of the udder of cow II.

Left Rear Quarter.

The finding is in general similar to the corresponding finding in the right front quarter of the udder of cow I; these changes, however, generally appear in a smaller degree and they are slighter, and the cornification of the epithelium of the changed lactiferous ducts proves to be absent.

The macroscopically observable cystiform formations in the right rear quarter, as regards the histological finding (fig. 31), are similar to the corresponding cystiform formations in the udder of cow I; the majority of these formations, however, resemble those described

in a, and in their extremely changed epithelium, foci of epithelioid cells are to be observed, with a giant-cell of the Langhans' type in some of them. The blood-vessels in the connective tissue in the neighbourhood of several cystiform formations are often surrounded by lymphocytes, and to a smaller degree by plasma cells. In the above-mentioned cystiform formations I found *Br. abortus* in the histological sections.

Table 17.

Extent of the Changes in the Quarters of the Udder according to Histological Studies.

Cow III.

Regions inquired	Approximate Percentage of the Changes of the Tissue in the Studied Regions (Scheme 1, Page 41)										Approximate Average Percentage of the Changes in the Whole Quarter [not considering Region 10 (Teat)]
	1	2	3	4	5	6	7	8	9	10	
Right front quarter	—	10	80	2	10	10	12·5	100	87·5	—	34·7
Right rear quarter	10	6·6	40	60	87·5	75	100	12·5	25	—	46·3
Left front quarter	10	12·5	75	40	50	40	33·3	40	33·3	—	37·1
Left rear quarter	—	—	—	75	20	10	100	12·5	12·5	—	25·5

The dash in table 17 means that there were no changes.

Lymph Nodes.

The supramammary and deep inguinal lymph nodes show a similar finding to that in the corresponding lymph nodes of cow I, only in places the changes are slighter in extent.

Uterus.

The uterus does not show any pathological changes.

Ovaries.

The ovaries do not show any pathological changes.

Cow IV — "Lehik".

The cow "Lehik" was 10 years of age (born in 1921), a thoroughbred Estonian red "angler", bought from the M. farm. Her weight was 495 kg. The cow was sold because of her rapidly diminishing milk

production and non-pregnancy, and was slaughtered on January 27, 32 in the town abattoir of Tartu.

aa. Preliminary Data.

"Lehik" had calved normally the first time on May 18, 24 and then on December 3, 25, December 22, 26, March 24, 28, January 29, 30, and on January 24, 31. After the last parturition she had been bred repeatedly on May 10, 31, August 9, 31, November 10, 31, and according to the explanations of the owner, several times which were not registered, but she never conceived.

On the M. farm there had not occurred any abortion caused by *Br. abortus* till 1930. According to the blood examinations by *Prof. Dr. F. Laja* in 1928, not one of the cows was infected. The cows on the M. farm probably took the infection from cattle bought in addition in 1929; in March 1931 the first abortion in consequence of brucellosis occurred.

According to the serological examinations made on March 18, 31 at the Bacteriological Station of the University of Tartu, the cow proved to be brucellous (agglutination titer 1:160), after that her agglutination titer continuously rose till on May 5, 31 it was 1:640+.

"Lehik" had not been given either living or killed cultures of *Br. abortus*.

Data concerning "Lehik's" milk production are presented in table 18.

Table 18.

Month	1924		1925		1926		1927		1928		1929		1930		1931	
	Milk kg	Butter-fat kg	Milk kg	Butter-fat kg	Milk kg	Butter-fat kg	Milk kg	Butter-fat kg	Milk kg	Butter-fat kg	Milk kg	Butter-fat kg	Milk kg	Butter-fat kg	Milk kg	Butter-fat kg
January	—	—	113	4·6	450	18·0	406	14·21	60	2·64	273	12·28	76	3·19	97	4·36
February	—	—	107	4·3	404	16·0	341	12·62	dry		245	11·51	434	19·19	622	27·99
March	—	—	164	6·2	404	15·9	364	12·11	70	2·80	260	11·44	409	19·60	707	28·28
April	—	—	156	5·9	320	12·4	309	10·51	450	16·20	234	9·36	446	17·39	506	20·75
May	88	3·1	162	6·5	332	10·9	312	12·56	440	14·52	201	8·65	466	19·11	498	20·42
June	222	8·0	174	6·4	312	10·8	302	11·84	427	14·52	196	7·64	454	18·16	417	17·51
July	222	7·1	176	7·4	298	11·92	290	11·02	395	14·61	207	8·07	362	14·74	479	20.12
August	204	6·9	156	6·7	260	10·40	305	11·59	383	14·17	240	9·84	418	17·18	350	15·70
September	194	6·6	142	6·1	240	9·84	327	13·08	418	17·56	238	9·76	348	14·62	289	13·58
October	145	5·0	dry		213	8·73	270	10·26	380	15·20	247	9·88	313	13·77	221	11·05
November	150	5·1	"		80	3·76	205	10·34	314	12·56	210	9·45	289	13·00	101	10·56
December	139	5·7	382	15·2	95	3·86	170	7·65	300	12·30	112	5·60	83	4·23	213	11·29
Total	1364	47·5	1732	69·3	3408	132·45	3631	137·79	3637	137·08	2663	113·48	4179	174·08	4590	201·61

bb. Results of Inquiries concerning Milk and Blood Sera.

I examined "Lehik's" milk on January 17, 32, to find out whether her udder was infected with *Br. abortus*, and to make sure if any other agents causing diseases were present. The results of these inquiries are presented in table 19.

According to table 19, "Lehik's" udder was infected with *Br. abortus*, but by a bacteriological examination of the milk no other bacteria were detected.

Before the slaughter (on January 27, 32) I once more examined the milk from each quarter individually, to find out which of the quarters were infected, and whether meanwhile there had intruded other agents causing diseases. Table 20 presents the results of these inquiries.

According to table 20, brucellous infection had intruded into all the quarters of her udder; no other bacteria, however, were detected by repeated bacteriological examinations.

The milk from each quarter, after being centrifuged, showed a good deal of sediment which contained a number of cellular elements, the proportion of the latter being approximately the same as that of the sediment in "Koidu's" milk. The agglutination of *Br. abortus* with the milk serum was intensive to an extraordinary degree. The agglutination titer with the milk serum of the right rear and left rear quarters was 1 : 12800. The aspect of the milk was normal, but according to the data of the examinations made on January 17, 32 and presented in table 19, it was changed in all the quarters. The average quantity of chlorine in all the quarters was twice as large as is normal.

cc. Macroscopical Finding at the Time of Slaughter.

General Condition.

"Lehik's" general condition was above the mediocre. The results of an exact inspection were as follows:

Udder.

The udder weighed 7980 g, was normal in shape, and of an even and unusually dense and fleshy consistency.

The right front quarter weighed 1930 g; the surface of the section was parti-coloured, showing in the intersection spots which had an indistinct border-line, and an alternately lighter and darker reddish-yellowish-grey colour. Almost all of the lobules

Table 19.

Milk examined on January 17, 32	Agglut. of *Br. Abortus* with Milk Serum	Aspect of Milk	Quantity of Sediment	Microscopical Finding in Sediment			Bacteriological Finding in Sediment		Agglut. of *Br. Abortus* with Blood Serum of Guinea-pigs injected with Sediment of Milk from all Quarters + Cream	Culture of *Br. Abortus* from the Spleen of Injected Guinea-pigs	Examination of Injected Guinea-pigs for Tuberculosis
				Cellular Elements	Microbes		Saccharose Plates	Brown's Plates			
					Gram's Staining	Ziehl-Neelsen's Staining					
Right front quarter	positive (1 : 1280)	milk watery with little cream	abundant above the standard	abundant	negative	negative	negative	negative	positive (1 : 160)	positive	negative
Right rear quarter	" (1 : 1280)	"	"	"	"	"	"	"			
Left front quarter	" (1 : 1280)	milk watery with very little cream	"	"	"	"	"	"			
Left rear quarter	" (1 : 1280)	milk watery with little cream	"	"	"	"	"	"			

had a lardaceous aspect, and they rose slightly above the surface. In places there were lobules which contained certain spots that were lighter and indistinct in border-line. The interstitial connective tissue was in general very considerably proliferated, and it often disappeared slowly in the lobules, replacing there the parenchyma of the usual lobule. In the border portion of the quarter, in many places of the lactiferous ducts and lobules, there were cystiform formations which were of the same qualities as those in the udder of "Koidu".

The right rear quarter weighed 2000 g and had the same qualities as the right front quarter, except that the lardaceous lobules were here even more abundant.

The left front quarter weighed 1400 g, but its other qualities were similar to those of the right rear quarter.

The left rear quarter weighed 2650 g. It resembled, in general, the right front quarter, except that there were more marked red spots in the intersection, also the interstitial connective tissue was more intensely proliferated. In places the interlobular connective tissue had extensively dammed individual lobules, and had apparently isolated them from the udder tissue.

Lymph Nodes.

There were two right supramammary inguinal lymph nodes which were very considerably dilated, and had a round shape. The bigger weighed 130 g and was 11·2×8×3·1 cm large. The smaller weighed 10·4 g and was 3×2·6×2 cm large. Their other qualities resembled those of the same lymph nodes of "Koidu", only the surface of the sections became more abundantly covered with succus and, in general, the connective tissue was more intensely proliferated.

There were two left supramammary inguinal lymph nodes, both round and very intensely dilated. One of them weighed 125 g and was 11×8·2×2·9 cm large. The other weighed 12·3 g and was 3·2×2·7×2 cm large. Their other qualities resembled those of the right supramammary inguinal lymph nodes.

The right deep inguinal lymph node weighed 50 g and was 8×7·1×2 cm large; all its other qualities were similar to those of the right deep inguinal lymph node of "Koidu".

Table 20.

Milk examined on January 27, 32	Agglut. of *Br. Abortus* with Milk Serum	Aspect of Milk	Quantity of Sediment	Microscopical Finding in Sediment			Bacteriological Finding in Sediment		Agglut. of *Br. Abortus* with Blood Serum of Guinea-pigs injected with Sediment of Milk + Cream	Culture of *Br. Abortus* from the Spleen of Injected Guinea-pigs	Examination of Injected Guinea-pigs for Tuberculosis	Quantity of Chlorine in Milk %
				Cellular Elements	Microbes							
					Gram's Staining	Ziehl-Neelsen's Staining	Saccharose Plates	Brown's Plates				
Right front quarter	positive (1 : 6400)	normal	abundant	very many	negative	negative	negative	negative	positive (1 : 640)	negative	negative	0·196
Right rear quarter	„ (1 : 12800)	„	„	„	„	„	„	„	„ (1 : 640)	„	„	0·229
Left front quarter	„ (1 : 6400)	„	„	abundant	„	„	„	„	„ (1 : 1280)	„	„	0·295
Left rear quarter	„ (1 : 12800)	„	„	very many	„	„	„	„	„ (1 : 1280)	„	„	0·234

The left deep inguinal lymph node weighed 45 g and was 7·9×7×2 cm large. The dark-red colour (haemorrhage) of the border portion extended as far as 3·2 and 1·9 cm. All other qualities resembled those of the right deep inguinal lymph node.

The Thyroid Gland.

The right lobe of the thyroid gland was 6·6×4·5×1·2 cm large and weighed 15·7 g, and the left lobe was 6·4×4·6×1·3 cm large and weighed 16·2 g. Both the lobes showed in intersections a little darker-reddish-brown colour than usual. There were noticed single follicles, of a 1·6 mm diameter, on the surface of the section.

Ovaries.

The right ovary was 3·9×2·7×2 cm large. It was of a denser consistency than is usual and was slightly wrinkled, showing a rough outer surface. In the section there were to be seen single second and third rate follicles, between which the connective tissue was intensely proliferated. There were also two half-involved corpora lutea.

The left ovary was 3·2×3×1·8 cm large; its other qualities resembled those of the right ovary, except that there was one half-involved corpus luteum.

Lungs.

In the lungs there were single yellowish-whitish-grey tubercles which were calcified (old tubercles of tuberculosis) and had a diameter of about 1·4 cm.

Hygroma of the Knee.

On the right knee-joint there was a hygroma 15×10·1×7 cm big; its capsule consisted of a dense connective tissue about 0·5 cm thick. It contained about 0·5 litre of a reddish-yellowish-grey turbid and sticky liquid, and about the same quantity of a porridge-like mass which was partly gelatinous, partly of a denser consistency and of a reddish-yellowish-grey colour. The inner surface of the capsule was covered with fringy cords of connective tissue, and some of them connected the polar walls of the capsule.

No macroscopical changes were noted in any other organ.

dd. Bacteriological Findings and Results of Injections, in Animals used for Experiments.

I carried out my inquiries exactly in the same manner as I did in examining the material taken from the cow "Koidu", only there were many more organs examined. The results of the inquiries are presented in table 21.

According to table 21, brucellosis had infected not only all the quarters of the udder, but also the supramammary and deep inguinal lymph nodes on both sides, the thyroid gland, the spleen, and the hygroma of the knee. Other bacteria, however, were not detected in the examined organs and parts of the body, except the lungs where there was revealed *Mycobacterium tuberculosis.*

Table 21.

Material	Cultures		Agglutin. of *Br. Abortus* with Blood Serum of Injected Guinea-pigs	Culture of *Br. Abortus* from the Spleen of Injected Guinea-pigs	Examination of Injected Guinea-pigs for Tuberculosis
	Saccharose Plates	Brown's Plates			
Lln. ing. sup. dext.	sterile	sterile	positive (1 : 160)	positive	negative
Lln. ing. sup. sin.	„	„	„ (1 : 160)	„	„
Ln. ing. prof. dext.	„	„	„ (1 : 640)	„	„
Ln. ing. prof. sin.	„	„	„ (1 : 160)	„	„
Gl. thyreoidea	„	„	„ (1 : 1280)	„	„
Ln. cervic. sup. dext.	„	„	negative	negative	„
Pulmones	„	„	„	„	positive
Hepar	„	„	„	„	negative
Lien	„	„	positive (1 : 320)	positive	„
Lln. mesenter.	„	„	negative	negative	„
Ren dext.	„	„	„	„	„
Ren sin.	„	„	„	„	„
Adren dext.	„	„	„	„	„
Adren sin.	„	„	„	„	„
Uterus	„	„	„	„	„
Ovar. dext.	„	„	„	„	„
Ovar. sin.	„	„	„	„	„
Hygroma	„	„	positive (1 : 1280)	positive	„

ee. Histopathological Finding.

Udder. Right Front Quarter.

In all regions but the 10th, which is not taken into account, there are to be found changes resembling those in the right front quarter of cow I, but the cornification of the proliferated and flattened epithelium is to be observed only in one lactiferous duct of region 8.

Besides, in each of the regions 1, 3, and 6 there is to be found one lobule, in region 4 there are two lobules, and in region 7 three lobules, in which polynuclear leucocytes appear in very great quantities in the lumina of the alveoli as well as in the interalveolar tissue; these polynuclear leucocytes are mostly fatty-degenerated to a greater or less degree. The lumina of the alveoli also show numerous desquamated epithelial cells (partly swollen and partly pyknotic), and single lymphocytes, decayed parts of cells, some fat globules, and a reticular mass of brownish-pale-red colour (according to van Gieson) in moderate quantities; the alveoli are partly dilated, and the epithelium is thoroughly desquamated in places; the epithelial cells which are in the usual posture are partly swollen, some of them being pyknotic. The tissue between the alveoli is dilated, and there appear lymphocytes and plasma cells in moderate quantities among the polynuclear leucocytes. The capillaries of the lobules in question are dilated and abundantly filled with blood.

Further, in regions 1, 2, 3, 4, 5, 6, 7, and 8 in some of the lobules, the interalveolar connective tissue is very intensely proliferated and dilated; the majority of the alveoli have perished. The preserved alveoli are of an indefinite shape, compressed and angular. The epithelium of some of the alveoli is atrophied and pyknotic, some of the alveoli show a proliferation of their epithelium and a swelling of some epithelial cells, and some of the alveoli show both the above-mentioned alterations simultaneously. In the lumina of the alveoli which are considerably diminished because of compression, there appear desquamated epithelial cells in varying amounts, polynuclear leucocytes, and lymphocytes in smaller quantities, accompanied by a reticular and granular mass of brownish-pale-red and yellowish-brownish-pale-red colour (according to van Gieson). In the interalveolar connective tissue there is to be found an infiltration of comparatively dense, diffuse and frequently focal lymphocytes, plasma cells, and polynuclear leucocytes; among this infiltration there are moderate numbers of fibroblasts and in places also some mast cells. Also the interlobular connective tissue is intensely proliferated and dilated in places, particularly in the neighbourhood of the changed alveoli; it shows an infiltration of varying density, similar to that in the changed interalveolar connective tissue.

In region 10 (teat) no pathological changes were to be observed.

The finding in the normal lobules resembles, in general, that in the corresponding lobules in the right front quarter of the udder of cow I, but the amyloid corpuscles appear in smaller quantities.

Right Rear Quarter.

The finding is generally similar to that in the corresponding right front quarter of the same cow, only the changes are in places greater and, in general, more spread. Thus, there appears a cornification of the proliferated and flattened epithelium of some lactiferous ducts in regions 3, 4, 5, 7, and 9. The amyloid corpuscles are also more numerous.

In region 10 (teat), in places, a slight subepithelial infiltration of lymphocytes and of plasma cells in smaller numbers appears in the teat canal.

I discovered *Br. abortus* in several places in the histological sections; they appeared singly and in groups in the degenerated cells, between the cells, and in the decayed mass (fig. 46), in one place even around the changed and partly decayed capillaries, in the endothelial cells, and even in the lumen of the capillary (fig. 45 and 45 a).

Left Front Quarter.

The finding resembles, in general, that in the right front quarter of the same cow, only the alterations are slighter in places, and they appear only over half the extent of the latter; the amyloid corpuscles, however, are more numerous.

No alterations are to be found in regions 5 and 10 (teat).

Left Rear Quarter.

The finding is similar to the corresponding finding in the right front quarter of the udder of the cow in question, not taking into account the diversity of the intensity and extent of the changes according to the regions (table 22). The changes of the lactiferous ducts are also generally more marked; in regions 2, 4, and 6 a cornification of the epithelium of some proliferated and flattened lactiferous ducts is to be found among other alterations.

In region 10 (teat) there is a subepithelial focus of infiltration in the teat canal, containing lymphocytes and plasma cells in moderate numbers and extending over almost three alveoli; other changes were not observed.

Table 22.

Extent of the Changes in the Quarters of the Udder according to Histological Studies.

Cow IV.

Regions inquired	Approximate Percentage of the Changes of the Tissue in the Inquired Regions (Scheme 1, Page 41)									Approximate Average Percentage of the Changes of the Tissue of the Whole Region [not considering Region 10 (Teat)].
	1	2	3	4	5	6	7	8	9	
Right front quarter	80	75	75	50	33·3	40	33·3	100	33·3	57·8
Right rear quarter	40	33·3	100	80	80	75	75	66·6	50	66·7
Left front quarter	75	20	25	10	—	10	25	80	10	28·3
Left rear quarter	20	60	80	60	50	75	100	66·6	40	61·3

The dash in table 22 means that no changes were to be found.

The finding in the macroscopically observable cystiform formations in all the quarters of the udder is similar to that in the cystiform formations in the udders of cows I and III.

Lymph Nodes.

The finding in the supramammary and deep inguinal lymph nodes on both sides resembles that in the corresponding lymph nodes of cow I, but it differs from the latter in that the lymph sinuses are, in general, much more dilated (fig. 36), and they also contain red corpuscles. Particularly acute changes are to be found in the left deep inguinal lymph node; there often appear diffuse haemorrhages in the cortical as well as in the medullary substance. Among the epithelioid cells, in one spot, there are two giant-cells of the Langhans' type side by side. I often found *Br. abortus* in the changed tissue and the lymph sinuses (fig. 49).

Thyroid Gland.

Examining the gland with a low powered microscope, it shows first of all a variety in the size of the follicles. Large follicles appear in very great quantities, the majority of which are about 1 mm in diameter, some of them being even

to about 1·6 mm in diameter. Between the above-mentioned large follicles there appear follicles of normal size singly and in groups. Over the whole thyroid gland there appear foci of different sizes, in the centre of which there is to be found an intense proliferation and desquamation of the epithelium, filling in places the lumina of the follicles. Among the epithelial cells there are to be found lymphocytes and plasma cells. A proliferation of the interfollicular connective tissue is to be observed in varying intensity; in places there appears an infiltration of lymphocytes, plasma cells, occasional polynuclear leucocytes, and mast cells in varying degrees, and moderately filled blood and lymph capillaries. An infiltration of lymphocytes and single plasma cells is to be found around some of the blood-vessels. An intense desquamation of the epithelium of the follicles is to be found in the border portions of the foci. In places there are follicles which show, besides a desquamation of single cells, also a desquamation of the epithelium to a large extent; the epithelial cells are still connected with each other, but they are pyknotic and hang either on the epithelium that has remained normal, or they occur quite freely in the lumina of the follicles. Colloid in varying quantities is to be found in the lumina of the follicles, but in some of the follicles it appears in very small quantities.

Furthermore, there appear foci which are similar to the foci described above, but no proliferation of the connective tissue is to be found in these foci; the blood and lymph capillaries are richly filled, and polynuclear leucocytes appear more frequently in the cellular infiltration.

Between these foci of the above-mentioned two types, there appear normal follicles in varying quantities, among which appear also the big follicles already mentioned, the lumina of the latter being richly filled with colloid.

Ovaries.

The finding in both ovaries, as to the changes, is similar to that in the ovary of cow I.

Hygroma of the Knee.

The capsule of the hygroma consists chiefly of a collagenic connective tissue; the latter shows an intense focal and diffuse infiltration of lymphocytes and plasma cells, these being particularly dense in the internal part of the capsule.

Lungs.

In the macroscopically observable tubercles in the lungs there are to be found typical tuberculous alterations in which I discovered *Mycobact. tuberculosis* in the histological sections.

In the other examined organs (uterus, kidneys, suprarenal glands, spleen, liver, left superficial lymph node of the cervix), no alterations were found on histological inquiries.

Cow V — "Klaara".

14 years of age (born in 1917); she was a thorough-bred Estonian red "angler", bought from the M. farm, the same from which the cow "Lehik" came. Her weight was 475 kg. She was sold and slaughtered on March 16, 32 in the town abattoir of Tartu because of her diminished milk production and open tuberculosis of the lungs.

aa. Preliminary Data.

The control data concerning "Klaara's" parturitions show that she calved normally on March 16, 23, February 13, 24, December 25, 24, December 3, 25, September 29, 26, August 14, 27, November 4, 28, November 14, 29, December 20, 30, and she aborted in July 1931. After that she was repeatedly bred, but she never conceived.

Serological examinations made on March 18, 31 at the Bacteriological Station of the University of Tartu showed brucellous infection of the animal in question (aggl. titer 1 : 160).

"Klaara" was not given either living or killed cultures of *Br. abortus*.

Data concerning the milk production of the above cow are presented in table 23.

bb. Results of Inquiries concerning Milk and Blood Sera.

"Klaara's" milk could not be investigated, because she was already in a dry period when I began examining her. Before the slaughter (March 16, 32), however, I still got about 40 ccm of a serum-like, half-turbid, viscous, yellowish-grey secretion. The results of the investigations of this secretion are presented in table 24.

The cellular elements in the sediment of the secretion from the udder of "Klaara" had about the same proportion as in the sediment of "Koidu's" milk.

Table 23.

Month	1923		1924		1925		1926		1927		1928		1929		1930		1931		Notes
	Milk kg	Butter-fat kg	Milk kg	Butter-fat kg	Milk kg	Butter-fat kg	Milk kg	Butter-fat kg	Milk kg	Butter-fat kg	Milk kg	Butter-fat kg	Milk kg	Butter-fat kg	Milk kg	Butter-fat kg	Milk kg	Butter-fat kg	
January	99	4·0	dry		285	9·1	444	16·0	312	10·63	315	10·39	387	13·54	464	16·24	537	18·79	At the time of slaughter (March 16, 32) "Klaara" was dry.
February	9	0·6	164	6·0	280	9·8	444	16·0	247	8·40	276	9·66	351	12·28	376	11·28	448	16·41	
March	275	8·5	296	10·7	222	7·8	454	14·5	280	9·24	256	9·98	351	10·53	402	14·07	477	16·22	
April	316	10·2	200	7·0	198	6·3	344	10·3	146	5·69	240	7·92	305	10·37	367	12·84	417	14·59	
May	236	6·6	283	7·3	234	7·7	324	10·4	dry		220	6·82	278	9·45	394	15·37	390	14·04	
June	217	7·2	165	5·4	258	9·2	288	9 5	„		198	7·33	265	8·48	361	13·0	406	15·42	
July	241	7·5	210	6·5	204	6·5	221	8·4	„		187	6·54	193	6·75	298	10·73	422	15·19	
August	276	8·9	203	6·9	184	6·4	138	5·38	140	4·9	173	6·4	160	6·08	317	12·68	280	11·20	
September	237	8·3	154	5·2	100	4·0	80	3·2	450	13·5	129	4·39	127	5·97	128	6·14	202	7·47	
October	171	5·6	90	4·0	dry		366	6·47	400	10·8	51	2·4	dry		dry		dry		
November	134	4·8	67	2·5	„		351	12·64	375	11·25	333	12·99	230	8·51	„	4·2	„		
December	91	4·7	38	1·2	488	19·5	350	13 30	349	14·66	465	17·06	490	16·66	120		„		
Total	2302	76·9	1870	62·7	2453	86·3	3804	136·09	2699	89·07	2843	101·88	3137	108·62	3227	116·55	3579	129·06	

Table 24.

Secretion examined on March 16, 32	Aspect of Secretion	Quantity of Sediment	Microscopical Finding in Sediment			Bacteriological Finding in Sediment		Agglut. of *Br. Abortus* with Blood Serum of Guinea-pig injected with Sediment	Culture of *Br. Abortus* from the Spleen of Injected Guinea-pig	Examination of Injected Guinea-pig for Tuberculosis
			Cellular Elements	Microbes		Saccharose Plates	Brown's Plates			
				Gram's Staining	Ziehl-Neelsen's Staining					
Secretion from all quarters	Serum-like, half-turbid, glutinous of yellowish-grey colour	abundant	abundant	negative	negative	negative	negative	positive (1 : 640)	positive	negative

On the day before the slaughter (March 15, 32), "Klaara's" blood serum was positive in a dilution of 1 : 1280 to the agglutination test for *Br. abortus*.

cc. Macroscopical Finding at the Time of Slaughter.

Nutritive State.

"Klaara" was in a mediocre state of nutrition. Further exact inspections revealed the following:

Udder.

The whole udder weighed 5800 g, having a normal shape, an even and unusually dense and fleshy consistency.

The right front quarter weighed 1190 g and had a speckled yellowish-reddish-grey colour. There were to be noticed porous lobules in small quantities, with lighter spots and mostly lardaceous. In general there was an intense proliferation of the interstitial connective tissue, and the intrusion of the latter into many lobules — beyond its normal limits. In the smaller lactiferous ducts there were in places longish and roundish elevations, rising about 1·5 mm above the surface of the ducts; these elevations consisted of a yellowish-grey brittle mass, and their outer surface was dull and slightly uneven. In the quarter there were numerous cystiform formations which were of various sizes (0·5 to 8 mm in diameter), and contained partly a glassy, half-turbid and glutinous, partly a yellowish-grey brittle mass. These cystiform formations were most abundant in the lower and middle portions of the quarter where they made the parenchyma of the udder look somewhat cavernous.

The right rear quarter weighed 1410 g and was exactly of the same qualities as the right front quarter.

The left front quarter weighed 1560 g; its other qualities resembled those of the right front quarter, only the cystiform formations were not as numerous here.

The left rear quarter weighed 1760 g; its other qualities were similar to those of the left front quarter.

Lymph Nodes.

There were two right supramammary inguinal lymph nodes, both swollen. The bigger weighed 99 g and was $10 \times 8 \times 2{\cdot}5$ cm large. The smaller weighed 14·2 g and

was 3·5 × 2·7 × 2 cm large. Their other qualities resembled those of "Lehik's" supramammary inguinal lymph nodes.

There was one left supramammary inguinal lymph node, which weighed 145 g and was 10·3 × 8·9 × 3 cm large; its other qualities resembled those of the right supramammary inguinal lymph nodes.

The right deep inguinal lymph node weighed 45 g and was 7·8 × 6·9 × 2 cm large. Its other qualities were similar to those of "Lehik's" right deep inguinal lymph node.

The left deep inguinal lymph node weighed 55 g and was 8·2 × 7 × 2·2 cm large. Its other qualities were similar to those of the right deep inguinal lymph node.

Uterus.

The uterus was similar to that of a normal, non-pregnant cow. The walls of the uterine horns were about 2·5 cm thick. The mucous membrane was reddish-grey, and it was covered with a light and slightly dull, sticky layer of a slimy substance.

Ovaries.

The right ovary was 3·8 × 3 × 2·1 cm large; it was of a denser consistency than usual, and its outer surface was furrowed and rough. In the intersection there was to be noticed first of all an interfollicular connective tissue, the second and third rate follicles being scarce; there were also three half-involved corpora lutea of various ages in the ovary.

The left ovary was 2·9 × 1·7 × 1·2 cm large; its other qualities resembled those of the right ovary, except that the corpora lutea were absent.

Lungs.

In the lungs there were in several places yellowish-whitish-grey tubercles (tubercles of tuberculosis) about 1·5 cm in diameter; some of them contained a yellowish-whitish-grey mass, resembling curdled milk; some of the tubercles were very considerably calcified.

The lymph nodes of the hilus of the lungs and mesentery revealed single coagulated tubercles about 0·4 cm in diameter. The preparations made from the above tubercles contained *Mycobacterium tuberculosis.*

No macroscopical changes were noted in any other organ.

dd. Bacteriological Findings and Results of Injections, in Animals used for Experiments.

The investigations were carried out in the same manner as in the case of the material taken from "Koidu", except that in this case more organs were examined. Table 25 presents the results of the inquiries.

From table 25 it follows that all the quarters of the udder, supramammary and deep inguinal lymph nodes on both sides, and the spleen of "Klaara" were infected with *Br. abortus*. No other types of bacteria were present in the material examined, except the infection of the mesenteric lymph nodes with *Mycobacterium tuberculosis*.

Table 25.

Material	Cultures		Agglut. of *Br. Abortus* with Blood Serum of Infected Guinea-pigs	Culture of *Br. Abortus* from the Spleen of Injected Guinea-pigs	Examination of Injected Guinea-pigs for Tuberculosis
	Saccharose Plates	Brown's Plates			
Right front quarter	negative	negative	positive (1 : 640)	positive	negative
Right rear quarter	"	"	" (1 : 640)	"	"
Left front quarter	"	"	" (1 : 640)	"	"
Left rear quarter	"	"	" (1 : 640)	"	"
Lln. ing. sup. dext.	"	"	" (1 : 640)	"	"
Lln. ing. sup. sin.	"	"	" (1 : 160)	"	"
Ln. ing. prof. dext.	"	"	" (1 : 320)	"	"
Ln. ing. prof. sin.	"	"	" (1 : 640)	"	"
Gl. thyreoidea	"	"	negative	negative	"
Lien	"	"	positive (1 : 640)	positive	"
Lln. mesenter.	"	"	negative	negative	positive
Medulla ossis metacarpi dext.	"	"	"	"	"
Uterus	"	"	"	"	"
Ovar. dext.	"	"	"	"	"
Ovar. sin.	"	"	"	"	"

ee. Histopathological Finding.

Udder. Right Front Quarter.

The finding resembles, in general, the corresponding finding in the right front quarter of the udder of cow IV, with the difference that the changes are more extensive and in places still more intensive, not taking into account the variety of the extent and intenseness of the changes according to the regions. The changes in the lactiferous ducts are in general severer: thus, among other

things, there is to be found a cornification of the epithelium of some proliferated and flattened lactiferous ducts in regions 1, 2, 7, and 8. Further, in region 5, many alveoli in several lobules and several lactiferous ducts are dilated to a very considerable degree; they are abundanty filled with a very finely granulated (almost homogeneous) mass of yellowish-reddish-brown colour (according to van Gieson), which contains in places numerous swollen and desquamated epithelial cells, mostly in the border portions of the lumina, and lymphocytes and polynuclear leucocytes in smaller numbers. The epithelium of the above-mentioned lactiferous ducts and the alveoli is observed to be partly proliferated, partly atrophied and pyknotic. Between the epithelial cells, and particularly under the epithelium and in its nearest surroundings, in places there appears an infiltration of lymphocytes and plasma cells in varying density, and that of a smaller number of polynuclear leucocytes, accompanied by numerous fibres of collagenic connective tissue and fibroblasts.

I discovered *Br. abortus* in several places in the changed portions of the histological sections.

The majority of the lobules that have preserved normally are in an inactive state, but in places some lobules resemble those in a state of small milk production; the epithelium of the alveoli in these lobules has a cubical shape, and in the epithelial cells, fat globules appear in places. In the lumina of the alveoli there appears slightly, in places even very slightly a reticular and granular mass of brownish-pale-red colour (according to van Gieson), containing in places numerous fat globules, single polynuclear leucocytes, and desquamated epithelial cells, the latter being very few in number.

Here and there in regions 1, 4, 6, 7, and 8 (fig. 35) there appear numerous amyloid corpuscles, in regions 2 and 9 they appear in small numbers, and in regions 3 and 5 they are totally absent.

In region 10 (teat), the teat canal shows in several places a subepithelial focal infiltration of lymphocytes and plasma cells; other changes are not to be observed in the region in question.

Right Rear Quarter.

The finding is in general similar to that in the corresponding right front quarter of the cow in question (including region 10), not taking into account the extent and the intensity of the changes according to the regions. Further, there do not appear such dilated

alveoli and lactiferous ducts in the quarter under examination as in the right front quarter of the udder of the cow in question in region 5. The amyloid corpuscles are generally less numerous than in the quarter of the udder that is being compared.

Left Front Quarter.

The finding is in general similar to the corresponding finding in the right rear quarter of the udder of the cow in question (including region 10), but the extent and the intensity of the changes according to the regions are not taken into consideration.

Left Rear Quarter.

The finding is in general similar to the corresponding finding in the right rear quarter of the udder of the cow in question (including region 10), only the alterations are in general more extensive and in places severer still.

Table 26.

Extent of the Changes in the Quarters of the Udder according to Histological Studies.

Cow V.

Regions inquired	Approximate Percentage of the Changes of the Tissue in the Inquired Regions (Scheme 1, Page 41)									Approximate Average Percentage of the Changes of the Tissue in the Whole Quarter [not taking into Account Region 10 (Teat)]
	1	2	3	4	5	6	7	8	9	
Right front quarter	80	75	60	33·3	40	33·3	100	100	75	66·3
Right rear quarter	66·6	66·6	100	40	100	33·3	33·3	60	75	63·9
Left front quarter	60	60	57·1	75	100	40	33·3	20	50	55·0
Left rear quarter	60	80	100	87·5	100	87·5	100	60	75	83·3

The macroscopically observable cystiform formations in all quarters of the udder are of three types, like those in the udder of cow I, but the cystiform formations as described in b and c occur more frequently. The finding in the cystiform formations resembles that in the cystiform formations in the udders of cows I and III.

Lymph Nodes.

The finding in the supramammary and deep inguinal lymph nodes on both sides resembles that in the corresponding lymph nodes of cow I, the difference being that the lymph sinuses are more dilated in places, and in the supramammary inguinal lymph nodes there are here and there focal haemorrhages.

Mesenteric lymph nodes show in places tuberculous alterations, where there are numerous *Mycobact. tuberculosis* in the histological sections.

Thyroid Gland.

In several places in the thyroid gland there are to be found foci where a very intense proliferation of the connective tissue has taken place; in the centre of the focus some of the follicles are compressed, and the epithelium of some of the follicles is proliferated and considerably desquamated; the majority of the epithelial cells of the mentioned follicles are atrophied and pyknotic. In the connective tissue and among the epithelial cells of the changed follicles there appear in places lymphocytes in moderate numbers, and very few plasma cells. In the border portions of the foci the desquamation of the epithelial cells proves to be intense in some of the follicles, in others, however, it is slight.

Uterus.

In both uterine horns, the mucous membrane in its transverse section is about half the thickness of the wall of the uterus. The majority of the glands in the mucous membrane are in an inactive state. In places, the subepithelial tissue shows an infiltration of lymphocytes, single plasma cells, and polynuclear leucocytes; this infiltration surrounds the glands and penetrates in places even between the epithelial cells. Fibroblasts and fibres of the collagenic connective tissue appear in varying quantities among the infiltration cells. In both oviducts, in that part of the subepithelial tissue that is towards the cornu of the uterus, there is to be found an infiltration which resembles that in the subepithelial tissue in the cornu of the uterus; in one place, the epithelium in the right oviduct is desquamated to a small extent, and substituted by a similar infiltration as described in the subepithelial tissue.

Ovaries.

As to the changes, the finding in both ovaries is similar to that in the ovaries of cow I.

The Lungs

show a similar finding as in the lungs of cow IV.

The Spleen and the Bone Marrow

(*metacarpus dext.*) do not show any pathological changes.

Cow VI — "Õunik".

The cow "Õunik" was 6 years of age (born in 1926), a cross-bred Estonian red "angler", bought from the J. estate, for which she had been bought from the "Veiber" colony in May 1930. Her weight was 430 kg. The cow was sold because of her diminished milk production, and was slaughtered on April 6, 32 at the town abattoir of Tartu.

aa. Preliminary Data.

According to the statement of the owner of the cow, she had calved twice before she was brought to the J. estate. On the J. estate she had aborted on March 7, 31, and *Br. abortus* was cultivated from the secundinae and from the abomasus of the calf. After that she had been repeatedly bred and had conceived at last, so that on the date of slaughter she contained a 6-months' fetus.

According to the serological examinations made on October 16, 30 at the Bacteriological Station of the University of Tartu, "Õunik" proved brucellous (agglutination titer 1:640), later, on

Table 27.

Month	1930		1931		1932		Notes
	Milk kg	Butter-fat kg	Milk kg	Butter-fat kg.	Milk kg	Butter-fat kg	
January	—	—	dry		113	4·63	At the time of slaughter (April 6, 32) "Õunik" was dry
February	—	—	dry		94	4·14	
March	—	—	240	7·2	54	3.43	
April	—	—	346	13·84	—	—	
May	41	1·35	289	9·83	—	—	
June	132	4·36	266	9·31	—	—	
July	143	6·29	255	7·9	—	—	
August	102	4·49	264	8·45	—	—	
September	118	4·60	212	7·84	—	—	
October	72	3·02	175	6·65	—	—	
November	37	1·74	152	5·47	—	—	
December	dry		116	4·76	—	—	
Total	645	25·85	2315	81·85	—	—	

March 9, 31 the agglut. titer was 1 : 8000, and on November 14, 31 it was 1 : 640 (weaker dilutions were not made).

"Õunik" had not been given either living or killed cultures of *Br. abortus*.

Data concerning her milk production are presented in table 27.

bb. Results of the Inquiries concerning the Milk and Blood Sera.

On March 10, 31 I examined "Õunik's" milk, to make sure whether the udder was infected with *Br. abortus*, and whether there were any other agents causing diseases. Table 28 presents the results of the examinations.

Table 28 indicates brucellous infection of "Õunik's" udder; further bacteriological examinations, however, did not reveal any other bacteria.

Before her slaughter (on April 6, 32) I took some secretion from each quarter of the udder individually, to find out which of the quarters were infected with *Br. abortus*, and to make sure if meanwhile no other bacteria had invaded the udder. "Õunik" had ceased to yield milk some days before the slaughter, therefore I got only about 25 cm of secretion from each quarter individually. Table 29 presents the results of the inquiries.

According to table 29, the secretion from only the right rear quarter of her udder revealed *Br. abortus* on the date of slaughter (April 6, 32). No other types of bacteria were detected in spite of repeated bacteriological examinations in any of the quarters. The sediment of the secretion from each quarter showed a good many cellular elements; the polynuclear leucocytes were most abundant, the lymphocytes and epithelial cells were fewer in number, and there were also a great many plasma cells.

At the time of slaughter (April 6, 32) the agglutination of the blood serum with *Br. abortus* was 1 : 3200 +.

cc. Macroscopical Finding at the Time of Slaughter.

General Condition.

"Õunik" was in a wellnourished condition.

Udder.

The whole udder weighed 3545 g, being normal in shape; it had an even and unusually dense and fleshy consistency.

Table 28.

Milk examined on August 10, 31	Agglut. of *Br. Abortus* with Milk Serum	Aspect of Milk	Quantity of Sediment	Microscopical Finding in Sediment			Bacteriological Finding in Sediment		Agglut. of *Br. Abortus* with Blood Serum of Guinea-pig injected with Cream + Sediment	Culture of *Br. Abortus* from the Spleen of Injected Guinea-pig	Examination of Injected Guinea-pig for Tuberculosis
				Cellular Elements	Microbes		Saccharose Plates	Brown's Plates			
					Gram's Staining	Ziehl-Neelsen's Staining					
Milk	positive (1 : 80)	normal	normal	abundant	negative	negative	negative	negative	positive (1 : 160)	positive	negative

Table 29.

Milk examined on April 6, 32	Aspect of Milk	Quantity of Sediment	Microscopical Finding in Sediment			Bacteriological Finding in Sediment		Agglut. of *Br. Abortus* with Blood Serum of Injected Guinea-pigs	Culture of *Br. Abortus* from the Spleen of Injected Guinea-pig	Examination of Injected Guinea-pig for Tuberculosis
			Cellular Elements	Microbes		Saccharose Plates	Brown's Plates			
				Gram's Staining	Ziehl-Neelsen's Staining					
Right front quarter	yellowish-grey, half-transparent, serum-like, glutinous	abundant	abundant	negative	negative	negative	negative	negative	negative	negative
Right rear quarter	"	very abundant	"	"	"	"	"	positive (1 : 320)	positive	"
Left front quarter	"	"	"	"	"	"	"	negative	negative	"
Left rear quarter	"	abundant	"	"	"	"	"	"	"	"

The right front quarter weighed 895 g, and in the intersection it was yellowish-reddish-grey. The surface of the section showed only single porous lobules, the greater number of them being lardaceous. In the border portions of the quarter there was a dense deposit of fat between the lobules. There was an intense proliferation of the interstitial connective tissue.

The right rear quarter weighed 850 g, and in the intersection it was yellowish-reddish-grey; in places red spots were more intense. First of all there were on the surface of the sections lardaceous lobules and a very considerably proliferated interstitial connective tissue; in places it was quite easy to detect with the naked eye that the connective tissue had also penetrated into the lobules. In the border portions a dense interlobular deposit of fat was noticed likewise.

The left front quarter weighed 826 g. Its other qualities resembled those of the right front quarter.

The left rear quarter weighed 974 g. Its other qualities resembled those of the right front quarter.

Lymph Nodes.

There were two right supramammary inguinal lymph nodes, both were swollen and roundish in shape. The bigger one weighed 55 g and was $8 \times 5{\cdot}5 \times 2$ cm large; the smaller node weighed 20 g and was $4 \times 3 \times 1{\cdot}2$ cm large. The other qualities of both the lymph nodes were similar to those of "Koidu".

There were two left supramammary inguinal lymph nodes, both were absolutely normal outwardly as well as in the intersection. The bigger weighed 41·5 g and was $6{\cdot}5 \times 5 \times 1{\cdot}7$ cm large; the smaller lymph node weighed 9 g and was $2{\cdot}5 \times 1{\cdot}2$ cm large.

The right deep inguinal lymph node was $7 \times 5 \times 1{\cdot}3$ cm large and weighed 45·5 g; its other qualities were similar to those of "Koidu's" right deep inguinal lymph node.

Uterus.

The right uterine cornu showed a 6-months' fetus. There were no pathological changes in the uterus and fetal membranes. The calf had developed normally.

The other organs which are not mentioned here were free from any pathological changes.

dd. Bacteriological Findings and Results of Injections, in Animals used for Experiments.

The inquiries were made exactly in the same manner as in the case of the material taken from "Koidu". The results of the inquiries are presented in table 30.

According to table 25, only the right rear quarter of the udder and the right supramammary inguinal lymph nodes of "Õunik" were infected with *Br. abortus*; no other bacteria were detected in the organs and parts of the body examined.

Table 30.

Material [1]	Cultures		Agglut. of *Br. Abortus* with Blood Serum of Injected Guinea-pigs	Culture of *Br. Abortus* from the Spleen of Injected Guinea-pigs	Examination of Injected Guinea-pigs for Tuberculosis	Notes
	Saccharose Plates	Brown's Plates				
Right front quarter	sterile	sterile	negative	negative	negative	
Right rear quarter	"	"	positive (1 : 160)	positive	"	
Left front quarter	"	"	negative	negative	"	
Left rear quarter	"	"	"	"	"	
Lln. ing. sup. dext.	"	"	"	"	"	
Lln. ing. sup. sin.	"	"	positive (1 : 160)	positive	"	
Ln. ing. prof. sin.	"	"	negative	negative	"	
Gl. thyreoidea	"	"	"	"	"	
Lien	"	"	"	"	"	
Uterus	"	"	"	"	"	
Ovar. dext.	"	"	"	"	"	
Ovar. sin.	"	"	"	"	"	
Medulla ossis metacarpi dext.	"	"	"	"	"	

ee. Histopathological Finding.

Udder.

The right front quarter does not show any pathological changes. The finding is in general similar to that in the left front quarter of cow I, but it differs from it in that the majority of the lobules are in an inactive state; in every region there are to be found lobules in varying quantities, which are in a state of low milk production. In the above lobules, fat globules very frequently appear in the epithelial cells. In places a fat tissue is to be found in great quantities in the interlobular connective tissue.

[1] The right deep inguinal lymph node could not be used for bacteriological inquiries, because the butcher had made an incision into it.

Right Rear Quarter.

The finding is in general similar to the corresponding finding in the right front quarter of cow I, but the changes appear to a greater extent, not taking into account the variety of the intensity and extent of the changes according to the regions. Besides, no cornification of the epithelium of the changed lactiferous ducts is to be found in the quarters of the udder in question; the lobules that have remained normal are mostly in an inactive state, and a small number of them are in a state of low milk production. Amyloid corpuscles appear in small numbers. In some regions, a fat tissue is to be found in places in great quantities in the interlobular connective tissue.

In several foci that appear in the parenchyma of the udder, and in the superficial epithelial cells of some considerably changed lactiferous ducts and between the cells, I found *Br. abortus* in the histological sections.

Left Front Quarter.

No pathological changes are to be found in the left front quarter. The finding resembles that in the right front quarter, but with the difference that in region 3 in the interalveolar connective tissue of two lobules that are in a state of inaction there are to be found some infiltration foci, of the size of an alveolus, of lymphocytes in moderate quantities, and of less numerous plasma cells.

The left rear quarter does not show any pathological changes. The finding resembles that in the right front quarter, but also in regions 2 and 5 there appear occasional infiltration foci which resemble those in the left front quarter in region 3.

Lymph Nodes.

The finding in the right supramammary and right deep inguinal lymph node is similar to that in the corresponding lymph nodes of cow I. In the left supramammary and the left deep inguinal lymph nodes there are no changes to be observed.

Thyroid Gland.

In the thyroid gland the finding is similar to that in the thyroid gland of cow V.

No pathological changes were observed in the other examined organs [uterus, ovaries, spleen, and bone marrow (*metacarpus dext.*)].

Table 31.

Extent of the Changes in the Quarters of the Udder according to Histological Studies.

Cow VI.

Regions inquired	Approximate Percentage of the Changes of the Tissue in the Inquired Regions (Scheme 1, Page 41)										Approximate Average Percentage of the Changes of the Tissue in the Whole Quarter [Region 10 (Teat) is not taken into Account]
	1	2	3	4	5	6	7	8	9	10	
Right front quarter	—	—	—	—	—	—	—	—	—	—	—
Right rear quarter	25	60	50	60	50	75	10	10	20	—	40·0
Left front quarter	—	—	—	—	—	—	—	—	—	—	—
Left rear quarter	—	—	—	—	—	—	—	—	—	—	—

Undiseased Cows.

Cow VII.

Judging by the circles on the horns, the cow had calved 5 times. She was a cross-bred Estonian red "angler". According to the information given by the owner, she had calved about 7 months before, and on the days previous to the slaughter she had yielded about 4 litres of milk a day. On the date of slaughter, April 29, 32, in the town abattoir of Tartu, the cow was pregnant and contained a 5-months' fetus.

I did not find any bacteria causing diseases in the milk samples taken from each quarter individually, previous to the time of slaughter, nor in the supramammary and deep inguinal lymph nodes, though I studied them by means of both the cultivation method and the guinea-pig experiment. No saprophytic microbes were to be found in the above-mentioned milk samples and the lymph nodes. The milk serum did not agglutinate *Br. abortus*. The appearance of the milk serum and the quantity of the sediment were normal. The chloride content of the milk was as follows:

in the milk of the right front quarter — 0·128 per cent.
" " " rear " — 0·135 "
" " left front " — 0·064 "
" " " rear " — 0·071 "

The blood serum taken at the time of slaughter did not agglutinate *Br. abortus*. No macroscopical changes were found in any of the organs and parts of the body.

The histological findings in the quarters were alternately as follows: most of the lobules were in the state of producing milk (fig. 1); the alveolar epithelium had a cubical and cylindrical shape; in the lumina of the alveoli there was a netlike and granular mass of varying consistency and of a reddish-yellowish-brown colour (according to van Gieson); in this mass the lumina of single alveoli of several lobules showed polynuclear leucocytes in moderate numbers; in the lumina of the alveoli there were some desquamated epithelial cells, but they were found very seldom. There was little of the interalveolar tissue to be found; it consisted of some strings of connective tissue, in which there appeared in places single lymphocytes and leucocytes. The interlobular connective tissue was of varying extent. One part of the lobules was in a state of inaction, in the very markedly proliferated interalveolar connective tissue of these lobules there were some infiltration foci of lymphocytes which were in some places small, in other places as large as middle-sized alveoli and of middle density; in these foci there were to be found in places some leucocytes and in rarer cases plasma cells. An involution was to be noticed in the alveoli. In the lumina of the alveoli there were in places amyloid corpuscles.

The findings in the supramammary and deep inguinal lymph nodes were similar to those described in the manuals of normal histology.

Cow VIII.

Judging by the circles on the horns, the cow had calved three times. She was a cross-bred animal, yielding about 6 litres of milk a day. On the date of slaughter, June 27, 32 in the town abattoir of Tartu, the cow was not pregnant.

Neither the milk samples taken from all the quarters previous to the time of slaughter, nor the supramammary and the deep inguinal lymph nodes removed after slaughter showed any bacteria causing diseases, though I studied them by means of both the cultivation method and the guinea-pig experiment. No saprophytic microbes were present in the above-mentioned milk samples nor in the lymph nodes. The milk serum did not

agglutinate *Br. abortus*. The appearance of the milk and the quantity of the sediment were normal. The chloride content of the milk was 0·134 per cent.

The blood serum of the cow taken at the time of slaughter did not agglutinate *Br. abortus*.

No macroscopical changes were observed in any of the organs and parts of the body.

The histological findings in the udder resembled those in the udder of cow VII, with the exception that the lumina of the alveoli contained a denser reddish-yellowish-brown mass (according to van Gieson), the epithelium of the alveoli was more often of a cylindrical shape, and there were no involved lobules.

The finding in the supramammary and the deep inguinal lymph nodes was similar to that in the corresponding lymph nodes of cow VII.

Cow IX.

Judging by the circles on the horns, the cow had calved 7 times. She was a cross-bred animal, and her daily milk production was about 5 litres. On the date of slaughter, April 26, 32 in the town abattoir of Tartu, cow IX proved non-pregnant.

Neither the milk sample taken from all the quarters previous to the time of slaughter, nor the supramammary and the deep inguinal lymph nodes removed after slaughter showed any bacteria causing diseases, though I made examinations by means of the cultivation method and the guinea-pig experiment; no saprophytes were detected in the above-mentioned milk sample nor in the lymph nodes. The milk serum did not agglutinate *Br. abortus*. The appearance of the milk and the quantity of sediment were normal. The chloride content of the milk was 0·137 per cent.

The blood serum taken at the time of slaughter did not agglutinate *Br. abortus*.

No macroscopical changes were observed in any of the organs and parts of the body.

The histological findings in all the quarters of the udder were similar to those in the quarters of cow VII, with the only exception that in region 5 of the right rear quarter in the lumina of some alveoli of two lobules there were polynuclear leucocytes in dense quantities, some of them being pyknotic with a slight fatty degeneration.

The finding in the supramammary and the deep inguinal lymph nodes was similar to that in the corresponding lymph nodes of cow VII.

Cow X.

Judging by the circles on the horns, she had calved 7 times. She was a cross-bred animal with low milk production. On the date of slaughter, December 2, 32 in the town abattoir of Tartu, the cow was pregnant and contained a 6-months' fetus.

I did not find any bacteria causing diseases either in the milk sample or in the supramammary and deep inguinal lymph nodes, though I made use of the cultivation method and the guinea-pig experiment; no saprophytic microbes were present in the above-mentioned milk sample nor in the lymph nodes. The milk serum did not agglutinate *Br. abortus*. The appearance of the milk and the quantity of the sediment were normal. The chloride content of the milk was 0·127 per cent.

The blood serum taken at slaughter did not agglutinate *Br. abortus*.

No macroscopical changes were found in any of the organs and parts of the body.

The histological findings in the quarters of the udder were similar to those in the udder of cow VII, except that many lobules were found in a state of inaction, and there were many amyloid corpuscles.

The histological findings in the supramammary and the deep inguinal lymph nodes resembled those in the corresponding lymph nodes of cow VII.

Cow XI.

Judging by the circles on the horns, the cow had calved 9 times. She was a cross-bred Estonian red "angler"; on the days previous to slaughter she had yielded about 3 litres of milk a day. On the date of slaughter, November 8, 32 the cow proved to be pregnant and contained a 6·5-months' fetus.

I did not find any bacteria causing diseases in the milk sample taken before the slaughter, nor in the supramammary and the deep inguinal lymph nodes removed after the slaughter, though I studied them by means of the cultivation method and the guinea-pig experiment; the above-mentioned milk sample and the lymph nodes did not show any saprophytic microbes.

The milk serum did not agglutinate *Br. abortus*. The appearance of the milk and the quantity of the sediment were normal. The chloride content of the milk was 0·140 per cent.

The blood serum of the cow taken at slaughter did not agglutinate *Br. abortus*.

No macroscopical changes were observed in any of the organs and parts of the body.

The histological findings in the quarters of the udder were similar to those in the udder of cow VII, except that the majority of the lobules were in a state of inaction.

The findings in the supramammary and the deep inguinal lymph nodes resembled those in the corresponding lymph nodes of cow VII.

Cow XII.

Judging by the circles on the horns, the cow had calved 12 times. She was a cross-bred Estonian red "angler". On the date of slaughter, December 2, 32, it appeared that the cow was not pregnant.

I did not find any bacteria causing diseases in the milk sample taken before slaughter, nor in the supramammary and deep inguinal lymph nodes removed after the slaughter, though I made use of both the cultivation method and the guinea-pig experiment; the above-mentioned milk sample and the lymph nodes did not show any saprophytic microbes. The milk serum did not agglutinate *Br. abortus*. The appearance of the milk and the quantity of sediment were normal. The chloride content of the milk was 0·120 per cent.

The blood serum of the cow taken at the time of slaughter did not agglutinate *Br. abortus*.

No macroscopical changes were observed in any of the organs and parts of the body.

The histological finding in the quarters of the udder was similar to that found in the udder of cow XI.

The histological findings in the supramammary and the deep inguinal lymph nodes resembled those in the corresponding lymph nodes of cow VII.

Cow XIII.

Judging by the circles on the horns, the cow had calved 11 times. She was a cross-bred Estonian red "angler" and in a dry

period. On the date of slaughter, November 7, 32, in the town abattoir of Tartu, the cow was pregnant and contained a fetus about 7 months old.

I did not find any bacteria causing diseases in the quarters of the udder, nor in the supramammary and deep inguinal lymph nodes, though I studied them by means of the cultivation method and the guinea-pig experiment; no saprophytic microbes were present in the examined material.

The blood serum of the cow taken at the time of slaughter did not agglutinate *Br. abortus.*

No macroscopical changes were observed in any of the organs and parts of the body.

The histological findings in the quarters resembled those in the udder of cow VII, except that the majority of the lobules were in a state of inaction (fig. 2), and in the lumina of single alveoli of some involved lobules there were dense foci of polynuclear leucocytes.

The histological finding in the supramammary and deep inguinal lymph nodes was similar to that in the corresponding lymph nodes of cow VII.

Cow XIV.

Judging by the circles on the horns, the cow had calved 7 times. She was a cross-bred animal in a dry period. On the date of slaughter, June 26, 32, in the town abattoir of Tartu, the cow proved non-pregnant.

I did not find any bacteria causing diseases in the quarters of the udder, nor in the supramammary and deep inguinal lymph nodes, though I studied them by means of the cultivation method and the guinea-pig experiment; no saprophytic microbes were present in the examined material.

The blood serum of the cow taken at the time of slaughter did not agglutinate *Br. abortus.*

No macroscopical changes were observed in any of the organs and parts of the body.

The histological findings in the quarters of the udder resembled those in the udder of cow XIII.

The histological findings in the supramammary and deep inguinal lymph nodes were similar to those in the corresponding lymph nodes of cow VII.

Cow XV.

Judging by the circles on the horns, the cow had calved 6 times. She was a cross-bred Estonian red "angler", in a dry period. On the date of slaughter, December 2, 32, in the town abattoir of Tartu, the cow was pregnant and contained a fetus about 7 months old.

I did not find any bacteria causing diseases in the supramammary and deep inguinal lymph nodes, though I studied them by means of the cultivation method and the guinea-pig experiment; nor were there any saprophytic microbes in the examined material.

The blood serum of the cow taken at the time of slaughter did not agglutinate *Br. abortus*.

No macroscopical changes were observed in any of the organs and parts of the body.

The histological findings in the quarters of the udder were similar to those in the udder of cow VII, except that the majority of the lobules were in a state of inaction.

The findings in the supramammary and deep inguinal lymph nodes resembled those in the corresponding lymph nodes of cow VII.

D. Estimation of the Results of the Inquiries.

The results of the inquiries when summed up and brought to a conclusion are as follows: Four out of six naturally infected cows (cows II, III, IV, V) had *Br. abortus* in all quarters, one cow (cow I) in three quarters, and one (cow VI) in one quarter. In addition to this, five cows (cows I, II, III, IV, V) had *Br. abortus* in the supramammary and deep inguinal lymph nodes on both sides, and cow VI in the right supramammary lymph nodes. Further, the above-mentioned microbe was detected in the spleen of cows IV and V and the thyroid gland of cow IV. No other microbes were present in the above-mentioned organs of the cows under examination, though they were examined bacteriologically, histologically, and by means of the guinea-pig experiment. Therefore, considering the character of the pathological changes noted in all the above-mentioned cases, the latter may be ascribed only to *Br. abortus*.

In studying the udders of the cows histologically, I found considerable pathological changes in all those quarters that eliminated *Br. abortus* with the milk (cow I from three quarters, cows II, III, IV and V from all quarters, and cow VI from one quarter), whereas those quarters that did not eliminate *Br. abortus* (cow I — one quarter and cow VI — three quarters) were pathologically unchanged. In this point the results of my inquiries differ from those of Friedemann (52). The latter failed to notice any pathological changes in the udder in cases (one case) where the above-mentioned microbe was eliminated with the milk.

The histo-pathological findings in the cows I investigated may be compared mostly with those obtained by Runnels and Huddleson from one naturally infected and two inoculated animals.

The results of similar investigations made by other authors are not to be compared with those of my inquiry for the following reasons:

In the cases studied by Smith, Orcutt, and Little (167), brucellosis of the udder was accompanied by infection with streptococci, so that it is impossible to say with any certainty whether and to what extent the histological changes observed were caused by *Br. abortus* or by streptococci; also in the cases studied by Sholl and Torrey (161), streptococcic and micrococcic infections of the udder were frequently observed together with *Br. abortus*; further, the technical defects of the researches made by Sholl and Torrey were such that there is no guarantee for the absence of other bacteria causing diseases of the udder, even in those cases when they had not found other bacteria besides *Br. abortus*. Like Runnels and Huddleson (147), in examining the udders of cows, I found that the changes caused by *Br. abortus* are acute, subacute, and chronic, and the latter predominate.

Like Runnels and Huddleson I found some changes of the epithelium of the alveoli, varying from a fatty degeneration and necrosis to disintegration. I found foci which showed in their centres a complete destruction of the shape of the alveoli (fig. 6, 14, 17, 20). The centres of the last-mentioned foci revealed dense, partly accumulated lymphocytes, plasma cells, frequently abundant polynuclear leucocytes (being mostly neutrophilic), smaller quantities of fibroblasts, and a few scattered epithelial cells. In the border

parts of the above-mentioned foci I found a degeneration of the epithelium of the alveoli of various intensity, partly a pyknosis of the cells and a desquamation of the epithelial cells; in the lumina of the alveoli, side by side with the desquamated epithelial cells there were in places polynuclear leucocytes in various quantities (in places in large numbers), lymphocytes in small numbers, rarely some plasma cells, and, in general, a granular and netlike substance in small quantities; between the alveoli I detected a focal and diffuse infiltration of plasma cells, lymphocytes, and in smaller numbers polynuclear leucocytes. The polynuclear leucocytes are fewer in those foci where the changes are subacute or chronic, whereas the border parts show a proliferation of the epithelium of the alveoli. The infiltration of the plasma cells and lymphocytes is in places even denser than in the acute foci; the subacute and chronic inflammatory foci show a large number of fibroblasts; in the centres of the foci the proliferation of the collagenic fibrous tissue between the infiltration cells is very intense, in places extremely intense. The changed parts generally show an increase in the number of capillaries (in places very dense), in places a purulent exudate in the lumina of the alveoli (a fatty degeneration of polynuclear leucocytes, *etc.*), and there is little perivascular infiltration of lymphocytes and plasma cells. Runnels and Huddleson detected the three last-described kinds of changes only in one inoculated cow and a heifer.

The proliferation of the interlobular interstitial connective tissue is of varying intensity, according to the changes in the parenchyma of the udder. In the proliferated interlobular interstitial connective tissue there appear varying amounts of focal and diffuse infiltration of lymphocytes, of plasma cells, of polynuclear leucocytes in smaller numbers, and of single mast cells, among which there are also fibroblasts in moderate quantities. I noticed considerable changes in the supramammary and deep inguinal lymph nodes on both sides of five cows (cows I, II, III, IV, and V), and in the right supramammary lymph nodes of one cow (cow VI), which resemble the findings by Runnels and Huddleson in the supramammary lymph nodes, *viz.* a thickening of the trabeculae in the cortical substance, diffuse haemorrhages (cow IV in the left deep inguinal lymph node), and small focal haemorrhages (cow V in the supramammary lymph nodes on both sides), a substitution of the connective tissue for the reticular tissue in the

medullary substance, and a diminution of the lymphoid tissue to a varying extent.

In the thyroid gland of cow IV I detected some acute and chronic inflammatory foci, and in the thyroid glands of cows V and VI — chronic inflammatory foci.

Further, I noticed changes caused by brucellosis in certain organs of the cows which, to the best of my knowledge, have never been described before. In the tissue of the udder, the supramammary and deep inguinal lymph nodes (fig. 10, 13, 15, 16, 37) I found foci of epithelioid cells which very often included giant-cells of the Langhans' type (fig. 10, 11, 37), and in the border parts of the foci there were dense lymphocytes and plasma cells, fibroblasts in smaller numbers, polynuclear leucocytes, single mast cells, and fibres of collagenic connective tissue singly and in groups; therefore these foci resemble those of early tuberculosis. The above-mentioned foci may be regarded as the most typical changes due to *Br. abortus* in udders of cows, because of their frequent occurrence and of the more or less regular shape they have. Similar changes have always been found in the organs of inoculated guinea-pigs [Schroeder and Cotton (152), Jaffe (81), my own investigations, *etc.*], and some authors have found such foci due to *Br. abortus* also in the testicles of bulls [Robinson (144), Ohlsson (132), *etc.*].

I noticed a variously extending necrosis of the above-mentioned foci of epithelioid cells in the udders, but these foci of epithelioid cells differed from tuberculous foci in that there was no caseation, nor a complete decay.

These foci of epithelioid cells due to *Br. abortus* in the udder, the supramammary and deep inguinal lymph nodes of cows are very similar to the foci noticed in the lymph nodes of men, due to the so-called climatic bubos and lymphogranulomatosis inguinalis, that have not as yet been explained (it is not known, whether brucellosis has been considered).

Further, I observed cystiform formations (fig. 31) of about 8 mm in diameter in the udders of four cows (cows I, III, IV, and V). These cystiform formations are evidently caused by the retention of the milk (retention cysts).

In all probability, in those parts of the udder where the alveoli, still capable of producing milk, get dilated, there is a retention of milk, because the lactiferous ducts are changed to such a degree

that a complete or a partial obstruction has taken place there. The above-mentioned cystiform formations appear mostly in the border parts of the udder, because there is less possibility for the milk to flow away, than in the central part of the udder.

No earlier authors have as yet in cases of brucellosis noted in their detailed descriptions of the findings in the histological sections a flattening and cornification of the normally cylindrical epithelium of the lactiferous ducts (fig. 21, 22, 23, 24, 25, 26, 30). Sholl and Torrey ascribe the cornification of the lactiferous ducts they had noted, to the streptococcic infection, but considering the technical defects in the researches of the above-mentioned authors (the presence of *Br. abortus* was ascertained only by means of the cultivation method), one cannot be sure whether there was also *Br. abortus* besides the streptococci in the cases they describe. The above-mentioned metaplasia is generally known to be the result of chronic infections, especially in human medicine (e. g. the flattening and cornification of the cylindrical epithelium of the trachea). I also found foci of epithelioid cells in the considerably changed walls of the lactiferous ducts, and in some of these foci there were giant-cells of the Langhans' type (fig. 27).

Varying amounts of amyloid corpuscles are frequently to be found both in the changed and in the normal parts of the udder in the lumina of the alveoli. The amyloid corpuscles are of no special consequence in case of brucellous infection, as they occur also in the udders of undiseased cows, but the abundance of the amyloid corpuscles in the changed places shows that they are partly connected with the changes caused by *Br. abortus*; just as in cases of chronic inflammations, they are present in various organs (the lungs, prostate, etc.).

According to the histological finding, it seems that the changes due to *Br. abortus* are usually healed and balanced by the organism itself through a considerable proliferation of the granulation tissue; in cases of chronic inflammation, however, it may grow fibrous, and in individual cases some deposit of lime (cystiform formations) may be found in the markedly changed places.

Very often I observed mast cells in the changed places and in their vicinity, *viz.* in the udders, supramammary and deep inguinal lymph nodes, very likely phagocytic in their nature, because in the same organs of the undiseased cows I did not notice any of the cells already mentioned.

I tried to ascertain the extent of the changes caused by *Br. abortus* in the udder only by means of the histological finding. I do not claim that the given data are absolutely true, because only a comparatively small part of the udder has been examined, and the extent of the changes in the histological sections cannot be determined with any mathematical accuracy. But considering the quantity of the material examined and its extraction from all parts of the udder, these data give a fairly general survey of the extent of the changes caused by *Br. abortus* infection in the udder of the cow. In fact, the extent of the changes may be even larger, because only the parts presented in scheme 1 were taken into account, without considering the teat, whereas the material taken from between the above-mentioned parts frequently showed macroscopical changes, but was not taken into account. I was also rather cautious in sketching the border-line of the changes as presented in scheme 1, page 41. The data concerning the extent of the changes in each udder are to be found in the tables at the end of the histological finding of the whole udder. The results of the inquiries show that in cases where *Br. abortus* is eliminated with the milk of certain quarters, the changes took place in about 25 per cent. to 83 per cent. of the quarters.

The changes are most frequently to be found in the alveoli and the interalveolar tissue, whereas the lactiferous ducts and the interlobular interstitial connective tissue show rarer changes. The changes due to *Br. abortus* may occur in all parts of the udder, *viz.* the basal part, the central part, and the milk cistern. The changes apparently take place first of all in the basal and central parts of the udder, that is to say, in places where there is more of the parenchyma of the udder. The original focus of the changes is evidently found in the parenchyma of the udder, from whence the process continuously extends to the interlobular connective tissue. This is proved by the fact that no changes take place in the interlobular interstitial connective tissue when there are acute changes only in the alveoli; reverse cases have never been observed. Further, according to the histological findings, the changes in the lactiferous ducts are in all probability different, and are caused by *Br. abortus* in the following way: the germs of the above-mentioned microbe are carried from the changed alveoli, together with the exudate and cells, into the lactiferous ducts, where they produce a pathogenic effect in the epithelial cells first of all, then further, in

the subepithelial tissue, and the tissue lining the ducts. There are, of course, other ways by which the infection and process take their origin in the lactiferous ducts: 1) the disease may be conveyed per continuum from the parenchyma, and 2) *Br. abortus* may arrive at the lactiferous ducts either haematogenically or lymphogenically. The first mode is theoretically possible, but to judge from the histological finding, it occurs very seldom indeed. It is impossible to determine how often the lactiferous ducts get infected in the haematogenic or lymphogenic way, but, evidently, it happens rarely. The epithelium of the lactiferous ducts is most frequently infected, being continuously in contact with the germs of *Br. abortus* — hence the changes in the lactiferous ducts.

Runnels and Huddleson state that the changes caused by *Br. abortus* are progressive; the infection takes place first of all in the parenchyma of the udder, and then in the interstitial connective tissue.

The histological findings in the udders of undiseased cows (9 cases) do not reveal any sudden or very marked changes which might be taken for changes caused by *Br. abortus*, either during the lactation or in the dry period. At the end of lactation in several parts of the udder in the lumina of the alveoli there appear polynuclear leucocytes, which increase in number when the cow is about to cease yielding milk. At the end of lactation there is to be noticed in a part of the lobules an extension of the interlobular and interalveolar interstitial connective tissue, and in those regions where the alveoli become first of all inactive, the interalveolar tissue shows the presence of lymphocytes, either singly or in small groups, together with single plasma cells. The last-mentioned infiltration is to be found sometimes over the whole of an inactive udder, and in the lumina of the alveoli there appear in places also polynuclear leucocytes in considerable numbers. With advancing years the interlobular interstitial connective tissue of the udder extends accordingly.

I cannot state that I have ever noted the presence of leucocytes in the udder of any undiseased cow, either in her lactation or in her dry period, in such quantities as described by Pfaundler (194), who declares in his general description of the mammary gland that the number of leucocytes in the interstitial connective tissue, the epithelium, and the lumina of the alveoli

is so large as completely to cover the corresponding tissue, whereas the shape of the latter is entirely lost.

It is doubtful whether the pathological processes that develop slowly in the udder, have as yet been sufficiently taken into consideration in studying the tissue of the udder normal-histologically, or whether they have been at all considered, especially in the studies made in those days when there was as yet no knowledge of the presence of *Br. abortus* in the bovine udder.

Works on the subject reveal the fact that no authors have as yet succeeded in ascertaining *Br. abortus* in the histological sections of the udder and the supramammary lymph nodes of the cow. Also only a few authors (Ohlsson, Christiansen, Witte, *etc.*) have discovered the above-mentioned microbe in other organs, such as in the histological sections of the testicles that in the case of *Br. abortus* have shown very considerable changes; even in some of these cases it is not quite clear whether *Br. abortus* was found in the histological sections, or in the smears taken from the altered parts.

I made experiments with a great many staining methods, because for the purpose of determining *Br. abortus* in the histological sections, no special methods are as yet known; the descriptions of the experiments I made are to be found under the heading "Course of Inquiries and Methods". After much experimenting, I succeeded in staining and finding *Br. abortus* in the histological sections from the udder, supramammary and deep inguinal lymph nodes, the fetal membranes of brucellous cows, and from the spleen and the lymph nodes of guinea-pigs. *Br. abortus* proved to be present in the altered portions of the udder, as well as in the supramammary and deep inguinal lymph nodes, sprinkled in the exudate between the cells, in parts of the decayed cells, and in the altered cells (fig. 45, 46, 47, 48); the microbe may be found also among the altered cells and in the necrotic foci in small and dense groups (fig. 47, 49); in places there appear cells densely packed with *Br. abortus* as usually noticed in the fetal membranes. The bacterium is often to be found in the fetal membranes in great quantities and large groups (fig. 43), which when examined with a low powered microscope appear like spots of a dark colour.

In one changed portion of the udder I detected *Br. abortus* surrounding damaged capillaries (fig. 45, 45a), occurring also in swollen

endothelial cells, and even in the lumina of capillaries, which suggests that a direct haematogenic infection with *Br. abortus* from changed foci is possible.

Having tried various methods of staining the histological sections, I found that I obtained some results by using the Löffler's methylene blue and carbolfuchsin staining methods, but the Giemsa method, as modified by me, proved to be the best. I stained sections in Giemsa's azure-eosin-methylene-blue solution (2 drops of the stock solution: 1 ccm of double distilled water) for 4 to 24 hours, the temperature in the room being about 30° C; I repeatedly changed the staining solution during the process of staining. After staining I rinsed the preparations for a short while in distilled water, and I decolorized them in 0.25 per cent. of acetic acid for 5 to 30 min., according to the duration of the staining and the thickness of the preparations, but usually until the preparation lost its dark-blue colour and assumed a reddish hue in the ground blue. Further, I rinsed the preparations in several distilled waters, and at last, I drew them through one 70° alcohol and two absolute alcohols (in all for 1 to 2 minutes); this was followed by xylol and Canada balsam.

It is already known that preparations stained according to Giemsa's method lose their colour when left in alcohol for a longer while; that is the reason why I did not draw the preparations at first through alcohol and xylol, but I decolorized them longer in acetic acid, and after rinsing them in distilled water, I laid them out to dry at 30° C, which was followed by Canada balsam and a cover-glass. The results obtained by this method proved sometimes sufficient, but very often the cleanness and the clearness of the preparations left much to be desired. Therefore I made further experiments with acetone-xylol, by drawing the preparations, after they were rinsed, through several acetone-xylols, increasing the quantity of xylol, and decreasing that of acetone; at last I drew the preparations through three clean xylols, then followed Canada balsam. This method, however, did not yield desirable results either, for the preparations were not clean and clear enough; I made further experiments in the above-described manner by drawing the preparations through alcohol and xylol. The preparations obtained by this method always proved good. The stain of the preparations did not suffer from being drawn speedily through alcohol (in 1 to 2 minutes), because all the parts of the

tissue, especially *Br. abortus*, were stained very intensively during the long period of staining. The germs of *Br. abortus*, when stained according to the Giemsa method, as modified by me, become partly pale-blue and partly pale-violet-blue, the gram-positive microbes become dark-blue (as e. g. in the fetal membranes), the nuclei of cells become dark-blue, and the protoplasma red. For the purpose of detecting and ascertaining *Br. abortus* in the changed tissue of the histological sections, the above-described method has proved very appropriate, because of the variety of the stain, just like the Andersen-Torbjörnsen method for staining the smears. I compared Löffler's methylene blue and carbolfuchsin methods with the previous method and found them to be insufficient, because of the monotony of their staining.

The staining of the germs of *Br. abortus* in cultures is of various intensity, especially in the preparations made from older cultures; they are likewise stained in various strengths in the histological sections. They become mostly pale-violet-blue, less frequently pale-blue, and some of the germs turn almost dark-blue. This variety in the staining is somewhat characteristic of the microbe in question.

For the purpose of staining *Br. abortus* in the histological sections, the material may be fixed equally well either in alcohol or in formalin.

The germs of *Br. abortus* in the histological sections are of similar shape and size to those found in old cultures; they have mostly the shape of cocci, whereas the short rod-like forms occur very rarely in the udder and in the changed portions of the supramammary and deep inguinal lymph nodes. There are also very frequently to be found diplococci-like formations. In the fetal membranes I have found cocci-like forms predominant, but very often also short rods. A fresh culture very seldom shows cocci-like forms, the majority being middle-sized rods, and single ones above middle-size.

The size of the germs of *Br. abortus* is dependent partly on the colouring, as is generally known of the staining of bacteria. The germs of *Br. abortus* in the histological sections of the udder, the supramammary and deep inguinal lymph nodes when stained according to the Giemsa-method, as modified by me, are of 0·4 to 0·8 by 0·4 to 1·2 microns.

In the supramammary and deep inguinal lymph nodes of both the undiseased and the brucellous cows, there are very often to be found a number of roundish and also angular pieces of decayed nuclei and granules of pigment (fig. 44), which may mislead, when the determination of *Br. abortus* in the histological sections is not performed by a thoroughly experienced and dexterous person. On further consideration the pieces of nuclei and the granules of pigment seem to vary in size, but they are, in general, many times larger than the germs of *Br. abortus*; the pieces of nuclei may be stained with all the usual nuclear stains.

According to the description of the histological findings, the changes caused by brucellosis are very considerable and extensive indeed. Among the considerable changes, there is very often to be noticed a complete disappearance of the alveoli, which must bring about a decrease of milk production as noted by several authors, in comparing the milk production of unaffected cows with that of cows infected with *Br. abortus*. Of course the decrease of the milk production of cows eliminating *Br. abortus* with their milk varies; it depends on the intensity of the changes in the udder and also on whether the infection has spread to the whole udder or to some of the quarters only. A little while after the infection of the udder with *Br. abortus* has begun, milk production may even increase, which fact is of great interest. In all probability the presence of *Br. abortus* in the udder causes an irritation hyperaemia, which then causes a markedly increased milk production.

The increase of milk production as apparently caused by irritation hyperaemia has been ascertained in two cases of the examined cows (cows IV and V).

The precise date of the infection of cows IV and V is not known, but according to the data given, these animals were most probably infected at the end of 1930 or at the beginning of 1931. In any case the blood serum of the above-mentioned cows agglutinated *Br. abortus* on March 18, 31.

Cow IV, being 10 years of age, having been regularly in milk all the time, and her condition having been more or less good and always regular, had probably attained and lost her highest milk production period. According to table 18, the highest monthly milk production of this cow was in June 1930 before she was infected with *Br. abortus*, when she yielded 454 kg of milk

and 18·16 kg of butterfat. But after she was infected with the above microbe in March 1931 (the udder became infected probably at that time) the milk production of the cow increased very considerably in comparison with her milk production of the previous years; she yielded in that month 707 kg of milk and 28·28 kg of butterfat. But this remarkable increase of milk production of cow IV was followed by a rapid decrease of her milk production when compared with that of the previous year, taking each month individually. This case is analogous to that of cow V, the latter being of even more interest, because at the time of slaughter she was 14 years old; she had been regularly in milk, and her condition had been good. According to table 23, the cow had produced most in December 1929, 490 kg of milk — 16·66 kg of butterfat; that was probably before she had been infected with *Br. abortus*; the highest year production of cow V was in 1926, being 3804 kg of milk — 136·09 kg of butterfat. But in January 1931, when cow V had probably been infected already, she produced 537 kg of milk which yielded 18·79 kg of butterfat; then followed a decrease in her milk production till she was dry in October 1931.

Like Bang and Bendixen (9), I have observed an increase in the chloride content and sediments in the milk of cows eliminating *Br. abortus* with their milk. Several quarters showed twice as much chloride as was found in the milk of unaffected cows in the same lactation period.

Like Cooledge (33), Tweed (180), Runnels and Huddleson (147), *etc.*, I also noted a considerable increase of the cellular elements in the milk of cows eliminating *Br. abortus*. In examining the preparations of the milk sediment, it appeared that of the cellular elements, the polynuclear leucocytes are most abundant in the milk, the epithelial cells, lymphocytes, and plasma cells occur in smaller numbers.

It is commonly asserted that *Br. abortus* does not remain very long in a non-pregnant uterus. After abortion the uterus contains very many germs of *Br. abortus* which, however, disappear in a short time. According to Klimmer (95), the uterus does not show any germs of *Br. abortus* three weeks after abortion. But this does not always seem to be the case after calving; the germs of the above-mentioned microbe cause changes in the mucous membrane of the uterus, and they are to be found there, even a year after calving. In this respect cows I and II are of great interest.

Cow I that had calved on February 17, 30 was slaughtered on January 27, 31, the time between the last calving and the slaughter being above eleven months. Cow II had calved on March 22, 30, and was slaughtered on January 28, 31, thus, more than ten months after her last calving. At the slaughter I found inflammatory changes in the mucous membranes of the uteri of both the above cows, and I got a culture of *Br. abortus* from the uteri of both cows.

The literature surveyed shows that up to 100 per cent. of cows vaccinated with a living culture of *Br. abortus*, eliminate the germs of *Br. abortus* with their milk for several years. This result shows as high a percentage of cows eliminating *Br. abortus*, or even higher, as in cases of natural infection. As the changes caused by *Br. abortus* in the udder are very considerable in cases of natural infection, there is no reason to suppose that the microbe would remain for years in the udder as a loyal commensal in the cases of vaccination (artificial infection) with an unweakened living culture of *Br. abortus*.

Runnels and Huddleson found that the pathological changes are not very different, whether the udder is naturally infected or whether it is inoculated with *Br. abortus*. The changes were even more considerable in udders of inoculated cows than in those of naturally infected animals.

I had no opportunity of examining the udders of the cows that were vaccinated with an unweakened living culture of *Br. abortus* (and were unaffected before the injection). But one cow (cow II), naturally infected with *Br. abortus*, was given subcutaneously an injection of an unweakened living culture of *Br. abortus* used for vaccination, and the histological finding in the udder of this cow showed a most marked necrosis, in comparison with the udders of other brucellous cows I investigated. The direct reason of that fact is not known, but considering the finding of Runnels and Huddleson in cases of artificial infection, such an extensive necrosis in the udder is very likely due to injecting the latter with a living culture of *Br. abortus*.

Also the majority of the clinical and bacteriological investigations have shown that vaccination with a living culture of *Br. abortus* does not bring about a cure — which statement is even now often made — but is rather a dissemination of *Br. abortus*.

Therefore, according to the data by Klimmer (95), vaccina-

tion with a living culture of *Br. abortus* is prohibited already in Palestine; in Australia this medical treatment has not been made use of for 11 years; neither is vaccination with a living culture of *Br. abortus* recommended by the Committee of the Veterinary Associations in the U. S. A. (1929), and the newest data show that this method of fighting brucellosis is not used any more in the U. S. A. Further, Hungary, Norway, Japan, and Russia have abandoned vaccination with a living culture of *Br. abortus* altogether.

Considering the very extensive changes in the udder caused by vaccination with a living culture of *Br. abortus,* and the danger of a general spread of brucellosis among cattle, it is most urgent that also in our country vaccination with a living culture of *Br. abortus* should be prohibited by legislative action, in order to prevent the great economical losses caused by this infection.

E. Summary.

1) These bacteriological and histological inquiries dealt with various parts and organs of six cows naturally infected with *Brucella abortus*, and of nine undiseased cows.

2) Four out of six naturally infected cows had *Brucella abortus* in all quarters, one cow in three quarters, and one in one quarter. Five cows had *Brucella abortus* in the supramammary and deep inguinal lymph nodes on both sides, one cow in the right supramammary lymph nodes, two cows in the spleen, and one in the thyroid gland. No other microbes causing diseases were present in the organs mentioned.

3) The histological examination revealed considerable pathological changes in all those organs where the bacteriological studies had shown *Br. abortus*, whereas those organs that had no *Br. abortus* were unchanged.

4) Of the tissue of the infected quarters of those cows that eliminated *Br. abortus* with their milk, about 25 per cent. to 83 per cent. was altered.

5) Changes due to *Br abortus* may occur in all the quarters of the udder in the lower and central parts, as well as in the milk cisterns regions.

6) Changes caused by *Br. abortus* take place in the parenchyma of the udder, the lactiferous ducts, and the interstitial tissue.

7) Changes are most frequent in the alveoli and the interalveolar connective tissue, the lactiferous ducts and the interlobular connective tissue showing fewer traces of change.

8) The pathological changes due to *Br. abortus* are acute, subacute, and chronic, the latter predominating.

9) In the parenchyma infected with *Br. abortus* there occur regressive changes (varying from fatty degeneration and necrosis to disintegration) and progressive ones (proliferation and dense cellular infiltration of the interalveolar connective tissue and of the epithelium of the alveoli).

10) The acute changes in the parenchyma are accompanied by fatty degeneration or even complete disintegration of the epithelium of the alveoli, by desquamation, infiltration of polynuclear leucocytes, appearance of purulent exudate, particularly in the lumina of the alveoli, and occasional destruction and necrosis of groups of alveoli. Usually these changes are also accompanied by symptoms of chronic inflammation (infiltration of lymphocytes and plasma cells).

11) The changes in the subacute inflammatory foci of the parenchyma of the udder resemble those in the acute foci, but the infiltration of polynuclear leucocytes is weaker and there are symptoms of chronic inflammation (proliferation of the epithelium of the alveoli, denser infiltration of lymphocytes and plasma cells, fibroblasts in small numbers, fibres of collagenic connective tissue, and an increase in the number of capillaries).

12) The changes in the chronic inflammatory foci in the parenchyma of the udder resemble those in the subacute foci, but polynuclear leucocytes occur only singly, the proliferation of the fibrous tissue is intense, in places very intense indeed, and there is little perivascular infiltration of plasma cells and lymphocytes.

13) In the chronic, and partly also in the subacute inflammatory foci of the udder the author found epithelioid cells due to *Br. abortus* — a fact never before described —, including giant-cells of the Langhans' type, and sometimes with necrosis, chiefly in the centre of the foci, but these foci of epithelioid cells differed from tuberculous foci in that there was no caseation.

14) The changes in the lactiferous ducts are of varying intensity. They begin with a slight proliferation of the epithelium, intumescence and moderate interepithelial and subepithelial infiltration (of lymphocytes, plasma cells, and smaller numbers of polynuclear leucocytes), and end in very intense proliferation, flattening, necrosis, and partial cornification of the epithelium of the lactiferous ducts — processes observed for the first time in connection with brucellous infection and often causing obstruction of the lumen.

15) In the very considerably changed epithelium of the lactiferous ducts, particularly in the subepithelial tissue, a certain amount of connective tissue is observed, the extent of which depends on the duration of the changes, and there is a dense, somewhat accumulated cellular infiltration of lymphocytes, plasma cells, smaller quantities of fibroblasts, and polynuclear leucocytes.

16) In the very markedly changed walls of the lactiferous ducts there are in places also foci of epithelioid cells with giant-cells of the Langhans' type.

17) Four cows had macroscopically observable cystiform formations proving to be dilated, and in many cases considerably altered lactiferous ducts, and dilated and confluent alveoli (retention cysts).

18) The interlobular connective tissue proliferates in varying degrees, according to the extent to which changes have taken place in the parenchyma of the udder.

19) In the interlobular connective tissue, in which proliferation has taken place, focal and diffuse infiltration (of lymphocytes, plasma cells, and single mast cells, a moderate amount of fibroblasts, and comparatively few leucocytes), indicative of chronic inflammation is observed in varying intensity.

20) The original focus of the changes of the udder is apparently found in the parenchyma of the udder, the changes in the interlobular connective tissue and in the lactiferous ducts being of secondary nature.

21) Varying amounts of amyloid corpuscles appear both in the changed and in the normal parts of the udder in the lumina of the alveoli.

22) The changes in the lymph nodes are predominantly chronic. There are also foci of epithelioid cells with giant-cells of the Langhans' type. Thickening of the trabeculae in the cortical substance, diffuse and focal haemorrhages, substitution of fibrous tissue for reticular tissue in the medullary substance, and some diminution of the lymphoid tissue are likewise to be noticed.

23) In the thyroid gland, infection with *Br. abortus* causes the appearance of acute and chronic inflammatory foci.

24) In the altered parts of the udder (the foci in the parenchyma and the altered lactiferous ducts), as well as in the lymph nodes, the author discovered *Br. abortus* in groups and sparse sprinklings between cells and in cells, by means of staining histological sections by the Giemsa method, as modified by the author, and by Löffler's methylene blue and the carbolfuchsin staining methods.

25) The *Br. abortus* found in the altered parts is of similar shape and size to that found in old cultures, and the staining is uneven.

26) The *Br. abortus* in the udder and in the lymph nodes, when stained in histological sections according to the Giemsa method, as modified by the author, mostly becomes pale-violet-blue, less frequently pale-blue, and in isolated cases almost dark-blue, the gram-positive microbes and nuclei of cells becoming dark-blue, and the protoplasma, red.

27) Direct haematogenic infection with *Br. abortus* from changed foci is possible, for *Br. abortus* was found by the author to surround damaged capillaries, occurring also in endothelial cells and even in the lumina of capillaries. No earlier authors have as yet succeeded in demonstrating the haematogenic spread of *Br. abortus* by means of histological sections.

28) The chief agent unfavourably affecting the milk production of cows eliminating *Br. abortus* with their milk is the change caused by *Br. abortus* in the udder.

29) Infection with *Br. abortus* nearly doubles the quantity of chlorine in the milk and multiplies the amount of cellular elements, as other authors have also proved.

30) A little while (some days or weeks) after the infection of the udder with *Br. abortus* has begun, milk production may even increase for some time (a few weeks), apparently owing to irritation hyperaemia.

31) In non-pregnant uteri, *Br. abortus* may be present for a considerable time, even for about a year (according to the earlier statements, this can happen for two months at most).

32) In the udder of undiseased cows, involution causes no changes that could be ascribed to brucellosis.

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ILLUSTRATIONS
with Explanation of Figures.

Fig. 1. Cow VII. Normal udder in a state of moderate milk production. van Gieson. Magnification: 60 ×. Microphotogram.

Fig. 2. Cow XIII. Normal udder in a state of inaction. van Gieson. Magnification: 60 ×. Microphotogram.

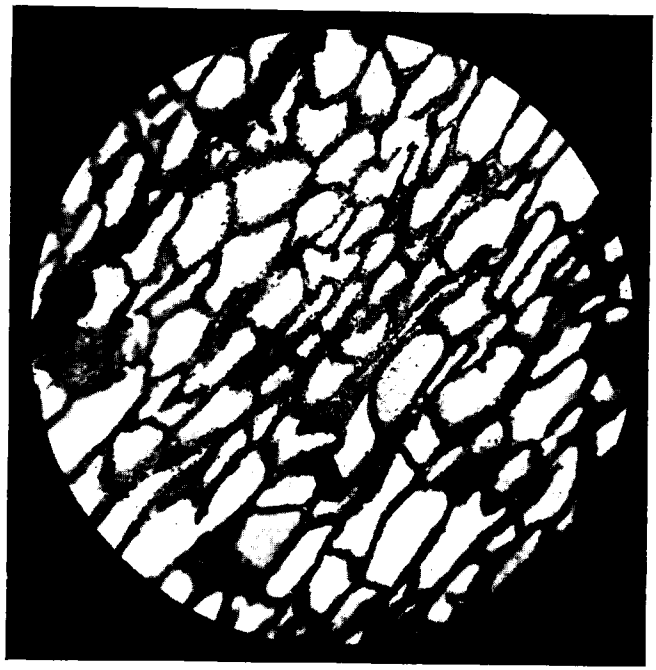


Fig. 1.

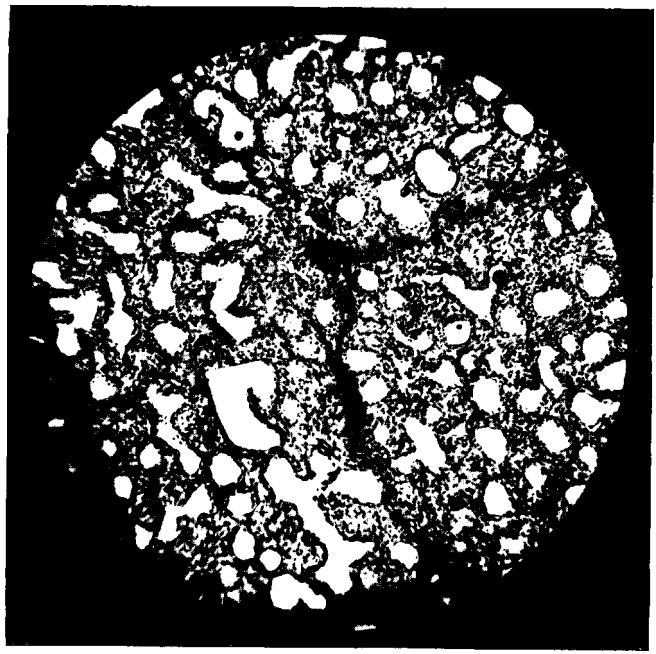


Fig. 2.

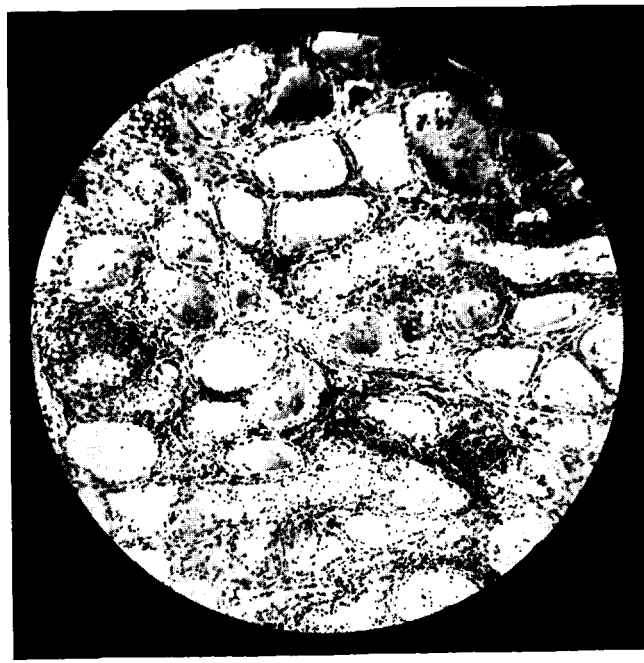


Fig. 3.

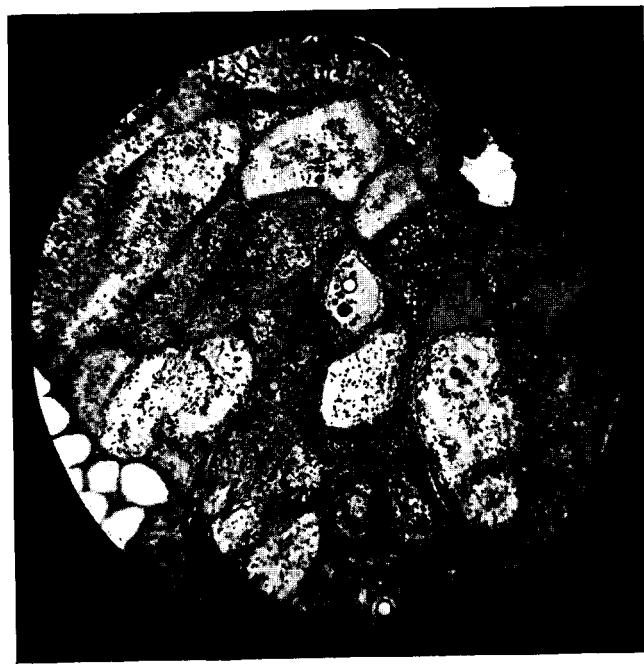


Fig. 4.

Fig. 3. Cow I. Udder, right rear quarter, region 2: considerable desquamation of the epithelium and infiltration of the interstitial tissue. van Gieson. Magnification: 80 ×. Microphotogram.

Fig. 4. Cow I. Udder, right rear quarter, region 3: very intense desquamation of the epithelium and infiltration of the interalveolar tissue. van Gieson. Magnification: 80 ×. Microphotogram.

Fig. 5. Cow II. Udder, right front quarter, region 7: destruction of the structure of the alveoli, and substitution of the inflammatory elements, in place of the structure of the alveoli. van Gieson. Magnification: 250 ×. Microphotogram.

Fig. 6. Cow I. Udder, right rear quarter, region 3: inflammatory elements (polynuclears, lymphocytes, plasma cells) and single desquamated epithelial cells, in place of the destroyed alveoli. van Gieson. Magnification: 500 ×. Microphotogram.

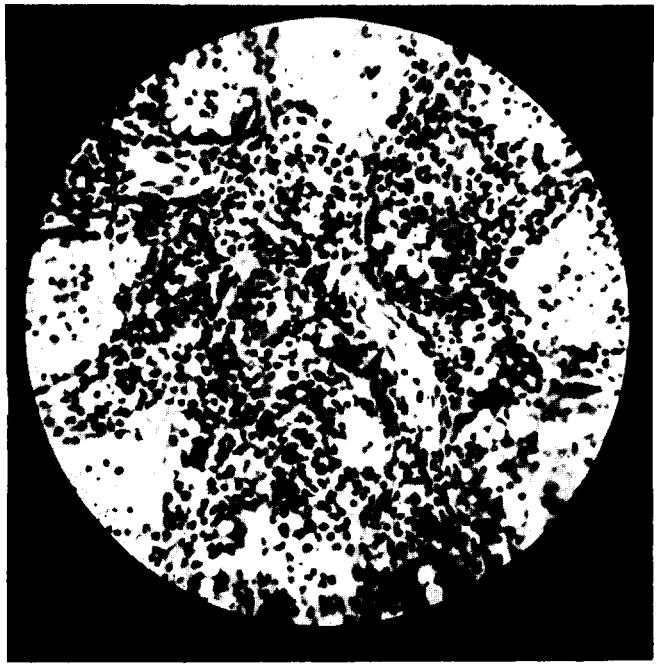
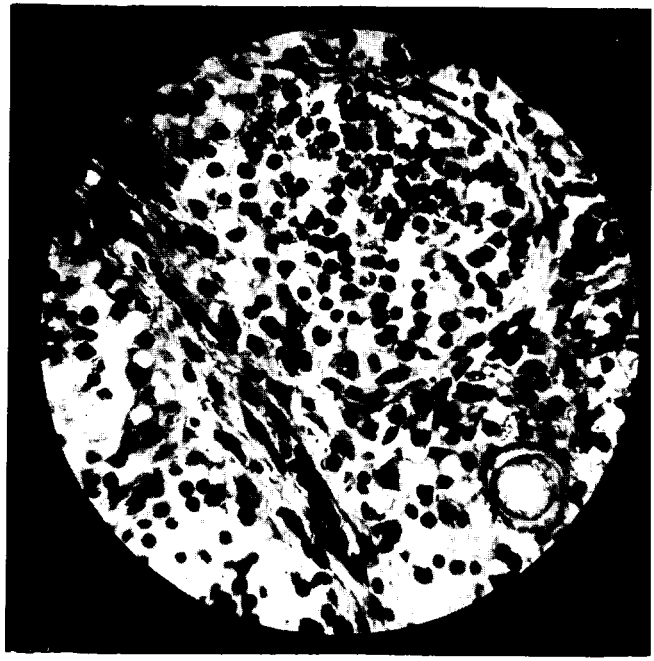


Fig. 5.



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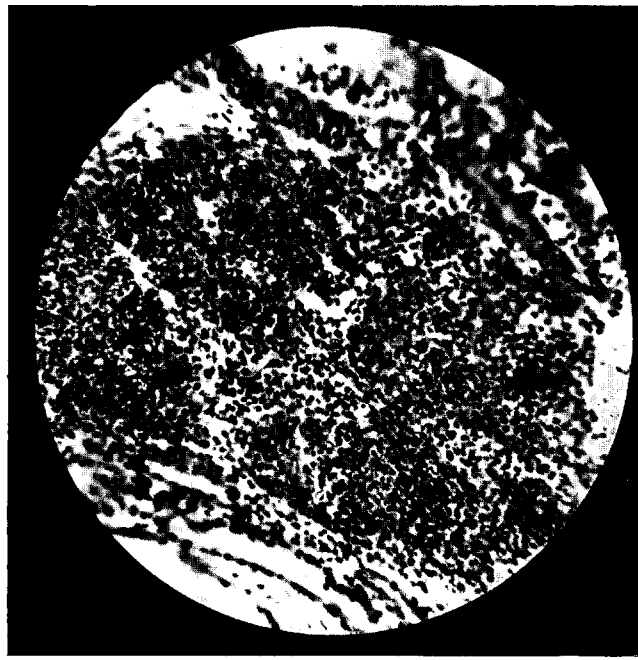

Fig. 7.

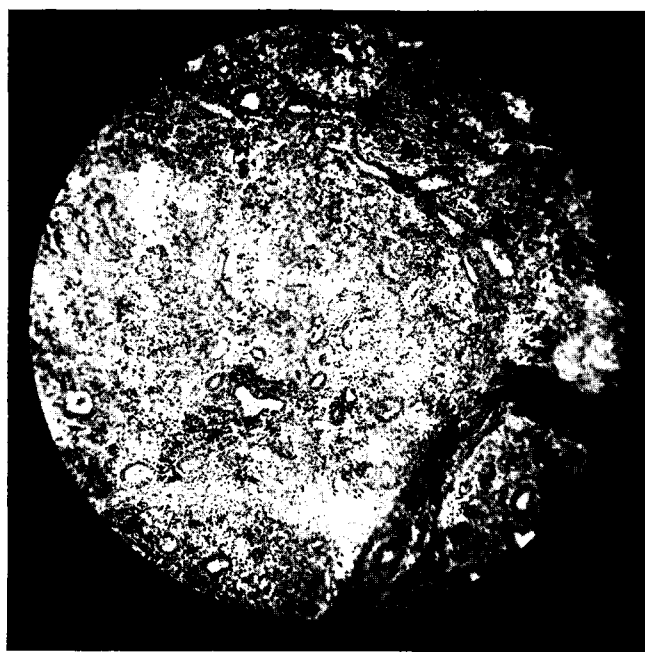

Fig. 8.

Fig. 7. Cow II. Udder, right front quarter, region 3: acute inflammatory foci with numerous purulent corpuscles. van Gieson. Magnification: 250 ×. Microphotogram.

Fig. 8. Cow II. Udder, right front quarter, region 2: necrosis of lobules caused by inflammation. van Gieson. Magnification: 60 ×. Microphotogram.

Fig. 9. Cow II. Udder, right rear quarter, region 1: necrosis of tissue caused by inflammation. van Gieson. Magnification: 60 ×. Microphotogram.

Fig. 10. Cow II. Udder, right front quarter, region 2: focus of the epithelioid cells with a giant-cell, and necrosis. Magnification: 420 ×. Photo from a drawing.

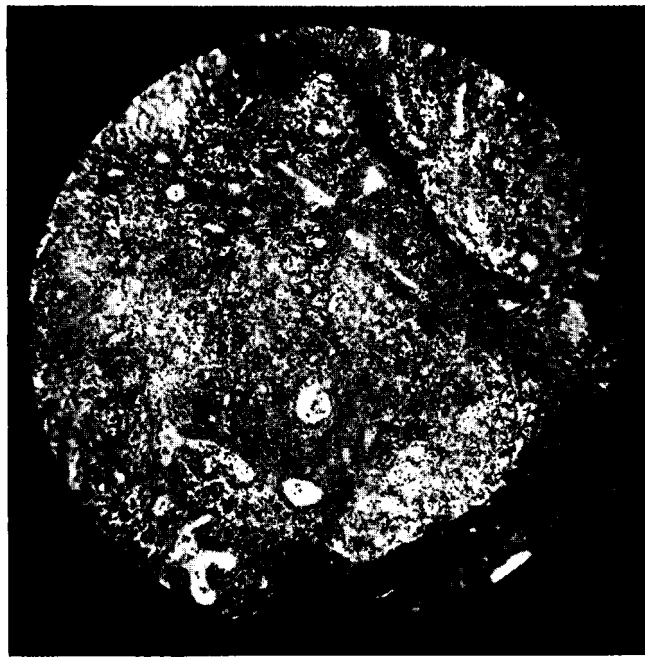

Fig. 9.

Fig. 10.

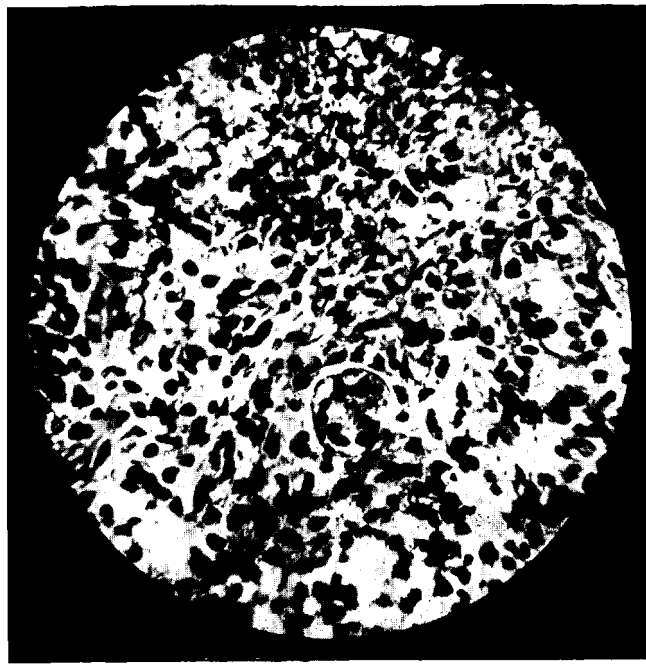

Fig. 11.

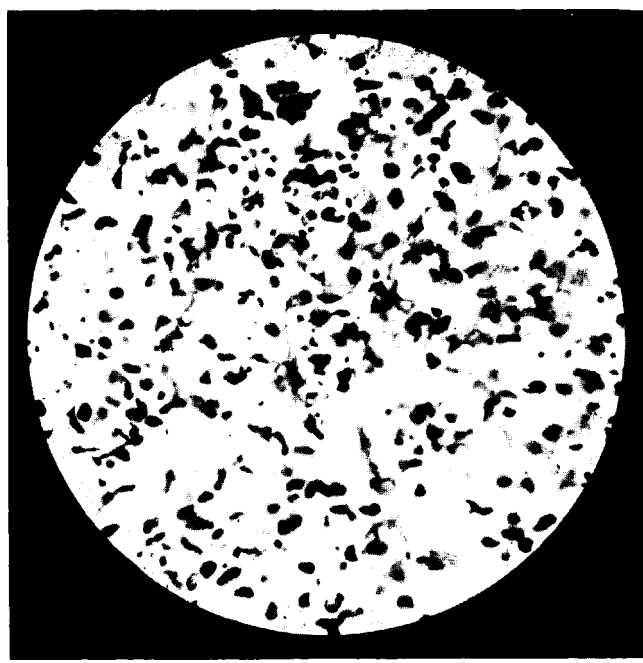

Fig. 12.

Fig. 11. Cow II. Udder, right rear quarter, region 1: focus of the epithelioid cells with a giant-cell. van Gieson. Magnificatcn: 500 ×. Microphotogram.

Fig. 12. Cow I. Udder, right rear quarter, region 6: necrosis. van Gieson. Magnification: 500 ×. Microphotogram.

Fig. 13. Cow II. Udder, right rear quarter, region 1: groups of epithelioid cells and necrosis. van Gieson. Magnification: 370 ×. Microphotogram.

Fig. 14. Cow II. Udder, right rear quarter, region 2: alveolus filled with desquamated epithelial cells and inflammatory elements. van Gieson. Magnification: 500 ×. Microphotogram.

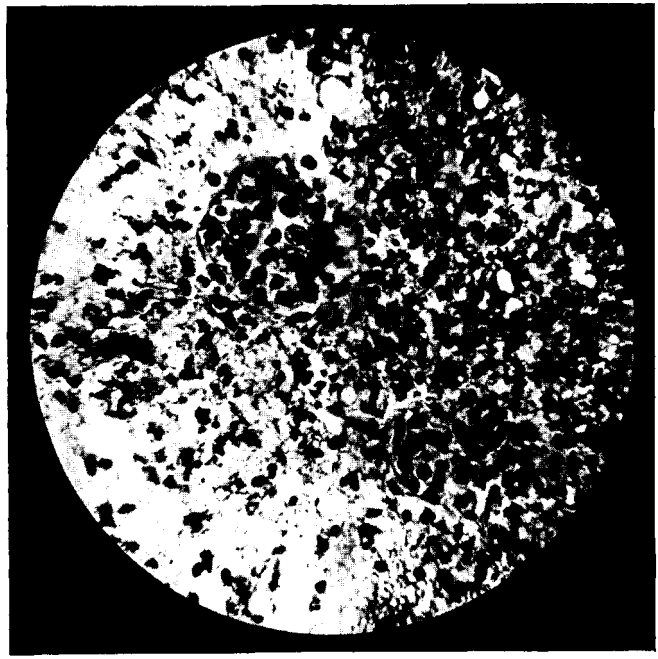

Fig. 13.

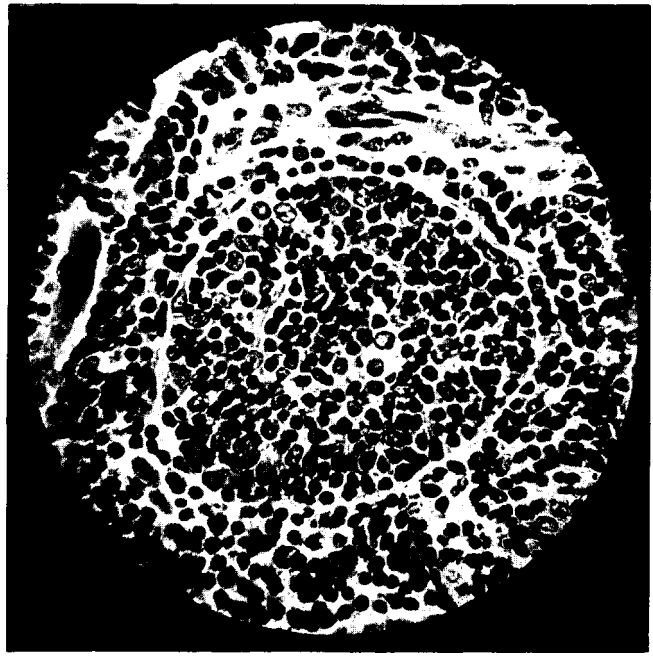

Fig. 14.

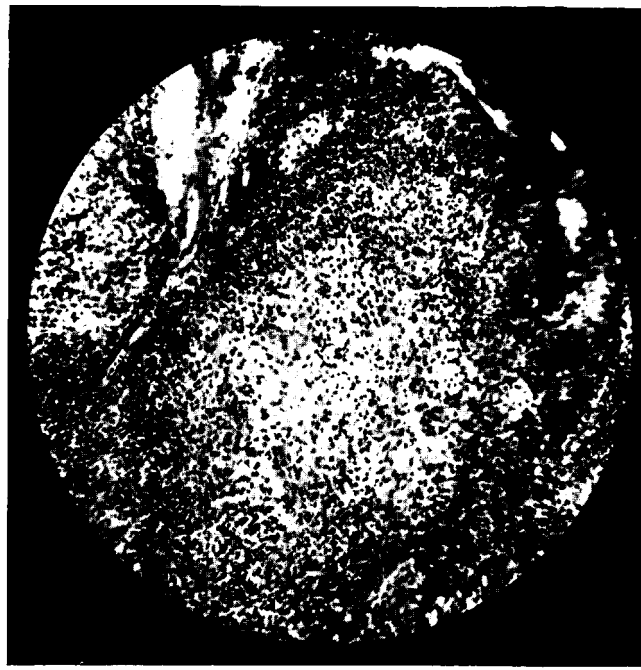

Fig. 15.

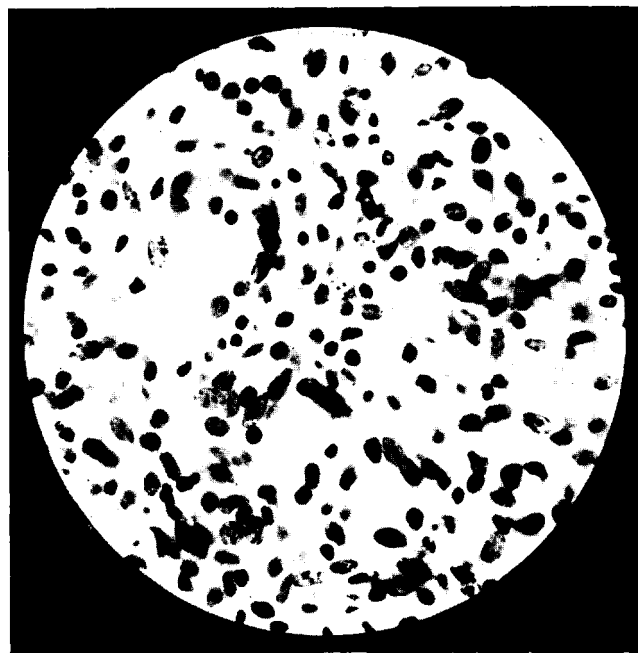

Fig. 16.

Fig. 15. Cow I. Udder, left rear quarter, region 4: focus of epithelioid cells. van Gieson. Magnification: 120 ×. Microphotogram.

Fig. 16. Cow. II. Udder, right front quarter, region 7: epithelioid cells. van Gieson. Magnification: 350 ×. Microphotogram.

Fig. 17. Cow I. Udder, right rear quarter, region 5: granulation and connective tissue with round-celled infiltration, in place of the alveoli. van Gieson. Magnification: 56 ×. Microphotogram.

Fig. 18. Cow II. Udder, right front quarter, region 7: connective and adipose tissue instead of the destroyed alveoli. van Gieson. Magnification: 56 ×. Microphotogram.

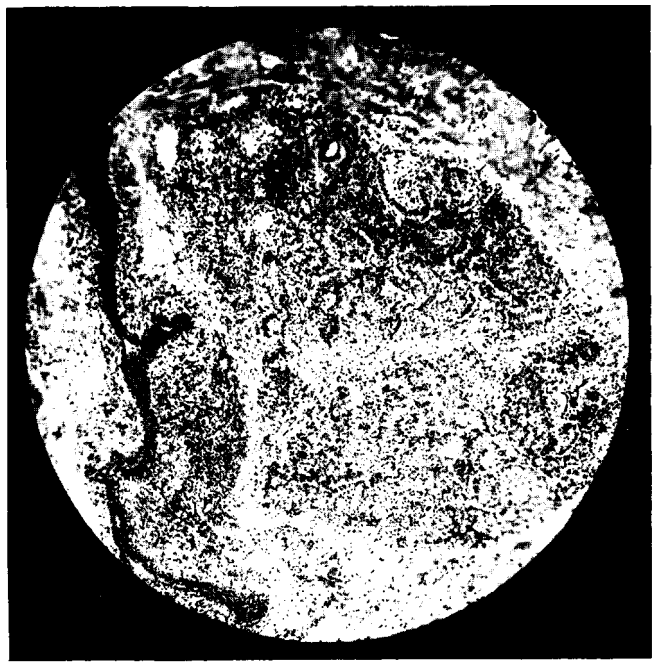

Fig. 17.

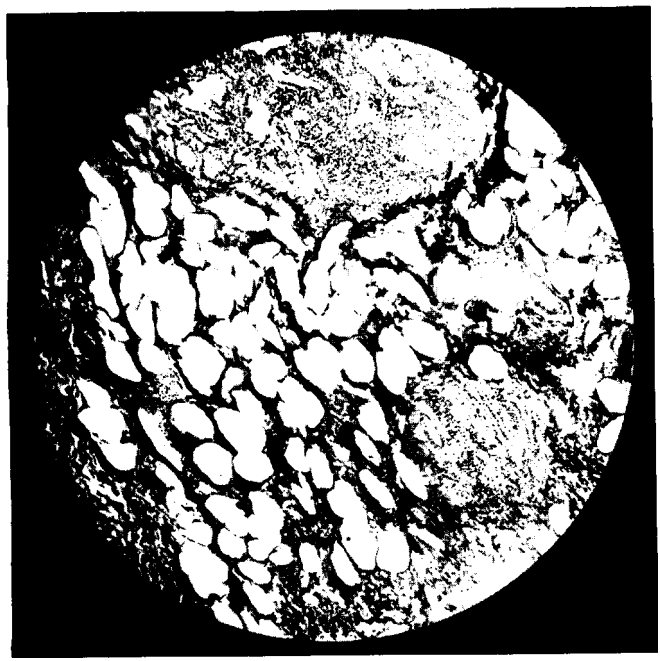

Fig. 18.

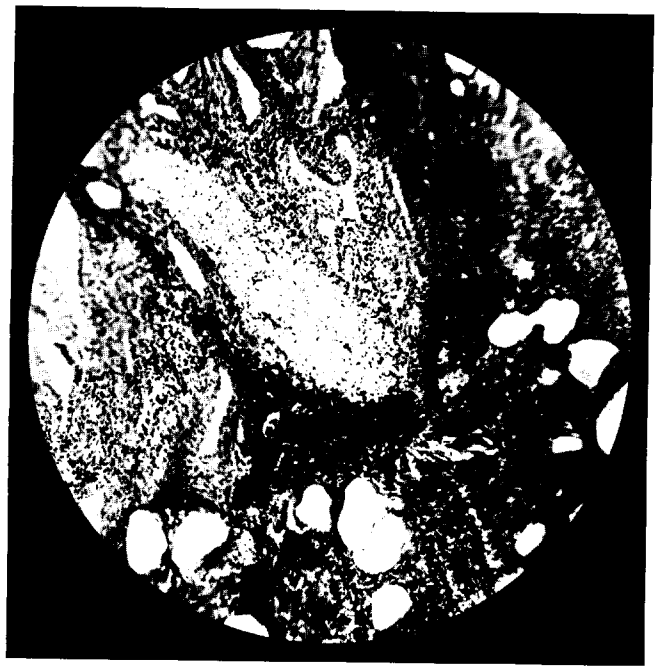

Fig. 19.

Fig. 20.

Fig. 19. Cow II. Udder, right front quarter, region 7: proliferation beginning in the fibrous connective tissue, in place of the destroyed alveoli. van Gieson. Magnification: 80 ×. Microphotogram.

Fig. 20. Cow II. Udder, left rear quarter, region 8: intensely proliferated fibrous connective tissue (with infiltration), in place of the destroyed alveoli. van Gieson. Magnification: 30 ×. Microphotogram.

Fig. 21. Cow II. Udder, right rear quarter, region 2: proliferation of the epithelium of the lactiferous duct and desquamation with obstruction of the alveolus, inflammatory infiltration in the epithelium and surroundings of the duct. van Gieson. Magnification: 80 ×. Microphotogram.

Fig. 22. Cow II. Udder, right rear quarter, region 2: granulation tissue in lactiferous ducts very intensely changed by inflammation. van Gieson. Magnification: 80 ×. Microphotogram.

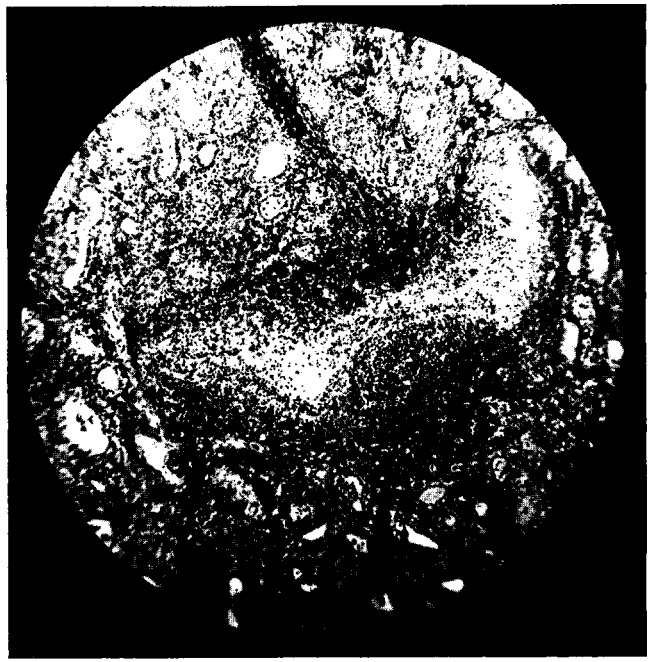

Fig. 21.

Fig. 22.

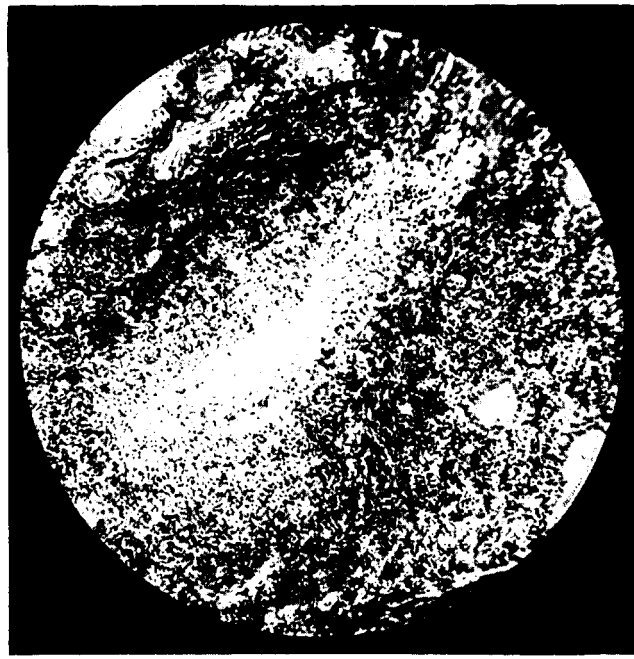

Fig. 23.

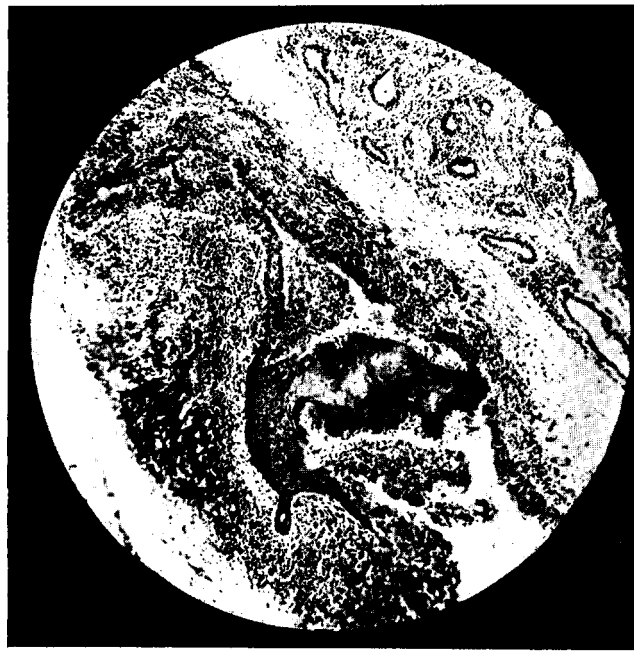

Fig. 24.

Fig. 23. Cow II. Udder, right rear quarter, region 2: destroyed lactiferous duct. van Gieson. Magnification: 120 ×. Microphotogram.

Fig. 24. Cow I. Udder, right rear quarter, region 2: flattening and partial cornification of the epithelium of the lactiferous duct. van Gieson. Magnification: 56 ×. Microphotogram.

Fig. 25. Cow I. Udder, left rear quarter, region 5: proliferation and flattening of the epithelium of the lactiferous duct, partial cornification, and obstruction of the lumen. van Gieson. Magnification: 350 ×. Microphotogram.

Fig. 26. Cow II. Udder, right rear quarter, region 2: proliferation and flattening of the epithelium of the lactiferous duct, partial cornification, and obstruction of the lumen. van Gieson. Magnification: 350 ×. Microphotogram.

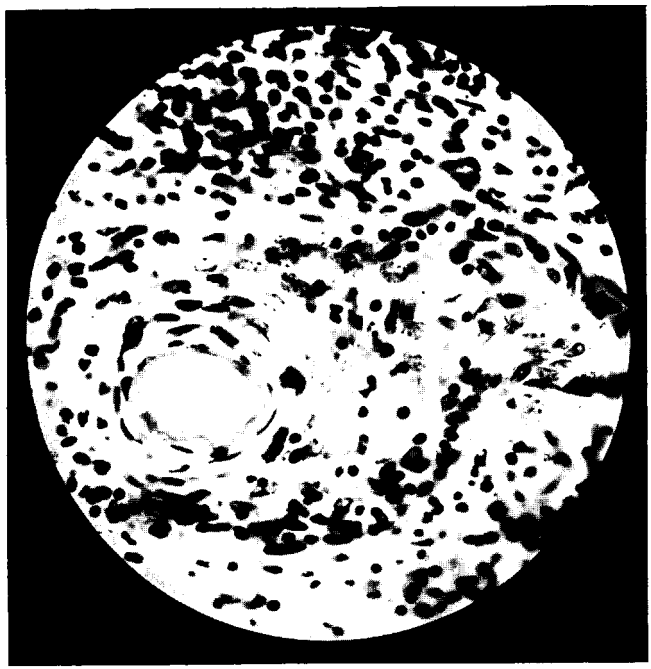

Fig. 25.

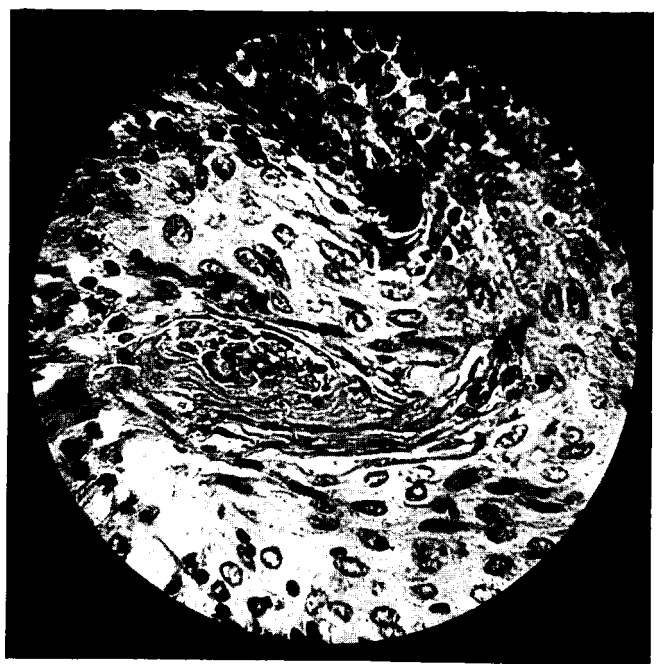

Fig. 26.

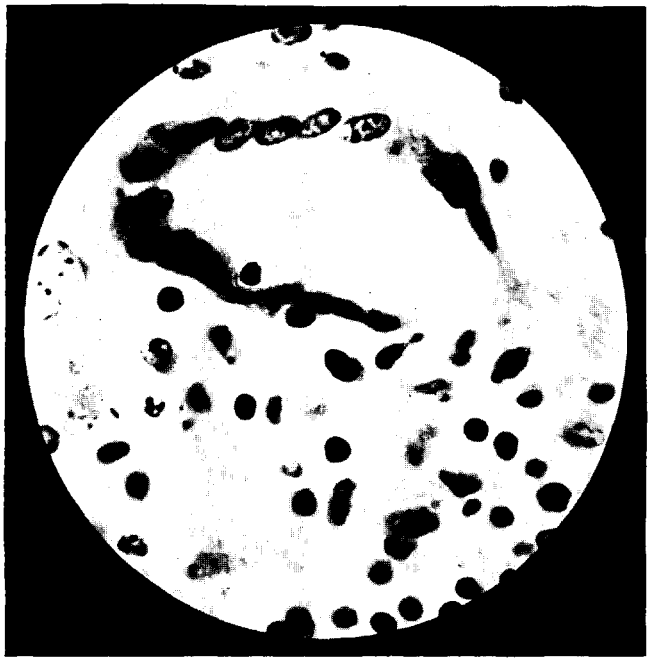

Fig. 27.

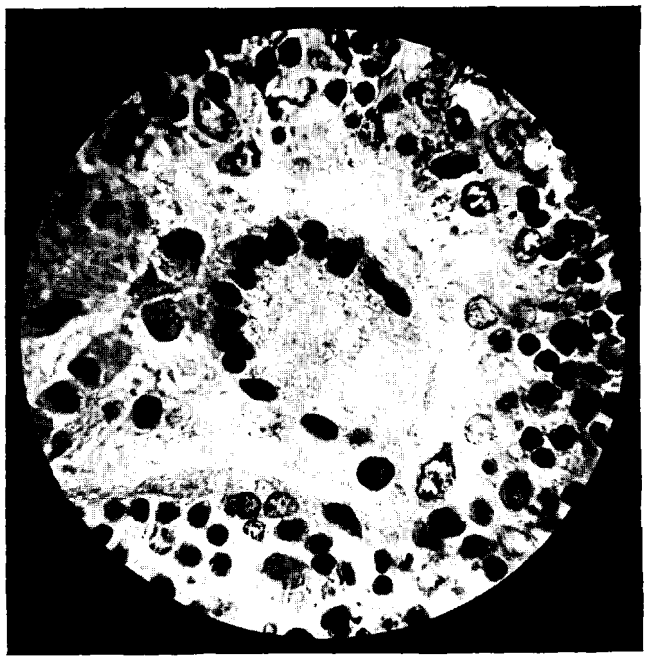

Fig. 28.

Fig. 27. Cow I. Udder, right rear quarter, region 5: focus of the epithelioid cells including a giant-cell in the very considerably changed lactiferous duct. van Gieson. Magnification: 900×. Microphotogram.

Fig. 28 Cow II. Udder, right rear quarter, region 2: epithelioid cells and one giant-cell in the epithelium of the destroyed lactiferous duct. van Gieson. Magnification: 900×. Microphotogram.

Fig. 29. Cow II. Udder, right rear quarter, region 2: numerous plasma cells in the epithelium of the destroyed lactiferous duct. van Gieson. Magnification: 900 ×. Microphotogram.

Fig. 30. Cow III. Udder, right rear quarter, region 7: obstruction of the lactiferous duct, and in the centre a cornified mass with a deposit of lime. Flattened epithelium round the cornified mass. van Gieson. Magnification: 60 ×. Microphotogram.

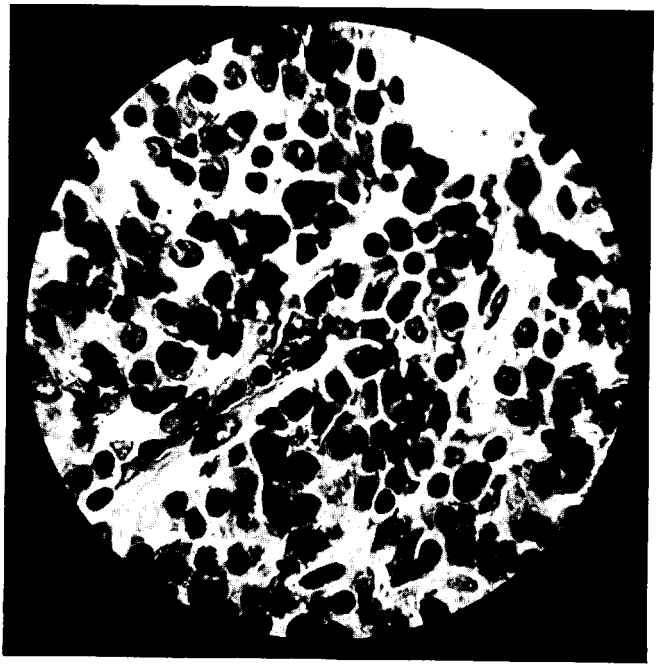

Fig. 29.

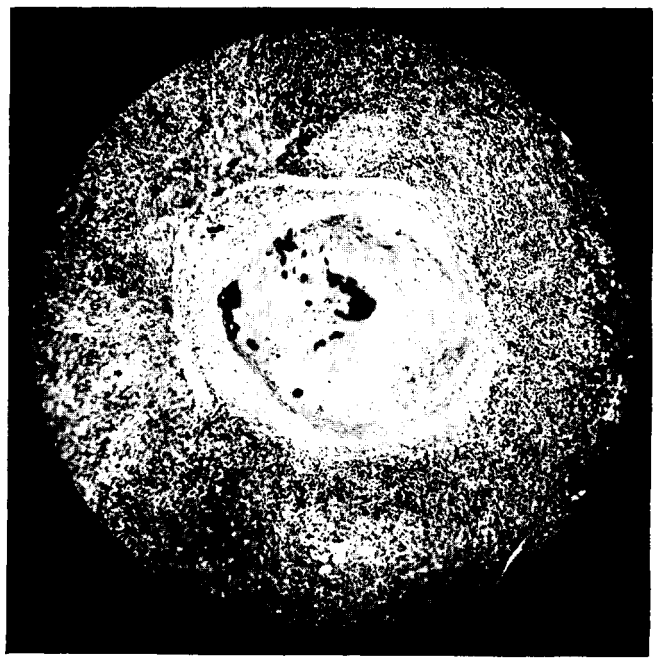

Fig. 30.

Fig. 31.

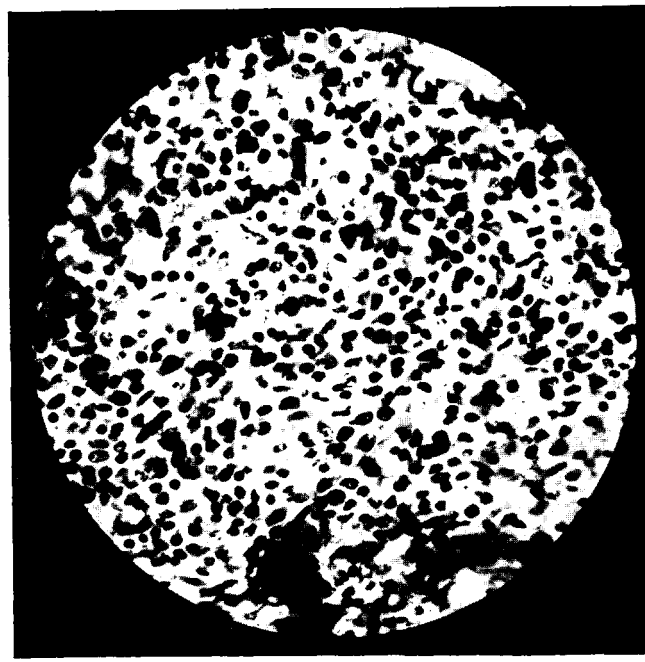

Fig. 32.

Fig. 31. Cow III. Udder, a part of the cystiform formation. van Gieson Magnification : 60 ×. Microphotogram.

Fig. 32. Cow II. Udder, right front quarter, region 7 : dense inflammatory infiltration in place of the destroyed alveoli, and intense proliferation of the connective tissue. van Gieson. Magnification : 350 ×. Microphotogram.

Fig. 33. Cow II. Udder, right rear quarter, region 2, numerous plasma cells in the interlobular interstitial connective tissue, changed by inflammation. van Gieson. Magnification: 500 ×. Microphotogram.

Fig. 34. Cow I. Udder, right front quarter, region 1: mast cell in the dilated interalveolar tissue. van Gieson. Magnification: 900 ×. Microphotogram.

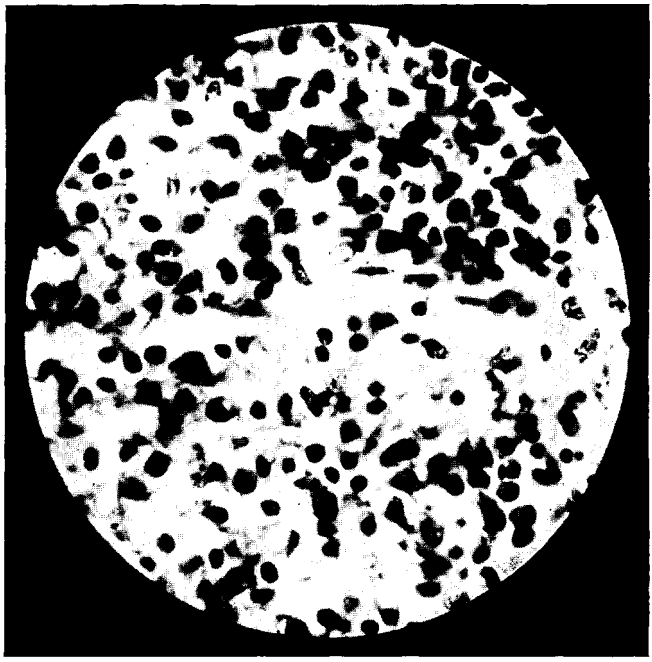

Fig. 33.

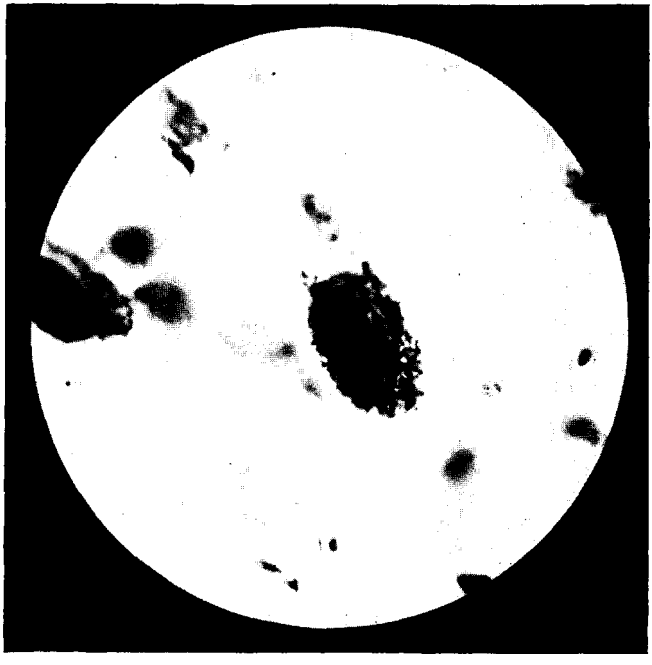

Fig. 34.

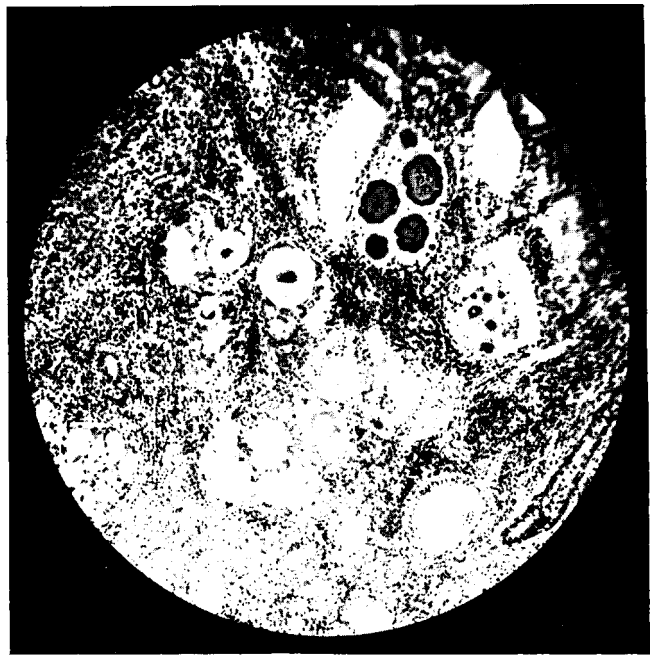

Fig. 35.

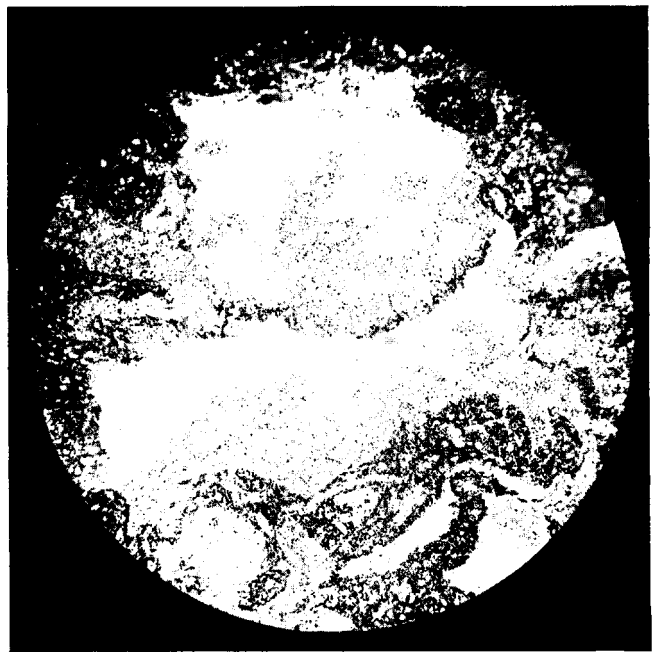

Fig. 36.

Fig. 35. Cow V. Udder, right front quarter, region 8: numerous amyloid corpuscles in the intensely changed lumina of the alveoli, and a dense interalveolar infiltration. Haematoxylin-eosin. Magnification: 60 ×. Microphotogram.

Fig. 36. Cow VI. Left deep inguinal lymph node: considerable dilatation of the lymph sinus containing masses of decaying cells and portions of decayed cells. van Gieson. Magnification: 60 ×. Microphotogram.

Fig. 37. Cow I. Right supramammary inguinal lymph node: focus of epithelioid cells with one giant-cell. van Gieson. Magnification: 900 ×. Microphotogram.

Fig. 38. Cow I. Right supramammary inguinal lymph node: connective tissue with infiltration of focal plasma cells and lymphocytes instead of the reticular tissue. Magnification: 60 ×. Microphotogram.

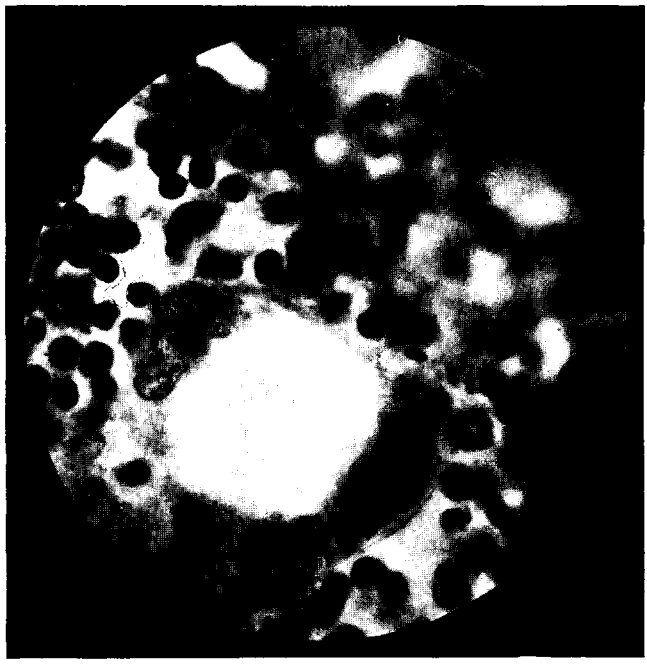

Fig. 37.

Fig. 38.

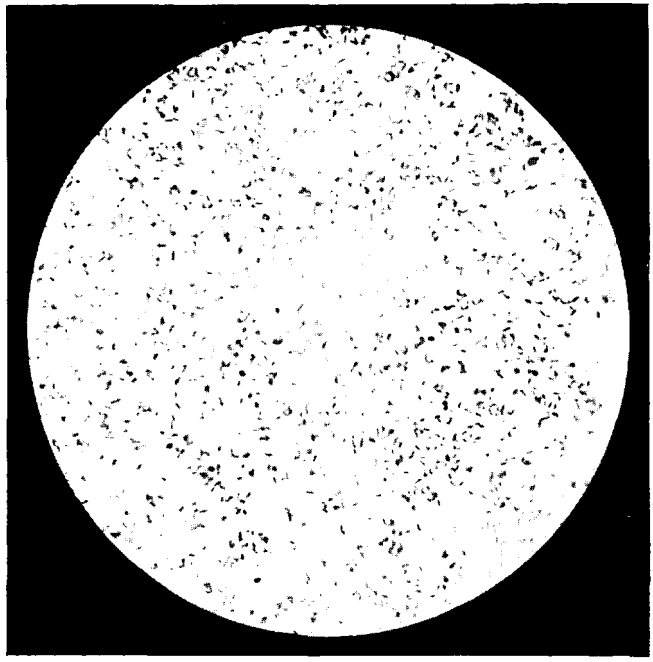

Fig. 39.

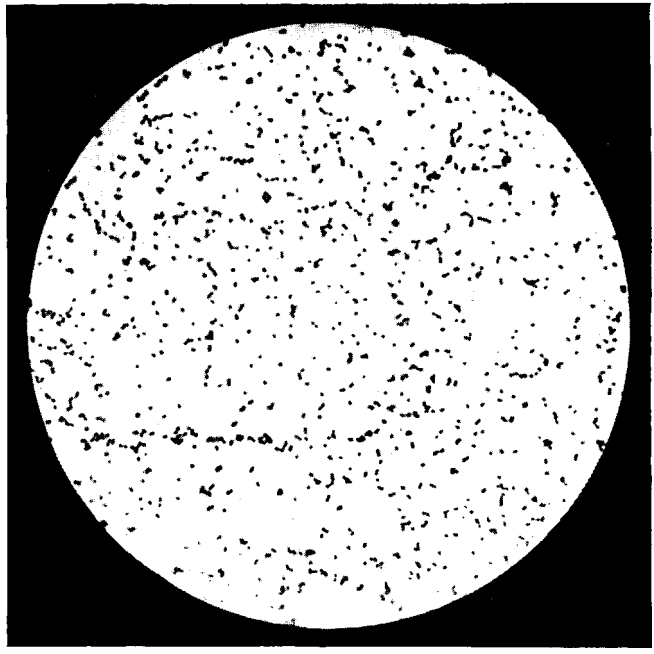

Fig. 40.

Fig. 39. Smear preparation from a 24 hours' old culture of *Br. abortus*. Carbolfuchsin. Magnification: 1100 ×. Microphotogram.

Fig. 40. Smear preparation from a 14 days' old culture of *Br. abortus*. Carbolfuchsin. Magnification: 1100 ×. Microphotogram.

Fig. 41. Histological section of a lymph node, showing a 14 days' old culture of *Br. abortus* injected into the lymph node. Giemsa method as modified by me. Magnification: 1100 ×. Microphotogram.

Fig. 42. *Br. abortus* in a histological section of the spleen of a brucellous guinea-pig. Giemsa method as modified by me. Magnification: 1100 ×. Microphotogram.

Fig. 41.

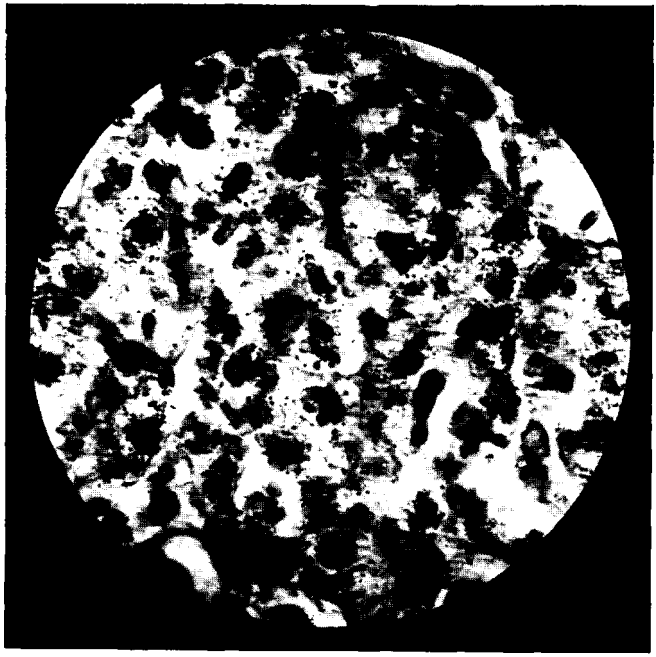

Fig. 42.

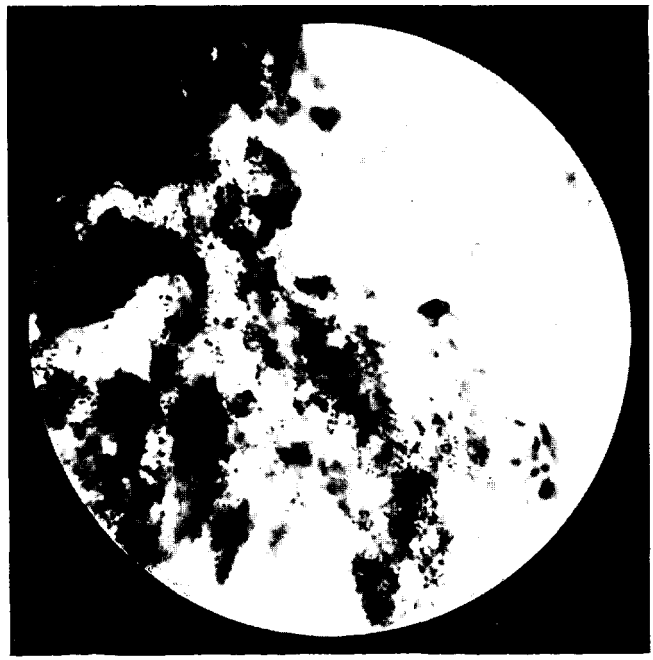

Fig. 43.

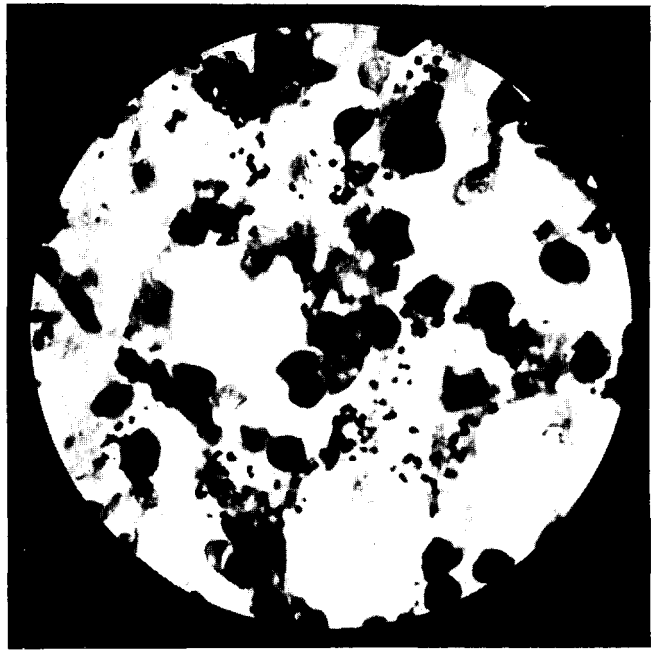

Fig. 44.

Fig. 43. *Br. abortus* in a histological section of fetal membranes of a brucellous cow. Giemsa method as modified by me. Magnification: 1100×. Microphotogram.

Fig. 44. Cow IV. Right supramammary lymph node: pieces of nuclei and granules of pigment between cells. Giemsa method as modified by me. Magnification: 1100×. Microphotogram.

Fig. 45. Cow IV. Udder, right rear quarter, region 3: *Br. abortus* in an intensely changed inflammatory focus, round the capillary, in endothelial cells and in the lumen of the capillary. Giemsa method as modified by me. Magnification: 1100 ×. Microphotogram.

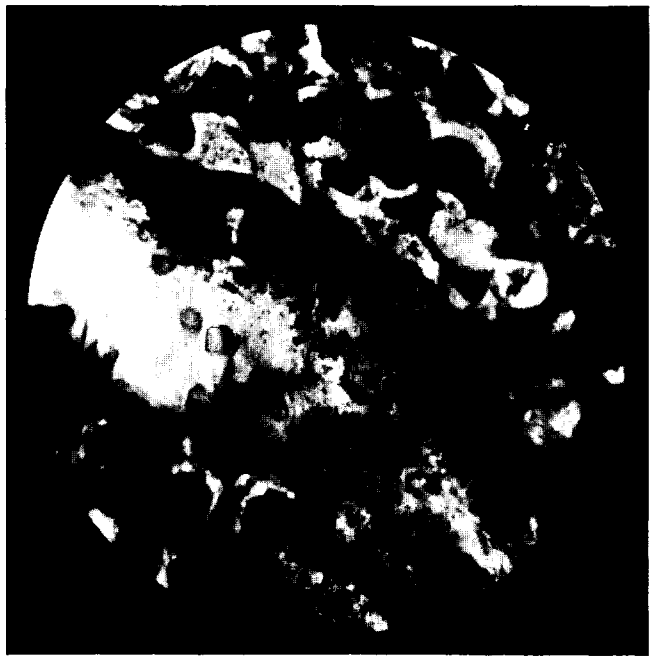

Fig. 45.

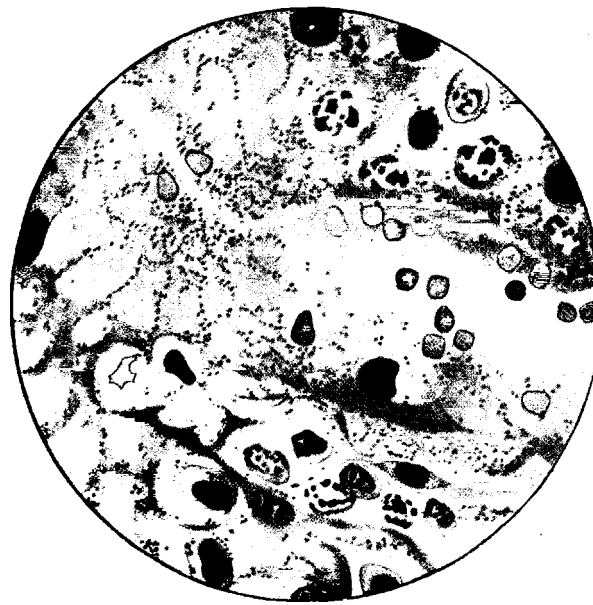

Fig. 45-a.

Fig. 45-a — like fig. 45. Drawing.

Fig. 46. Cow IV. Udder, right rear quarter, region 3: *Br. abortus* in a histological section of an intensely changed parenchyma, singly between cells and in cells. Giemsa method as modified by me. Magnification: 1100 ×. Microphotogram.

Fig. 47. Cow. I. Udder, left rear quarter, region 4: *Br. abortus* in the focus of epithelioid cells, between cells and in cells. Giemsa method as modified by me. Magnification: 1100 ×. Microphotogram.

Fig. 46.

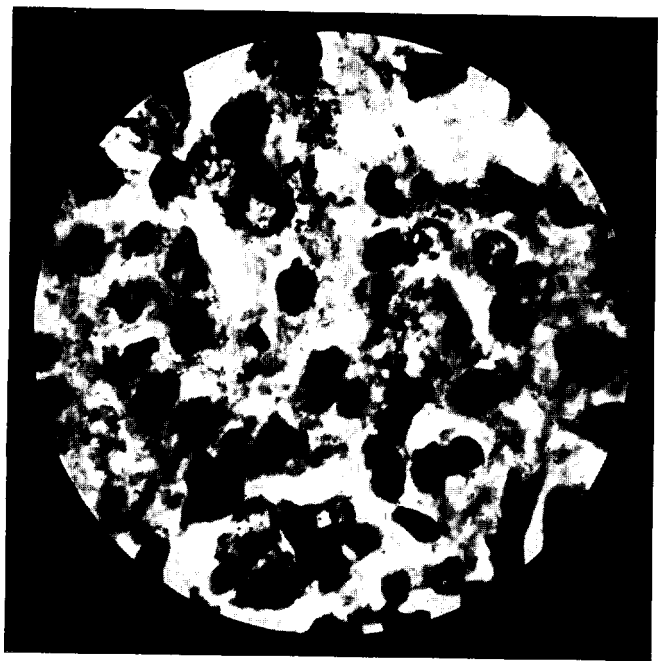

Fig. 47.

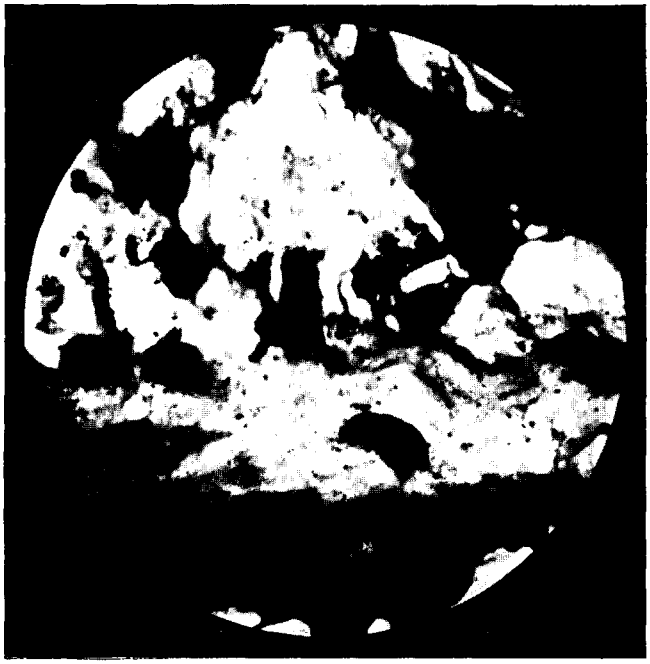

Fig. 48.

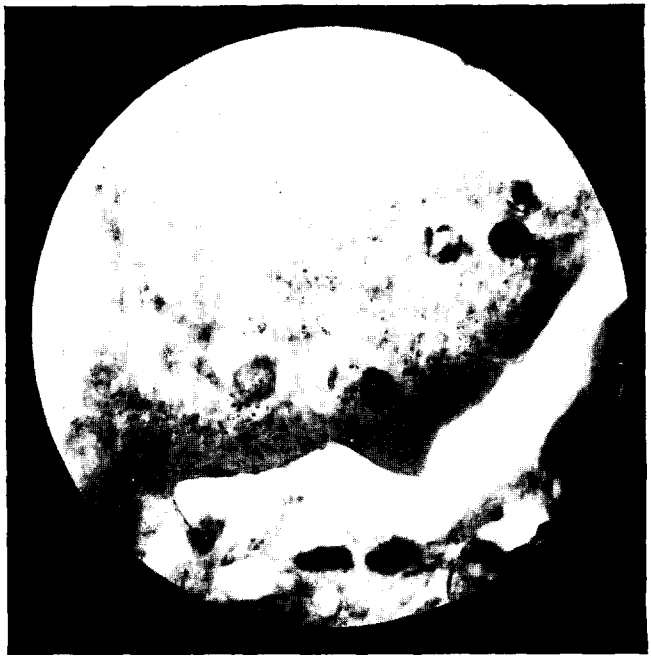

Fig. 49.

Fig. 48. Cow II. Right supramammary lymph node: *Br. abortus* in an inflammatory focus, between cells and in cells. Carbolfuchsin. Magnification: 1100 ×. Microphotogram.

Fig. 49. Cow IV. Left supramammary lymph node: *Br. abortus* in the lymph sinus. Giemsa method as modified by me. Magnification: 1100 ×. Microphotogram.