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A. D. MELVIN, CHIEF OF BUREAU.

TESTS CONCERNING TUBERCLE BACILLI
IN THE CIRCULATING BLOOD.

BY

E. C. SCHROEDER, M. D. V.,
Superintendent of Experiment Station,
AND
W. E. COTTON,
Expert Assistant at Experiment Station.

WASHINGTON:
GOVERNMENT PRINTING OFFICE.
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U. S. Department of Agriculture,  
Bureau of Animal Industry,  

Sir: I have the honor to transmit herewith, and to recommend for publication in the bulletin series of this Bureau, the accompanying manuscript, entitled "Tests Concerning Tubercle Bacilli in the Circulating Blood," by Dr. E. C. Schroeder and Mr. W. E. Cotton, of the Experiment Station of this Bureau.

The tests described in the bulletin were undertaken upon the appearance of a recent paper in which were recorded a large number of microscopic examinations of the blood of tuberculous individuals, the result of which, it was stated, proved that tuberculosis in all its forms was a bacteriemia.

This conclusion was entirely contrary to the views of the authors of this bulletin, based upon their work in the Bureau for a long series of years; and as the matter was of great importance in its bearing on the tuberculosis question, it was considered advisable to put it to a thorough test. Accordingly inoculation experiments were made with the blood of a large number of tuberculous cattle, and the results have demonstrated that tuberculosis is not in any sense a bacteriemia, and that if tubercle bacilli ever float in the blood of tuberculous animals this is an exceedingly rare condition.

Respectfully,

A. D. Melvin,  
Chief of Bureau.

Hon. James Wilson,  
Secretary of Agriculture.
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TESTS CONCERNING TUBERCLE BACILLI IN THE CIRCULATING BLOOD.

INTRODUCTION.

In a paper dealing with the occurrence of tubercle bacilli in the circulating blood, read before a medical society some months since and soon afterwards published in a medical journal, there were recorded the results of microscopic examinations of the blood of 125 tuberculous individuals, some of whom were affected with only incipient tuberculosis, and the statement was made that tubercle bacilli were found in the blood of every one of them. In some cases only a few bacilli were seen, but "they were mostly in large numbers, and clumps of 30 to 40 bacilli were not unusual, especially in cases of acute miliary tuberculosis." From his observations the author of that paper formulated the conclusion: "It appears that tuberculosis in all its forms is a bacteriemia."

That tubercle bacilli occasionally float in the blood stream is hardly open to question, because of the occurrence of isolated lesions in the bodies of otherwise tuberculous as well as otherwise healthy individuals located in regions remote from the various channels that communicate with the exterior. The same is true when we consider cases of more or less generalized tuberculosis with many lesions in widely separated portions of the body and cases of miliary tuberculosis with innumerable lesions of approximately, if not precisely, the same age and stage of development. But such occasional presence of tubercle bacilli in the circulating blood is a very different condition from their constant occurrence in it in sufficient numbers to justify the classification of tuberculosis as a bacteriemia. Hence, Rosenberger's conclusion was received with considerable surprise and doubt.

Although the conclusion seemed sufficiently incredible because of the simple fact that a constant occurrence of tubercle bacilli in the blood of all tuberculous individuals could hardly have been overlooked by the host of investigators who have studied tuberculosis

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with no greatly different technique than Rosenberger used, we did not feel warranted in opposing it without offering some specific evidence. Because of the important bearing of the matter on the tuberculosis problem, the experiments hereinafter reported were carried out.

Rosenberger stated that he found tubercle bacilli on microscopic examination in the blood of every one of the 125 cases of tuberculosis he studied, notwithstanding that some of the cases were incipient and failed to show tubercle bacilli in the sputum. It was, therefore, almost taken for granted that the microscopic examination of blood, according to his method, of animals affected with advanced and long standing tuberculosis and animals that were expelling tubercle bacilli from their bodies in large numbers would reveal at least a few tubercle bacilli. A considerable number of such microscopic examinations were made, but not a tubercle bacillus was found in our blood preparations, and hence we have to record wholly negative results with the blood of tuberculous animals. Similar negative results were obtained with the blood of tuberculous persons in two large New York hospitals.  

It is not uncommon for virulent tubercle bacilli to be present in animal substances in numbers too small to serve for their detection by optical methods. For example, at the Experiment Station we found the intraabdominal injection of guinea pigs with suspected milk to be a test for tubercle bacilli that has fully fifty times the delicacy of a microscopic examination. Furthermore, tinctoral and optical methods of distinguishing between tubercle bacilli and other acid-fast bacteria are not wholly satisfactory, hence we concluded to inject a sufficient number of guinea pigs with blood from a sufficient number of certainly tuberculous cattle to show conclusively that tubercle bacilli either are or are not commonly present in such blood.  

Incidently it appears that Doctor Rosenberger failed to confirm adequately by animal experiments his surprising microscopic observations, which, if correct, would have been of the greatest value alone for the early and certain diagnosis of tuberculosis. In all he inoculated only two guinea pigs, of which he gives a record: one with blood from a tuberculous person who was expelling tubercle bacilli per rectum, and one with blood from a case of acute miliary tuberculosis. The development of tuberculosis in the latter guinea pig can not be regarded as a remarkable phenomenon. There is nothing about the fact that a guinea pig contracted tuberculosis after an injection of blood obtained from a case of acute miliary tuberculosis that necessitates a modification of our currently accepted views on the presence of tubercle bacilli in the circulating blood. That is to say, we need not look upon tuberculosis as a bacteriemia because tubercle  

bacilli were demonstrated in blood of a kind in which we have long considered that they might sometimes occur.

This leaves one guinea pig that may have some evidential value, but we must not lose sight of the fact that it was injected with blood obtained from a person who was expelling tubercle bacilli from his body and hence to some extent infecting his environment. We must also bear in mind that guinea pigs are highly susceptible to tubercle bacilli injected into their bodies, and that it is often impossible for an investigator who handles much tuberculous material, who is in frequent contact with tuberculous persons, and whose environment may be characterized as containing tubercle bacilli, to eliminate all danger of extraneous tuberculous infection sufficiently to make a test satisfactory when he seeks to verify the tuberculous character of some material from a tuberculous individual by the injection of one, and only one, guinea pig.

SUMMARY OF PRESENT TESTS.

Our own tests were made entirely with the blood of tuberculous cattle. In every case the blood was drawn from the jugular vein of the tuberculous animal and injected in its fresh, naturally warm state into the peritoneal cavity of a guinea pig. The tuberculous cattle, as their records show, may be divided into four distinct lots, according to their tuberculous condition:

Lot 1. Four cattle, the precise tuberculous condition of which is known, because they were killed and examined post-mortem shortly after blood was drawn from them for guinea-pig injections.

Lot 2. Six cattle, known to be tuberculous because they had reacted with tuberculin, because tubercle bacilli were found in their feces on microscopic examination, and because their feces were proven to be infectious by animal experiments.

Lot 3. Nineteen cattle, known to be tuberculous because they had reacted with tuberculin and because tubercle bacilli were found in their feces on microscopic examination.

Lot 4. Thirteen cattle, known to be tuberculous because they had reacted with tuberculin.

We made no attempt to treat the blood used for the injections in any way, because we assumed that the best results would be obtained with it by transferring it as rapidly as possible from the tuberculous cattle to the peritoneal cavities of the guinea pigs. It was learned from the injections that guinea pigs tolerate a relatively large quantity of bovine blood in their peritoneal cavity. The guinea pigs that died shortly after as the result of the blood injections (about 15 per cent of all injected) with few exceptions showed extreme impaction and some inflammation of the large bowel, associated in several instances with invagination of the colon.
The possibility exists that the intraperitoneal injection of from 3 to 5 cubic centimeters of fresh, warm blood from tuberculous cattle induces an immunity in guinea pigs to the tubercle bacilli the blood may contain. Though this view is purely hypothetical and we know of nothing to sustain it, we have carried out an investigation to prove or disprove it, and will give the results later in this paper.

The total number of cattle from which blood injections were made was 42, and these, as their records show, represent a considerable variety relative to the severity and extent of the tuberculous disease with which they were affected. They ranged from animals that would not have been suspected to be diseased without a tuberculin test to a cow so badly affected that a calf of which she became the mother a little less than a year before her blood was used for guinea-pig injections was born affected with tuberculosis contracted from ante-partum exposure to her tuberculous body.

The total number of guinea pigs injected was 104. Of these, 16 died within a few days after the injection and no doubt as a result of it. Three died of intercurrent affections, but not until a sufficient period of time had passed for lesions of tuberculosis to become clearly manifest. The remaining 85 lived until they were killed after a lapse of from seven and one-half to eleven weeks, or an average for all of seventy days after they were injected. The three guinea pigs that died of intercurrent affections showed no lesions of tuberculosis on post-mortem examination, and 84 of the 85 guinea pigs that lived until they were killed showed no lesions of any kind on autopsy. One guinea pig of the 85 showed lesions very slightly resembling tuberculosis, but these were proved by microscopic examinations and guinea-pig inoculation tests to be free from tubercle bacilli.

A detailed record of the cattle and guinea pigs used in our tests follows:

**RECORDS OF CATTLE AND RESULTS OF GUINEA-PIG INOCULATIONS.**

The 42 cattle included in the records below are all that were available for this investigation among the tuberculous cattle kept for various purposes at the Bureau of Animal Industry Experiment Station. The general condition of the cattle is briefly defined as good, fairly good, fair, or poor; and as these terms are used somewhat arbitrarily, it is desirable to specify more precisely what they are intended to convey. The word "good" is used in connection with cattle that were really to all appearances in excellent physical condition, and of which no one would suspect that they were diseased. The words "fairly good" are used to mean that condition commonly found among dairy cows of the better class. "Fair" is used to desig-
nate a condition which the average dairyman regards as satisfactory, and “poor” is applied to cattle that are thin or that show visible symptoms of disease.

LOT 1.

Rull 333, general condition very good, had been affected with tuberculosis a year or more; was killed and examined post-mortem April 8, 1909. The autopsy revealed only one small tuberculous lesion located in one of the superficial inguinal glands.

On February 5, 1909, two guinea pigs, Nos. 2891 and 2892, received each an intraabdominal injection of 2 c. c. of blood from the bull. One guinea pig, No. 2891, died on February 14, 1909, affected with invagination of the bowel. The other guinea pig remained healthy until April 13, 1909 (sixty-seven days after injection), when it was killed and found on post-mortem examination to be free from lesions of disease.

Cow 533, general condition poor, had been affected with tuberculosis two years or more; was killed and examined post-mortem April 24, 1909. The autopsy revealed the following conditions: The principal lobe of the right lung contained a cavity about 3 inches in diameter, partly filled with pasty, necrotic tuberculous material. This cavity was in direct communication with a large bronchial tube, which contained a considerable amount of material discharged from the cavity. Sprinkled throughout the lungs generally were a number of smaller tuberculous foci, in a completely broken-down condition. The mediastinal and mesenteric lymph glands and the liver were sprinkled with tuberculous foci, some of which were as much as half an inch in diameter. Prior to the cow’s death her feces were examined microscopically on nine different days and on six of these days were found to contain tubercle bacilli.

On February 3, 1909, two guinea pigs, Nos. 2859 and 2860, received each an intraabdominal injection of 3 c. c. of blood of the cow. The guinea pigs remained healthy until April 13, 1909 (sixty-nine days after injection), when they were killed and found on post-mortem examination to be free from lesions of disease.

Cow 549, general condition poor, had been affected with tuberculosis several years. On March 27, 1908, she gave birth to a calf affected with congenital tuberculosis. The cow was killed April 8, 1909, and on autopsy was found to be affected with advanced, generalized tuberculosis. The lungs contained lesions varying from quite recent tuberculous disease to large tuberculous cavities that had discharged most of their contents through the bronchial tubes. No tests were made relative to the infectious character of the feces before death.

On February 3, 1909, two guinea pigs, Nos. 2863 and 2864, received each an intraabdominal injection of 3 c. c. of blood from the cow. The guinea pigs were killed April 13, 1909 (sixty-nine days after injection), and on post-mortem examination were found to be free from lesions of disease.

Cow 552, general condition poor, had been affected with tuberculosis several years; was killed April 1, 1909. The autopsy revealed a fairly generalized tuberculosis with lesions of greater or less magnitude in the lungs and in the pharyngeal, bronchial, and mesenteric lymph glands. Prior to the cow’s death her feces were examined microscopically on ten different days and on six of these days were found to contain tubercle bacilli.

Hogs that were fed feces from the cow contracted tuberculosis, and guinea pigs inoculated subcutaneously with small masses of her feces likewise contracted tuberculosis.
Guinea pigs were injected intraabdominally with blood from this cow as follows:

January 25, 1900, guinea pig 2785 received 5 c.c.
January 25, 1900, guinea pig 2786 received 5 c.c.
January 25, 1900, guinea pig 2782 received 2½ c.c.
January 25, 1900, guinea pig 2784 received 2½ c.c.
January 25, 1900, guinea pig 2781 received 1 c.c.
January 25, 1900, guinea pig 2782 received 1 c.c.
February 3, 1900, guinea pig 2861 received 3 c.c.
February 3, 1900, guinea pig 2862 received 3 c.c.
February 5, 1900, guinea pig 2880 received 2½ c.c.
February 5, 1900, guinea pig 2890 received 2½ c.c.

Guinea pigs 2780 and 2890 died prematurely as a result of the blood injections, and the remaining 8 were killed on the following dates and on autopsy were found to be free from lesions of disease: Guinea pigs 2781 and 2782, killed March 27, 1900 (sixty-one days after injection); guinea pigs 2783 and 2784, killed April 13, 1900 (seventy-eight days after injection); guinea pig 2785, killed April 12, 1900 (seventy-eight days after injection); guinea pig 2880, killed April 13, 1900 (sixty-seven days after injection); guinea pigs 2861 and 2862, killed April 13, 1900 (sixty-nine days after injection).

LOT 2.

Cow 511, general condition poor, had been affected with tuberculosis eighteen months or more. Microscopic examinations of the feces on seven different days revealed tubercle bacilli on three days. A hog fed with feces from the cow contracted tuberculosis.

On February 1, 1900, two guinea pigs, Nos. 2820 and 2830, received each an intraabdominal injection of 3 c.c. of blood from the cow. The guinea pigs remained healthy until April 13, 1900 (seventy-one days after injection), when they were killed and found on autopsy to be free from lesions of disease.

Cow 537, general condition fairly good, had been affected with tuberculosis more than two years. Microscopic examination of the feces on fifteen days revealed tubercle bacilli on eleven days. Guinea pigs inoculated with small masses of feces contracted tuberculosis.

Guinea pigs were injected intraabdominally with blood from the cow as follows:

February 4, 1900, guinea pig 2871 received 3 c.c.
February 4, 1900, guinea pig 2872 received 3 c.c.
February 19, 1900, guinea pig 3062 received 3 c.c.
February 19, 1900, guinea pig 3063 received 3 c.c.

Guinea pig 2872 died prematurely as a result of the injection. Guinea pig 2871 was killed April 13, 1900 (sixty-eight days after injection), and on autopsy was found to be free from lesions of disease. Guinea pigs 3062 and 3063 were killed April 13, 1900 (fifty-three days after injection), and on autopsy were found to be free from lesions of disease.

Cow 538, general condition very poor, had been affected with tuberculosis two years or longer. Microscopic examinations of feces on eleven different days revealed tubercle bacilli on nine days. Guinea pigs inoculated with small masses of feces and hogs fed feces of this cow contracted tuberculosis.

On February 3, 1900, two guinea pigs, Nos. 2851 and 2852, received each an intraabdominal injection of 3 c.c. of blood from the cow. On April 13, 1900 (sixty-nine days after injection), the guinea pigs were killed and on autopsy were found to be free from lesions of disease.
Cow 555, general condition fairly good, had been affected with tuberculosis more than two years. Microscopic examinations of feces on five different days revealed tubercle bacilli on two days. A hog fed feces from the cow contracted tuberculosis.

On January 30, 1909, two guinea pigs, Nos. 2811 and 2812, received each an intraabdominal injection of 3 c. c. of blood from the cow. Guinea pig 2811 died prematurely as a result of the injection. Guinea pig 2812 was killed April 12, 1909 (seventy-two days after injection), and on post-mortem examination was found to be free from lesions of disease.

Cow 567, general condition good, had been affected with tuberculosis at least two and one-half years. Microscopic examinations of feces on ten different days revealed tubercle bacilli on five days. A hog fed with feces from the cow contracted tuberculosis.

On February 4, 1909, two guinea pigs, Nos. 2869 and 2870, received each an intraabdominal injection of 3 c. c. of blood from the cow. The guinea pigs were killed April 12, 1909 (sixty-seven days after injection), and on autopsy were found to be free from lesions of disease.

Cow 646, general condition fair; had been affected with tuberculosis for some time, but just how long was not known. Microscopic examinations of feces on two different days revealed tubercle bacilli on one day. Guinea pigs inoculated with her feces contracted tuberculosis.

On February 2, 1909, two guinea pigs, Nos. 2847 and 2848, received each an intraabdominal injection of 3 c. c. of blood from the cow. The guinea pigs were killed April 13, 1909 (seventy days after injection), and on autopsy were found to be free from lesions of disease.

LOT 3.

Cow 503, general condition good; had been affected with tuberculosis at least two and one-half years. Microscopic examinations of feces on two days revealed tubercle bacilli on one day.

On January 30, 1909, two guinea pigs, Nos. 2805 and 2806, received each an intraabdominal injection of 3 c. c. of blood from the cow. The guinea pigs were killed April 30, 1909 (seventy-three days after injection), and on autopsy were found to be free from lesions of disease.

Cow 510, general condition fairly good; had been affected with tuberculosis about three years. Microscopic examinations of feces on three days revealed tubercle bacilli on two days.

Guinea pigs were injected intraabdominally with blood from the cow as follows:

January 29, 1909, guinea pig 2791, received 5 c. c.
January 29, 1909, guinea pig 2792, received 5 c. c.
February 4, 1909, guinea pig 2881, received 3 c. c.
February 4, 1909, guinea pig 2882, received 3 c. c.

Guinea pigs 2791 and 2792 died prematurely as a result of the injections. Guinea pigs 2881 and 2882 were killed April 13, 1909 (sixty-eight days after injection), and on autopsy were found to be free from lesions of disease.

Cow 512, general condition good; had been affected with tuberculosis eighteen months or longer. Microscopic examinations of feces on six different days revealed tubercle bacilli on four days.

On February 11, 1909, two guinea pigs, Nos. 2823 and 2824, received each an intraabdominal injection of 3 c. c. of blood from the cow. The guinea pigs were killed April 12, 1909 (seventy days after injection), and on autopsy were found to be free from lesions of disease.
Cow 513, general condition fairly good, but had greatly enlarged throat glands; had been affected with tuberculosis eighteen months or longer. Microscopic examinations of feces on four different days revealed tubercle bacilli on two days.

On February 2, 1909, two guinea pigs, Nos. 2835 and 2836, received each an intraabdominal injection of 3 c. c. of blood from the cow. Guinea pig 2835 died prematurely as a result of the injection. Guinea pig 2836 was killed April 12, 1909 (sixty-nine days after injection), and on autopsy was found to be free from lesions of disease.

Cow 514, general condition poor; had been affected with tuberculosis about three years. Microscopic examinations of feces on three different days revealed tubercle bacilli on one day.

On January 30, 1909, two guinea pigs, Nos. 2815 and 2816, received each an intraabdominal injection of 3 c. c. of blood from the cow. Guinea pig 2816 died of an intercurrent affection March 3, 1909 (thirty-two days after injection), and on autopsy was found to be free from lesions of tuberculosis. Guinea pig 2815 was killed April 12, 1909 (seventy-two days after injection), and on autopsy was found to be free from lesions of disease.

Cow 515, general condition fair, had been affected with tuberculosis eighteen months or longer. Microscopic examinations of feces on six different days revealed tubercle bacilli on four days.

On February 1, 1909, two guinea pigs, Nos. 2821 and 2822, received each an intraabdominal injection of 3 c. c. of blood from the cow. The guinea pigs were killed April 12, 1909 (seventy days after injection), and on autopsy were found to be free from lesions of disease.

Cow 516, general condition fairly good, had been affected with tuberculosis eighteen months or longer. Microscopic examinations of feces on seven different days revealed tubercle bacilli on four days.

On February 1, 1909, two guinea pigs, Nos. 2831 and 2832, received each an intraabdominal injection of 3 c. c. of blood from the cow. The guinea pigs were killed April 13, 1909 (seventy-one days after injection), and on autopsy were found to be free from lesions of disease.

Cow 536, general condition poor, had been affected with tuberculosis two years or longer. Microscopic examinations of feces on eleven different days revealed tubercle bacilli on eight days.

On February 3, 1909, two guinea pigs, Nos. 2865 and 2866, received each an intraabdominal injection of 3 c. c. of blood from the cow. The guinea pigs were killed April 13, 1909 (sixty-nine days after injection), and on autopsy were found to be free from lesions of disease.

Cow 551, general condition fairly good, had been affected with tuberculosis two years or longer. Microscopic examinations of feces on nine different days revealed tubercle bacilli on three days.

On February 1, 1909, two guinea pigs, Nos. 2833 and 2834, received each an intraabdominal injection of 3 c. c. of blood from the cow. The guinea pigs were killed April 12, 1909 (seventy days after injection), and on autopsy were found to be free from lesions of disease.

Cow 553, general condition fairly good, had been affected with tuberculosis two years or longer. Microscopic examinations of feces on three different days revealed tubercle bacilli every day.

On February 1, 1909, two guinea pigs, Nos. 2827 and 2828, received each an intraabdominal injection of 3 c. c. of blood from the cow. The guinea pigs were killed April 13, 1909 (seventy-one days after injection), and on autopsy were found to be free from lesions of disease.
Cow 620, general condition good, had been affected with tuberculosis a year or longer. Microscopic examinations of feces on four different days revealed tubercle bacilli on one day.

On February 3, 1909, two guinea pigs, Nos. 2855 and 2856, received each an intraabdominal injection of 3 c. c. of blood from the cow. The guinea pigs were killed April 13, 1909 (sixty-nine days after injection), and on autopsy were found to be free from lesions of disease.

Cow 629, general condition fair, had been affected with tuberculosis at least one year. Microscopic examinations of feces on five different days revealed tubercle bacilli on two days.

On February 3, 1909, two guinea pigs, Nos. 2857 and 2858, received each an intraabdominal injection of 3 c. c. of blood from the cow. The guinea pigs were killed April 13, 1909 (sixty-nine days after injection), and on autopsy were found to be free from lesions of disease.

Cow 631, general condition fair, had been affected with tuberculosis at least one year. Microscopic examinations of feces on five different days revealed tubercle bacilli on two days.

On February 4, 1909, two guinea pigs, Nos. 2877 and 2878, received each an intraabdominal injection of 3 c. c. of blood from the cow. The guinea pigs were killed April 13, 1909 (sixty-eight days after injection), and on autopsy were found to be free from lesions of disease.

Bull 635, general condition good, had been affected with tuberculosis over two years. Microscopic examinations of feces on three different days revealed tubercle bacilli on one day.

On January 20, 1909, two guinea pigs, Nos. 2817 and 2818, received each an intraabdominal injection of 3 c. c. of blood from the bull. Guinea pig 2818 died prematurely as a result of the injection. Guinea pig 2817 was killed April 12, 1909 (seventy-two days after injection), and on autopsy was found to be free from lesions of disease.

Cow 636, general condition fairly good, had been affected with tuberculosis at least one year. Microscopic examinations of feces on five different days revealed tubercle bacilli on two days.

On January 30, 1909, two guinea pigs, Nos. 2803 and 2804, received each an intraabdominal injection of 3 c. c. of blood from the cow. The guinea pigs were killed April 12, 1909 (seventy-two days after injection), and on autopsy were found to be free from lesions of disease.

Cow 638, general condition fairly good, had been affected with tuberculosis over two years. Microscopic examinations of feces on four different days revealed tubercle bacilli on all four days.

Guinea pigs were given intraabdominal injections of the blood of this cow as follows:

January 29, 1909, guinea pig 2795 received 5 c. c.
January 29, 1909, guinea pig 2796 received 5 c. c.
February 4, 1909, guinea pig 2879, received 3 c. c.
February 4, 1909, guinea pig 2880 received 3 c. c.

Guinea pig 2795 died prematurely as a result of the injection. Guinea pig 2796 was killed April 12, 1909 (seventy-three days after the injection), and on autopsy was found to be free from lesions of disease. Guinea pigs 2879 and 2880 were killed April 12, 1909 (sixty-seven days after injection), and on autopsy were found to be free from lesions of disease.

Cow 639, general condition good, had been affected with tuberculosis at least one year. Microscopic examinations of feces on four different days revealed tubercle bacilli on one day.
Guinea pigs were given intraabdominal injections of the blood of this cow as follows:

January 29, 1909, guinea pig 2801 received 5 c. c.
January 29, 1909, guinea pig 2802 received 5 c. c.
February 5, 1909, guinea pig 2887 received 3 c. c.
February 5, 1909, guinea pig 2888 received 3 c. c.

Guinea pig 2801 died prematurely as a result of the injection. Guinea pig 2802 was killed April 12, 1909 (seventy-three days after injection), and on autopsy several small necrotic foci were found in the liver and spleen. The lesions were not at all like the conditions caused by the tubercle bacillus, and microscopic examinations failed to reveal tubercle bacilli. Some of the abnormal tissue was used to make subinoculations into guinea pigs; the sub-inoculated guinea pigs failed to show tuberculosis.

Guinea pigs 2887 and 2888 were killed April 13, 1909 (sixty-seven days after the injection), and on autopsy were found to be free from lesions of disease.

Cow 640, general condition good, had been affected with tuberculosis over two years. Microscopic examinations of feces on three different days revealed tubercle bacilli on all three days.

On January 29, 1909, two guinea pigs, Nos. 2799 and 2800, received each an intraabdominal injection of 5 c. c. of blood from the cow. The guinea pigs were killed April 12, 1909 (seventy-three days after injection), and on autopsy were found to be free from lesions of disease.

Cow 642, general condition good, had been affected with tuberculosis at least one year. Microscopic examinations of feces on three different days revealed tubercle bacilli on two days.

Guinea pigs were injected intraabdominally with blood from this cow as follows:

January 29, 1909, guinea pig 2793 received 5 c. c.
January 29, 1909, guinea pig 2794 received 5 c. c.
February 5, 1909, guinea pig 2885 received 3 c. c.
February 5, 1909, guinea pig 2886 received 3 c. c.

Guinea pig 2794 died prematurely as the result of the injection. Guinea pig 2886 died March 5, 1909 (twenty-eight days after injection), of an intercurrent affection, and the autopsy revealed no lesions resembling tuberculosis. Guinea pig 2793 was killed April 13, 1909 (seventy-three days after injection), and on autopsy was found to be free from lesions of disease. Guinea pig 2885 was killed April 13, 1909 (sixty-seven days after injection), and on autopsy was found to be free from lesions of disease.

LOT 4.

Cow 479, general condition fair, but had greatly enlarged throat glands, had been affected with tuberculosis about three years.

On February 4, 1909, two guinea pigs, Nos. 2873 and 2874, received each an intraabdominal injection of 3 c. c. of her blood. The guinea pigs were killed April 13, 1909 (sixty-eight days after injection), and on autopsy were found to be free from lesions of disease.

Bull 508, general condition good, had been affected with tuberculosis about two and one-half years.

On February 4, 1909, two guinea pigs, Nos. 2867 and 2868, received each an intraabdominal injection of 3 c. c. of his blood. The guinea pigs were killed April 12, 1909 (sixty-seven days after injection), and on autopsy were found to be free from lesions of disease.

Cow 517, general condition good, had been affected with tuberculosis about two and one-half years.
On February 1, 1909, two guinea pigs, Nos. 2825 and 2826, received each an intraabdominal injection of 3 c. c. of her blood. Guinea pig 2825 died of an intercurrent affection April 5, 1909 (sixty-three days after injection), and on autopsy was found to be free from lesions of tuberculosis. Guinea pig 2826 was killed April 13, 1909 (seventy-one days after injection), and on autopsy was found to be free from lesions of disease.

Cow 639, general condition fair, had been affected with tuberculosis about two years.

On February 1, 1909, two guinea pigs, Nos. 2819 and 2820, received each an intraabdominal injection of 3 c. c. of her blood. The guinea pigs were killed April 12, 1909 (seventy days after injection), and on autopsy were found to be free from lesions of disease.

Cow 630, general condition fair, had been affected with tuberculosis about one year.

On February 3, 1909, two guinea pigs, Nos. 2853 and 2854, received each an intraabdominal injection of 3 c. c. of her blood. The guinea pigs were killed April 13, 1909 (sixty-nine days after injection), and on autopsy were found to be free from lesions of disease.

Cow 632, general condition good, had been affected with tuberculosis at least one year.

On January 30, 1909, two guinea pigs, Nos. 2807 and 2808, received each an intraabdominal injection of 3 c. c. of her blood. Guinea pig 2808 died prematurely as a result of the injection. Guinea pig 2807 was killed April 13, 1909 (seventy-two days after injection), and on autopsy was found to be free from lesions of disease.

Cow 633, general condition fair, had been affected with tuberculosis at least one year.

On January 30, 1909, two guinea pigs, Nos. 2813 and 2814, received each an intraabdominal injection of 3 c. c. of her blood. Guinea pig 2813 died prematurely as a result of the injection. Guinea pig 2814 was killed on April 12, 1909 (seventy-one days after injection), and on autopsy was found to be free from lesions of disease.

Cow 634, general condition fair, had been affected with tuberculosis at least one year.

On January 29, 1909, two guinea pigs, Nos. 2787 and 2788, received each an intraabdominal injection of 3 c. c. of her blood. The guinea pigs were killed April 12, 1909 (seventy-three days after injection), and on autopsy were found to be free from lesions of disease.

Cow 641, general condition fair, had been affected with tuberculosis over two years.

On January 29, 1909, two guinea pigs, Nos. 2707 and 2708, received each an intraabdominal injection of 5 c. c. of the blood of the cow, and on February 4, 1909, two guinea pigs, Nos. 2875 and 2876, received each a similar injection of 3 c. c. of blood. Guinea pig 2708 died prematurely as a result of the injection. Guinea pig 2707 was killed April 12, 1909 (seventy-five days after injection), and guinea pigs 2875 and 2876 were killed April 12, 1909 (sixty-seven days after injection). The last three guinea pigs were found to be free from lesions of disease.

Cow 644, general condition fair, had been affected with tuberculosis four months or more.

On February 2, 1909, two guinea pigs, Nos. 2849 and 2850, received each an intraabdominal injection of 3 c. c. of blood of the cow. The guinea pigs were killed April 13, 1909 (seventy days after injection), and on autopsy were found to be free from lesions of disease.
Cow 645, general condition good, had been affected with tuberculosis three months or more.

On February 2, 1909, two guinea pigs, Nos. 2845 and 2846, received each an intraabdominal injection of 3 c. c. of her blood. Guinea pig 2845 died prematurely as a result of the injection. Guinea pig 2846 was killed April 13, 1909 (seventy days after injection), and on autopsy was found to be free from lesions of disease.

Cow 648, general condition good, had been affected with tuberculosis for an unknown period of time. She was brought to the Experiment Station shortly before her blood was used for guinea-pig injections, and reacted with tuberculin.

On February 2, 1909, two guinea pigs, Nos. 2837 and 2838, received each an intraabdominal injection of 3 c. c. of her blood. The guinea pigs were killed April 12, 1909 (sixty-nine days after injection), and on autopsy were found to be free from lesions of disease.

Cow 657, general condition fair, had been affected with tuberculosis for an unknown period of time. She was brought to the Experiment Station shortly before her blood was used for guinea-pig injections, and reacted with tuberculin.

On February 2, 1909, two guinea pigs, Nos. 2841 and 2842, received each an intraabdominal injection of 3 c. c. of her blood. The guinea pigs were killed April 12, 1909 (sixty-nine days after injection), and on autopsy were found to be free from lesions of disease.

**DISCUSSION OF RESULTS.**

Among the 42 cattle enumerated above, 27, or 64 1/2 per cent, were shown by microscopic examinations to be discharging tubercle bacilli from their bowels—in most instances intermittently—and the infectious character of the feces in 7 cases, or 16 2/3 per cent, was demonstrated by animal experiments—that is, feeding and inoculation tests.

These two facts—that 27 of the cattle were shown by microscopic tests to be expelling tubercle bacilli per rectum, while only 7 were proved by animal experiments to be passing infected feces—must not be taken as being in any sense contradictory, as the feces of only a sufficient number of tuberculous cattle were tested by animal feeding and inoculation experiments to prove conclusively that the acid-fast bacilli found on microscopic examinations in the feces of tuberculous cattle are certainly live, virulent tubercle bacilli.

Relative to the expulsion of tubercle bacilli from the bowels of tuberculous cattle, all the evidence we have indicates that the bacilli have their origin in the lung and throat, from which regions they are coughed up, swallowed, and passed through and out of the intestinal canal without appreciable loss of pathogenic virulence. That a large proportion of the tubercle bacilli swallowed by cattle really pass through their bodies and out per rectum without a determinable loss of virulence was experimentally shown in some of our earlier work. We have absolutely no reason to believe that tubercle bacilli

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enter the intestinal canal from the lymph radicals or blood capillaries or by any complex and mysterious system of transportation from lesions of all descriptions and kinds in any or every portion of the body. It is our conviction that, unless an open tuberculosis is in more or less direct communication with the intestinal canal or there is a tuberculous disease of the intestine itself, which latter is rare among cattle, no tubercle bacilli will be expelled with the feces.

If tuberculosis in all its forms was a bacteriemia the expulsion of tubercle bacilli from the bowels of all tuberculous individuals, as well as with their urine, saliva, milk, and other bodily secretions, would follow as a natural consequence. Those who have carefully studied the secretions from the uninvolved organs of tuberculous subjects know how rarely tubercle bacilli are detected in them even with the application of the most delicate tests.

When we consider cattle like Nos. 533, 549, and 552—three of the four animals of which autopsy records are given—and note that they were so badly diseased that they would have been condemned on superficial examination as wholly unfit for use as food under the existing meat-inspection regulations, the absence of tubercle bacilli from their blood may be regarded as a sufficient reason for assuming that the possible occurrence of tubercle bacilli in the blood of tuberculous animals will almost invariably be associated with pathological conditions of a very marked character, or that the tubercle bacilli will be present in extremely small numbers and will speedily be filtered out of the blood stream. Cow 533 had been affected with tuberculosis two years or longer, was in poor condition as a result of the disease, and on autopsy was found to have an extensive, open tuberculosis of the lung and lesions of tuberculosis in the liver and in both the thoracic and abdominal lymph glands. Cow 549 was, if anything, even more severely and extensively affected, and had given birth to a congenitally tuberculous calf less than a year before her blood was injected into guinea pigs. Cow 552 was also affected with generalized, advanced, open tuberculosis, and prior to the use of her blood for the guinea-pig injections was found to be passing from her bowels large numbers of tubercle bacilli, which were proved by feeding tests to be virulent for hogs and by inoculation tests to be virulent for guinea pigs. With the blood obtained from these three cows 14 guinea pigs were injected, of which 2 died prematurely and 12 lived two months or more afterwards, until they were intentionally killed, when they were found on post-mortem examination to be wholly free from lesions of disease of any kind.

The possibility exists that tubercle bacilli introduced into the stomach and intestine by swallowing may be taken up by the lymph radicals, passed along the lymph channels, and emptied through the
great lymph ducts into the venous circulation. The investigations of Nicolas and Descos, Ravenel, Calmette and Guérin, Schlossman and Engle, and others speak for this; but such tubercle bacilli will not be very numerous and will no doubt be filtered out of the blood as soon as it reaches the lung through the heart and pulmonary arteries, to which it passes directly after it has received the lymph stream.

SUPPLEMENTAL TESTS REGARDING POSSIBLE IMMUNITY.

We have already stated that the possibility exists that the intra-peritoneal injection of from 3 to 5 c. c. of fresh, warm blood from tuberculous cattle induces an immunity in guinea pigs to the tubercle bacilli the blood may contain. Although we knew of nothing to uphold this theory, we considered it necessary to undertake an investigation to prove or disprove it, the results of which are now presented.

On April 24, 1909, blood and tuberculous material was obtained from cow 533 (see record of cow on p. 11) for a number of guinea-pig injections. The primary object of the injections was to prove that the blood of a tuberculous cow, when introduced into the peritoneal cavity of a guinea pig, has no retarding influence on the development of tuberculosis from tubercle bacilli that may be present in it.

Cow 533 was first bled from the jugular vein and then at once killed. As soon as she was dead a tuberculous mediastinal gland was removed from her body and 500 mg. of it emulsified with 2 c. c. of sterile, normal salt solution. Cover glasses of this emulsion, stained with carbolfuchsin and decolorized with 20 per cent sulphuric acid, revealed on microscopic examination, an average, two tubercle bacilli each. The emulsion was mixed with an additional quantity of sterile, normal salt solution, so that each cubic centimeter of the dilution represented a strength equal to one drop of the original emulsion.

The blood obtained from the cow prior to her death and the diluted emulsion made with the tuberculous mediastinal gland from her body were used to inject seven groups of guinea pigs, the records of which are given below.

The guinea pigs in the seven different groups were injected for the following purposes: Group 1, to serve as checks on the absence or presence of tubercle bacilli in the blood of the tuberculous cow that was used for the investigation; group 2, to show that the intra-abdominal injection of fresh, warm blood from a tuberculous cow can not protect against tubercle bacilli simultaneously introduced into the abdominal cavity; group 3, to show that the intraabdominal injection of fresh, warm blood from a tuberculous cow can not protect against tubercle bacilli introduced into other parts of the body than the abdominal cavity; groups 4 and 5, to show that the blood of
tuberculous cows has no special germicidal potency for tubercle bacilli; groups 6 and 7, to serve as guides relative to the amount of tuberculous disease to be expected in the bodies of the guinea pigs that were injected with both blood and emulsion of tuberculous material.

GROUP 1.

On April 24, 1909, eight guinea pigs, Nos. 3626 to 3633, inclusive, received each an intraabdominal injection of 3 c. c. of freshly drawn warm blood. On May 6, 1909, guinea pig 3627 died affected with congestion of the lungs. On autopsy no lesions of tuberculosis were found. On May 27 and 28, 1909, guinea pigs 3626, 3628, 3629, 3630, 3631, 3632, and 3633 were killed and examined post-mortem. No lesions of tuberculosis or other disease were found.

GROUP 2.

On April 24, 1909, eight guinea pigs, Nos. 3642 to 3649, inclusive, received each an intraabdominal injection of 3 c. c. of freshly drawn blood, followed as quickly as possible by an intraabdominal injection of 0.5 c. c. of tuberculous emulsion. On April 30, 1909, guinea pig 3642 died affected with inflammation of the large bowel. On May 27, 1909, guinea pigs 3643, 3644, 3645, 3646, 3647, 3648, and 3649 were killed and examined post-mortem. Every one of the seven was found to be affected with generalized tuberculosis of the abdominal and thoracic organs.

GROUP 3.

On April 24, 1909, eight guinea pigs, Nos. 3634 to 3641, inclusive, received each an intraabdominal injection of freshly drawn warm blood, followed as soon as possible by a subcutaneous injection into the right thigh of 0.5 c. c. of tuberculous emulsion. On May 27, 1909, guinea pigs 3636 and 3637 and on May 28, 1909, guinea pigs 3634, 3635, 3638, 3639, 3640, and 3641 were killed and examined post-mortem. The eight guinea pigs all showed more or less extensive lesions of tuberculosis at the seat of the subcutaneous injection, tuberculosis of the adjacent superficial inguinal gland, tuberculosis of the pelvic, lumbar, and gastro-hepatic glands, and a sprinkling of tuberculous foci in the liver and spleen.

GROUP 4.

On April 24, 1909, eight guinea pigs, Nos. 3658 to 3665, inclusive, received each an intraabdominal injection of 3 c. c. of a mixture of defibrinated blood and tuberculous emulsion. Each 3 c. c. of this mixture was equivalent to 0.5 c. c. of the diluted tuberculous emulsion described earlier. The mixture was two hours old at the time it was injected into the guinea pigs. On April 25, 1909, guinea pigs 3659, 3660, and 3661 died. The post-mortem examinations showed no lesions excepting a congested condition of the lungs and a quantity of unabsorbed blood in the peritoneal cavity. On May 12, 1909, guinea pig 3662 died affected with congestion of the lungs; no lesions of tuberculosis were found. On May 27, 1909, guinea pigs 3658, 3663, 3664, and 3665 were killed and examined post-mortem. Three of them were affected with completely generalized tuberculosis of the abdominal and thoracic organs, and the remaining one (No. 3665) with generalized tuberculosis of the abdominal organs only.

GROUP 5.

On April 26, 1909, eight guinea pigs, Nos. 3670 to 3677, inclusive, received each an intraabdominal injection of 3 c. c. of the same mixture of blood and
tuberculous emulsion used for the guinea pigs of group 4. The mixture was forty-five hours old at the time it was injected. On May 27, 1909, the eight guinea pigs were killed and examined post-mortem. Seven of them were affected with generalized tuberculosis of the abdominal and thoracic organs, and one (No. 3672) with generalized tuberculosis of the abdominal organs only.

GROUP 6.

On April 24, 1909, four guinea pigs, Nos. 3650 to 3653, inclusive, received each an intraabdominal injection of 0.5 c. c. of tuberculous emulsion. On May 6, 1909, guinea pig 3651 died affected with congestion of the lungs. On May 27, 1909, Nos. 3650, 3652, and 3653 were killed and examined post-mortem. No. 3650 showed tuberculous lesions of the spleen and omentum only, and Nos. 3652 and 3653 showed a fairly generalized tuberculosis of the abdominal and thoracic organs.

GROUP 7.

On April 24, 1909, four guinea pigs, Nos 3654 to 3657, inclusive, received each a subcutaneous injection, in the right thigh, of 0.5 c. c. of tuberculous emulsion. On May 27, 1909, the four guinea pigs were killed and examined post-mortem. Nos. 3654 and 3656 each showed a small tuberculous abscess at the seat of injection, a tuberculous condition of the superficial inguinal gland near the seat of injection, and a fairly generalized tuberculosis of the pelvic and abdominal organs. Guinea pigs 3655 and 3657 showed similar lesions with the exception of the abscesses at the seat of injection.

The autopsy records of the guinea pigs show, in a general way, very little difference between the animals that received only tuberculous emulsion and those that received both blood and emulsion. The guinea pigs that received both blood and emulsion into their abdominal cavities showed numerically more extensive lesions of tuberculosis than the guinea pigs that received only emulsion into their abdominal cavities. This condition would naturally be expected because the same number of tubercle bacilli contained in 3 c. c. of blood would be more widely separated and in better condition to start a large number of individual lesions than those in 0.5 c. c. of salt solution.

The use of an emulsion of tuberculous tissue from the tuberculous cow that supplied the blood for the supplemental injections was preferred to the use of a pure culture of tubercle bacilli, because it seemed desirable to us to use infectious material and blood in this instance from the same individual case of tuberculosis.

The total number of guinea pigs injected in this supplemental investigation was 48, of which 8 received blood only, 32 both blood and tuberculous material, and 8 tuberculous material only. Of the 32 that received both blood and tuberculous material and the 8 that received only tuberculous material, 6 died prematurely, and the remaining 34, when they were killed—thirty to thirty-one days after the injection—were all found to be affected with tuberculosis of a form that would have progressed to death in a short time.
Among the 8 guinea pigs that received an injection of fresh warm blood without the addition of tuberculous material, 1 died prematurely and the remaining 7 were found on autopsy to be free from lesions of disease. Since the cow that supplied the blood for the injections was affected, as her record shows, with extensive, advanced tuberculosis, the 7 guinea pigs make a strong addition to the 88 parallel cases of which the records have been previously given; and hence we have 95 guinea pigs as the total number that received injections of blood from tuberculous cattle into their peritoneal cavities—the most delicate test for tubercle bacilli available—and survived the injection long enough for tuberculosis to manifest itself clearly. Among this total of 95 guinea pigs not one case of tuberculosis developed.  

CONCLUSIONS.

1. We failed utterly to find tubercle bacilli in the blood of tuberculous cattle which we examined microscopically in accordance with the method described and used by Doctor Rosenberger.

2. The negative results of our microscopic examinations are confirmed by the negative results obtained with 95 guinea pigs, each of which received an intraabdominal injection of blood from a tuberculous cow or bull.

3. As the number of cattle from which blood was injected into the 95 guinea pigs was 42, and as these cattle represented practically all stages of tuberculosis from mildly affected recent cases to old and completely generalized cases, we feel that our work shows beyond the remotest doubt that tuberculosis is not to be classified, in any sense of the word, as a bacteriemia.

An independent investigation relative to the occurrence of tubercle bacilli in the circulating blood of cattle was made in the Bureau of Animal Industry by Dr. John R. Mohler, chief of the Pathological Division. Mohler examined the blood of 8 cattle microscopically, and with blood from each of these cattle injected 5 guinea pigs. The microscopic examinations and injections were made precisely in the manner described by Doctor Rosenberger. No tubercle bacilli were discovered microscopically, and not one of the 40 injected guinea pigs contracted tuberculosis. Two of the 8 cattle were in good condition, but were passing tubercle bacilli from their bowels; 2 of the cattle were in poor condition and were passing tubercle bacilli from their bowels; and 4 of the cattle were slaughtered for meat, but on inspection were found to be so extensively affected with tuberculosis that it was necessary to condemn and tank their carcasses under the Federal meat-inspection regulations. This evidence, kindly presented to us by Doctor Mohler, raises the number of tuberculous cattle from which blood was tested to 50, and the number of guinea pigs that received injections of blood from tuberculous cattle without contracting tuberculosis to 135.