

FURTHER STUDIES ON BACTERIUM ABORTUS AND RELATED BACTERIA

I. THE PATHOGENICITY OF BACTERIUM LIPOLYTICUS FOR GUINEA-PIGS

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The organism causing infectious abortion has been known as *Bacillus abortus*. But in accordance with the recommendation of the Committee of the Society of American Bacteriologists on Characterization and Classification of Bacterial Types,¹ the family Bacillaceae, including the genus *Bacillus*, is characterized by the production of endospores. Such a classification would exclude the abortion organism from that genus. The committee suggests that it be included in the genus *Bacterium*. Accordingly the name of the abortion organism becomes *Bact. abortus*. The generic name *Bacillus* has hitherto also been applied to two other organisms discussed in this series of papers which are similar in morphology to *Bact. abortus*. To be consistent in nomenclature therefore, the two organisms are called, respectively, *Bact. bronchisepticus* and *Bact. melitensis*.

In earlier publications^{2,3} it has been shown that there is commonly present in large numbers in milk, a variety of bacterium which agrees with Bang's⁴ description of *Bact. abortus*, but which differs markedly from the virulent strains of *Bact. abortus* which were available for comparison. The common milk variety was designated *Bact. abortus* var. *lipolyticus*⁵ because of its ability to break down the butterfat to fatty acids. The question arose as to whether it might possess disease-producing properties. Accordingly, a series of inoculation and feeding experiments with guinea-pigs was conducted to determine the point in question.

Inoculation Experiments.—The inoculation of the animals and postmortem examinations were made by Dr. G. M. Potter, formerly of the Pathologic Division of this Bureau. Thirteen guinea-pigs were inoculated with 2 c c of broth culture of *Bact. lipolyticus*. Three were inoculated with milk known to be naturally infected with the organism. They received 2½, 5, and 7½ c c, respectively. All inoculations were made intra-abdominally. Altogether, 16 guinea-pigs were inoculated.

After the death of the animals, cultures were made from the lungs, heart blood, kidneys, liver, and spleen, and in some cases from other organs, in plain-infusion agar and in agar to which 10% of sterile, raw cow's-blood serum had been added. Slope, shake, and plate cultures were made.

The results of the experiments were complicated by a contagious infection with a small, rod-shaped organism, similar in morphology to the abortion

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¹ Jour. Bacteriol., 1917, 2, p. 546.

² Evans: Jour. Infect. Dis., 1916, 18, p. 437.

³ Evans: Jour. Bacteriol., 1917, 2, p. 185.

⁴ Ztschr. f. Tier., 1897, 1, p. 241.

⁵ The name *Bact. abortus* var. *lipolyticus* is unwieldy. Although the organism is related to *Bact. abortus*, it is no more closely related to that organism than are several other types of bacteria which bear distinct species names. Therefore the fat-splitting bacterium will be called *Bact. lipolyticus* in this series of papers.

organism, but readily distinguishable by its power to produce gas in the agar shake cultures. Two of the inoculated animals died as a result of infection with the gas-producing organism. Of the remaining 14 animals, 7 died between the 49th and 67th day after inoculation, and from 3 of those animals which died, there was isolated a small, rod-shaped organism which resembled pathogenic strains of *Bact. abortus*. It was isolated from the lungs of one animal that died of pneumonia, 67 days after inoculation; from the heart blood of one, and from the lungs of another, both of which died 52 days after inoculation, with no gross lesions showing at postmortem.

Feeding Experiments.—Since the udder of the cow is the natural habitat of *Bact. lipolyticus*, the possibility suggested itself of infection by ingestion of large numbers of the organisms with the milk. Therefore a series of feeding experiments with guinea-pigs was planned. At the beginning of the feeding the weights of the animals ranged from 200-375 gm. The guinea-pigs soon learned to like the milk, which was poured over their oats, and they took considerable quantities of it. The milk was from a cow which was known to have eliminated large numbers of the organism for 2 years preceding the first feeding experiment. During the period of the experiment the numbers varied from a few thousand per cubic centimeter on some days to 70,000 on the day the largest count was made. Milk from the same cow was pasteurized for feeding control animals. At the time the first group was fed with the naturally contaminated milk, another group of guinea-pigs was fed with a pure culture isolated from the raw milk. The organisms were grown in mass on serum agar in plate cultures. A heavy growth was obtained by flooding the surface of the agar with a whole-milk culture and then incubating for 2 days. A water suspension containing the growth from 2 or 3 plates was fed with the oats every day to each guinea-pig.

In the first feeding experiment there were 3 groups of guinea-pigs, with 4 in each group. One group was given pasteurized milk, the second raw milk, and the third the pure culture. After 70 days of feeding, the experiment was discontinued, for at that time, of the groups which had been receiving raw milk or pure culture, there was only one animal living, whereas three of those fed with pasteurized milk were in good condition, one having died as a result of infection with the gas producer. No definite conclusions could be drawn from this first feeding experiment, for no results were obtained by incubating cultures from the organs. The circumstances indicated, however, that the ingested bacteria may have caused the death of the animals.

In the second feeding experiment there were 3 groups of guinea-pigs, with 10 in each group. One group was fed with pasteurized milk, the second was fed with raw milk, and the third group was fed with inoculated milk. The pasteurized and the raw milk was from the same source, and treated in the same manner as in the first experiment. The inoculated milk was sterilized milk which had been inoculated with a pure culture isolated from the raw milk. The milk was incubated until it contained about the same number of bacteria as the raw milk.

Of the 10 animals fed with pasteurized milk, 6 died as a result of infection with the gas produced before the experiment was discontinued, after 99 days feeding. The remaining 4 animals were then killed, and postmortem examinations were made. No gross lesions were found, and cultures from the organs showed no growth, or a few scattered colonies of contaminating bacteria.

Of the 10 guinea-pigs fed with raw milk, one died as a result of infection with the gas producer; one died as a result of a ruptured stomach; one died on the 39th day with no gross lesions, and with no growth developing in cultures made from the organs; one died of pneumonia on the 62nd day, and a small rod-shaped organism resembling *Bact. abortus* was isolated from the lungs. This was the

same organism which had been isolated from the organs of 3 of the inoculated guinea-pigs. It was isolated also from the lungs of one, and from the lungs and spleen of another of the guinea-pigs fed with raw milk which were killed on the 99th day, with no gross lesions appearing in postmortem examination. No results were obtained from the remaining 4 animals of this group, 3 of which were killed on the 79th, and 1 on the 99th day.

Of the 10 guinea-pigs fed with inoculated milk, 4 died as a result of infection with the gas producer; 1 died of pneumonia on the 94th day, but no growth could be obtained in cultures inoculated with the lungs and other organs. The remaining 5 animals were killed, 2 on the 79th, and 3 on the 99th days. The organs of all 5 appeared normal, and no growth could be obtained in cultures inoculated with them.

DISCUSSION

Organisms resembling *Bact. abortus* were isolated from the organs of 3 of the 16 guinea-pigs that were inoculated with *Bact. lipolyticus*, either in pure culture or in naturally contaminated milk. These 3 animals died between the 52nd and 67th days after inoculation. Four more of the 16 inoculated guinea-pigs died between the 49th and 67th days, but no cultures were obtained from the organs. The time of death of the animals agreed with the time required for *Bact. abortus* to develop lesions in guinea-pigs, as reported by Fabyan.⁶ This investigator states that the lesions in guinea-pigs inoculated with *Bact. abortus* usually appear between the 3rd and 6th week, the acute changes extending over a period of 10-20 weeks, after which reparative processes appear.

Twenty-eight guinea-pigs were fed with *Bact. lipolyticus* in naturally contaminated milk, or in inoculated sterile milk, or in mass of pure culture. The organism resembling *Bact. abortus* which was isolated from the organs of 3 of the inoculated animals was isolated also from the organs of 3 of the animals which had been fed, from the lungs of 1 which died of pneumonia on the 62nd day and from the apparently normal organs of 2 guinea-pigs which were killed on the 99th day.

These results were misleading, for it is now known that the abortion-like organism isolated from 3 of the inoculated animals and from 3 of the fed animals was another contagious infection which complicated the experiments. It was apparently a mere matter of circumstance that the infection prevailed in the cages with inoculated and fed animals, whereas the cages with the control animals escaped the infection.

The similarity of the infectious organism, which has been identified as *Bact. bronchisepticus*, with pathogenic strains of *Bact. abortus* will

⁶ Jour. Med. Research, 1912, 26, p. 441.

be demonstrated in the second paper of this series. They resembled one another so closely that it was hypothetically assumed that the lipolyticus variety of *Bact. abortus* had acquired the characteristics of pathogenic strains during the several weeks or months of life in the guinea-pig's bodies. This led to an erroneous conviction when cultures of the lipolyticus variety and of the recovered abortion-like bacteria were submitted to another investigator for serologic tests, and it was reported that the blood of a rabbit immunized with a strain of the lipolyticus variety strongly agglutinated the strains obtained from the guinea-pig organs. Subsequently, it was found that these results were due to an error in the reading of labels, and that the rabbit had not been immunized with the lipolyticus strain but with *Bact. bronchisepticus*.

If those animals are eliminated which died of one or the other of the contagious infections, including with those from whose organs the causal organism was isolated, 2 which died at about the same time but from whose organs no cultures were obtained, there remain 7 guinea-pigs which were inoculated with pure culture of *Bact. lipolyticus*. Four of these were killed; 2 on the 48th day, 1 on the 67th and 1 on the 70th day after inoculation. The appearance of all organs was normal, and no growth could be obtained in cultures heavily inoculated with the organs. The remaining 3 animals showed no signs of any ill effects resulting from the inoculations through several months of observation.

Eliminating in the same manner from the 28 guinea-pigs which were fed with *Bact. lipolyticus* those animals which died with one or the other of the contagious infections as the proved or suspected causal agent, there remain to be considered 12 guinea-pigs. Seven of the twelve were fed with raw milk. Three of these were killed on the 79th day, and 3 were killed on the 99th day. The organs appeared normal at post mortem, and the ingested organism could not be recovered. The other guinea-pig fed with raw milk was not killed. It never showed any ill effects resulting from the feeding. Five of the 12 guinea-pigs were fed with inoculated milk. Two were killed on the 79th, and 3 on the 99th days. The organs of these animals also appeared normal, and the ingested organism could not be recovered.

Although these experiments do not demonstrate the harmlessness of *Bact. lipolyticus* as clearly as could be desired, due to the complications with the two contagious infections, no evidence was found to show that it is pathogenic for guinea-pigs.