TUBERCLE BACILLI IN THE BLOOD

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In February, 1909, Rosenberger¹ published his announcement of the finding of tubercle bacilli in the blood of every one of 125 tuberculous patients examined. Previous to that time the prevailing opinion had been that only occasionally in cases of so-called localized tuberculosis² did bacilli escape into the blood-stream. It had been supposed that in the miliary form alone was the number of organisms sufficient to give any promise of detection, and that even in these cases the search was apt to be futile. The description by Landouzy³ of typho-bacillose, a stage of tuberculosis which precedes the formation of localized lesions and which is supposed by him to be a septicemia, had added interest to this subject.

After unsuccessful attempts to confirm Rosenberger's findings in a search of the blood of human beings, I undertook, in conjunction with C. M. Haring, D.V.M., Assistant Professor of Veterinary Science in the University of California, the systematic examination of the blood of tuberculous cattle. The work was done under the Department of Agriculture of the University of California. The investigation has a bearing on meat-inspection, for the blood-containing muscle of cattle with "localized" tuberculosis is now considered to be fit for food and is passed by the government inspectors.

Since this work was begun various reports have appeared, of which some confirm Rosenberger's work while some radically differ from it. Tubercle bacilli have frequently been found in the blood by Forsyth⁴ and by Petty and Mendenhall.⁵ Rosenberger has reported subsequent positive blood-findings, bringing his total to 300 cases, and has concluded that tubercle bacilli may exist free in the blood of even apparently healthy

1. Rosenberger, R. C.: The Presence of Tubercle Bacilli in the Circulating Blood in Tuberculosis. *Am. Jour. Med. Sc.*, 1909, cxxxvii, 267.

2. Pearce, R. M.: *Osler's Modern Medicine*, 1907, ii, 657.

3. Landouzy, L.: *Tr. Sixth Internat. Cong. on Tuberculosis*, 1908, i, part 2, p. 581.

4. Forsyth, C. E. P.: Occurrence of Tubercle Bacilli in the Blood in Tuberculosis. *Brit. Med. Jour.*, April 24, 1909.

5. Petty, O. H., and Mendenhall, A. M.: Tubercle Bacilli in the Blood. *Jour. Am. Med. Assn.*, 1909, liii, 867.

individuals.⁶ Failures to find the bacilli have been reported by Ravenel and Smith,⁷ Burnham and Lyons,⁸ and Dailey.⁹ Schroeder and Cotton¹⁰ have announced negative results in their search in the blood of cattle.

An isolated herd of 51 cattle at the University Farm at Davis, Cal., was available for study. In the herd were 42 3-year-old steers and cows. Of these, 22 had been twice inoculated intravenously, when young calves, with attenuated cultures of tubercle bacilli. Of the 42 animals, 12 (3 of the inoculated animals and 9 of the others) gave decided tuberculin reactions. These were selected to form Group B of this investigation. With the young stock were 9 old tuberculous cows which had been added to the herd to test the susceptibility of the others to tuberculosis. These old animals, most of them in advanced stages of the disease, constituted Group A.

The method of examination was essentially taken from Rosenberger. The search of the sediment from laked and centrifugalized blood was added for control and comparison. All glassware (test-tubes, pipettes, syringes, centrifuge tubes, slides, cover-slips, jars) were cleaned with hot washing-powder solution and then subjected for half an hour or more to concentrated nitric acid. The needles were cleaned with soap and water, syringed out in concentrated caustic soda solution, and finally boiled for ten minutes in the latter. The distilled water and the citrate-chlorid solution were examined microscopically and found to be free from organisms. (Attention has recently been called by Brem¹¹ to the liability of distilled water to contamination with acid-fast bacilli.) Test-tubes each containing 5 c.c. of 2 per cent. sodium citrate and 0.6 per cent. sodium chlorid solution were covered with larger test-tubes, inverted, instead of being closed with the usual cotton plugs. Tubes containing the syringes, needles and solution were placed in a covered jar, sterilized, and carried unopened to the field. The animal to be bled was thrown, and its neck was shaved and scrubbed. Through a needle introduced into the jugular

6. Rosenberger, R. C.: Presence of the Tubercle Bacillus in the Circulating Blood. *New York Med. Jour.*, 1909, lxxxix, 1250.

7. Ravenel, M. P., and Smith, K. W.: Detection of Tubercle Bacilli in the Blood. *Proc. State Med. Soc. of Wisconsin*, abst. in *Jour. Am. Med. Assn.*, 1909, liii, 649.

8. Burnham, M. P.: Tuberculosis—A Bacteriemia. *Jour. Am. Med. Assn.*, 1909, liii, 731.

9. Dailey, M. A.: The Presence of Tubercle Bacilli in the Blood in Tuberculosis. *Boston Med. and Surg. Jour.*, 1909, clxi, 318.

10. Schroeder, E. C., and Cotton, W. E.: Tests Concerning Tubercle Bacilli in the Circulating Blood. *THE ARCHIVES INT. MED.*, 1909, iv, 133.

11. Brem, W. V.: Investigation of Blood for Tubercle Bacilli; Contamination of Distilled Water with Acid-fast Organisms a Source of Error. *Jour. Am. Med. Assn.*, 1909, liii, 909.

vein 5 c.c. of blood were withdrawn, added to 5 c.c. of the citrate-chlorid solution, and shaken. Then 5 c.c. more of blood were withdrawn and added to another tube of solution, in order that more sediment might be obtained for inoculation into a guinea-pig. In this way the probability of infection was supposed to be increased. The tubes of blood-mixture were placed in the refrigerator from twenty-four to forty-eight hours. Then five preparations were made from one of the two tubes. Two smears were made from the uppermost part of the sediment and two from the bottom. These were dried and fixed on a copper plate with moderate heat. They were laked in distilled water for from fifteen minutes to three hours, and then stained with carbol-fuchsin (for two minutes steaming or one or two hours cold). One of the specimens from the top and one from the bottom were decolorized for twenty minutes with 1 per cent. rosolic acid in absolute alcohol. The other two specimens were decolorized with either 30 per cent. nitric acid or 20 per cent. sulphuric acid, followed by 95 per cent. alcohol. Methylene blue was used as counterstain.

The tube from which a small part of the sediment had been taken for smears was shaken and 5 c.c. of the mixture (almost 2.5 c.c. of blood) was added to 10 c.c. of distilled water in a centrifuge tube. Laking was complete. Centrifugalizing for thirty minutes brought down a small bit of tenacious reddish white sediment, which was teased out on a coverslip, dried, and fixed over a copper-plate, stained with carbol-fuchsin, decolorized with rosolic acid solution, and counter-stained with methylene blue. Each of the five specimens was searched at least thirty minutes. When the remaining blood-mixture in the tube had again settled, the sediment from both tubes was injected intraperitoneally into a guinea-pig, which was then placed alone in a cage. The amount of sediment received by the guinea-pig varied from 0.5 to 5.0 c.c. These animals had been previously used in standardizing diphtheria antitoxin. One of the guinea-pigs died a few hours after inoculation, but was replaced after a later bleeding of the animal which had furnished the fatal blood. The earliest of the examinations deviated from the method just described in unimportant details.

Each of the old cows in Group A was bled three times between March 5, 1909, and July 1, 1909, and the blood studied. The blood-sediment of one bleeding from each animal was injected intraperitoneally into a guinea-pig, which, in the case of all but one of the cows, later received the sediment from another bleeding from the same animal. None of the blood-preparations revealed any acid-fast bacilli. Each of the nine guinea-pigs was killed between ten and one-half and eleven and one-half

weeks after its first inoculation. The second inoculation received by eight of them had occurred between eight and one-half and ten and one-half weeks prior to autopsy. The animals were fat, and not one of them showed any macroscopic lesions suggesting tuberculosis.

Each of the 12 young animals of Group B was bled once. The blood-preparations were free from acid-fast bacilli. The blood sediment from each animal, except Steer 51, was inoculated intraperitoneally into a guinea-pig. These 11 guinea-pigs remained in good health until they were killed, ten or eleven weeks after inoculation. No macroscopic tuberculous lesions were found at autopsy. Positive results were scarcely to be expected after the work of Schroeder and Cotton,¹⁰ who injected 88 guinea-pigs with the blood of 42 tuberculous cattle without producing tuberculosis.

From the material from 39 bleedings of the 21 cattle 167 preparations were made and searched 110.9 hours; 133 of the slides, made from gravity sediments, were studied 88.9 hours. From the laked and centrifugalized blood 34 slides were prepared and searched for 22 hours. These examinations failed to reveal any tubercle bacilli.

In order to secure evidence as to whether the failure of guinea-pigs to contract tuberculosis, when inoculated with the blood of tuberculous cattle, was due to protection by the blood injected, the following experiment was performed: Each of three guinea-pigs of about equal weight was injected intraperitoneally with mixtures containing 0.2 c.c. of pus from an axillary abscess of Cow 3 of Group A. This pus contained so few tubercle bacilli that twenty to thirty minutes' search was necessary to find any. One of the guinea-pigs (No. 23) received the pus thoroughly mixed with 5 c.c. of fresh blood-citrate mixture from Cow 3, the source of the pus. Another (No. 25) received the sediment (1.2 c.c.) from a similar mixture of pus and blood. The third (No. 24) received the pus in 5 c.c. of the citrate-chlorid solution. Ten weeks later guinea-pig No. 23 died and the other two were killed. Autopsy showed all three to be emaciated and to be about equally and very generally tuberculous. Apparently no appreciable effect on the presence or severity of infection had been exerted by the blood.

In order to make a study of the methods of detecting tubercle bacilli in the blood, it was attempted to produce artificially the tuberculous bacteremia which we had failed to find. A cow suffering from advanced tuberculosis (No. 7 of Group A) and a plump 3-year-old steer (No. 18 of the normal animals) were selected for experiment. The cow had a submaxillary abscess from which tuberculous pus and a calcareous gland had been taken. The steer had been twice inoculated when a calf with

attenuated cultures of tubercle bacilli. There was no reaction to the tuberculin test. The animals received in their jugular veins quantities proportional to their weights of a suspension of live bovine tubercle bacilli in normal salt solution. No. 7 received 200 c.c. and No. 18, 250 c.c. The bacilli had been washed, dried, ground dry for ten minutes in a revolving Erlenmeyer flask containing glass balls, and had been rotated for several hours more after the addition of normal salt solution. About 200 mg. of tubercle bacilli were contained in 250 c.c. Smears of the suspension mixed with blood permitted the rough estimate to be made that there were many more free bacilli, and as many clumps, in the suspension injected into No. 18 as there were white corpuscles in the entire circulating blood of the animal. Inasmuch as many corpuscles had been found in almost every field of most of the blood-preparations of this investigation, it was expected that many bacteria would be found in each field of the slides of the steer's blood. No. 18 was bled from the jugular vein, which had not received the inoculation, thirty minutes, five hours, eleven hours, twenty-five hours, forty-six hours, and ten days after inoculation. The routine microscopic blood examination failed to reveal any tubercle bacilli.

Part of a mixture of the blood sediments of the first five bleedings was inoculated intraperitoneally into a guinea-pig, which was allowed to live eleven weeks. Another guinea-pig received the sediment of the sixth and last bleeding and was killed at the end of ten weeks. At autopsy both animals appeared to be entirely normal.

The steer's temperature rose rapidly to 105.5 after inoculation, but fell to normal the next morning. After a few days the temperature began to rise again, and four weeks after inoculation the animal died. Autopsy, performed twenty-four hours after death, showed the lungs to be crowded with miliary tubercles. The spleen contained on the average one miliary tubercle in each square centimeter of its surface. The kidneys contained a few miliary tubercles and the liver none. The mediastinal, inguinal, pelvic, and crural lymph-nodes were enlarged and soft. In the heart were a clot and some red turbid serum. The centrifugalized sediment of this serum showed many coarse bacilli and one group of three tubercle bacilli.

Cow 7 was bled one hour, five hours, eleven hours, twenty-five hours, forty-six hours and fourteen days after inoculation, and the blood was examined. One short structure resembling a tubercle bacillus was found in a smear from the top layer of the sediment drawn eleven hours after inoculation. This slide had been decolorized by the rosolic acid solution. All the other slides were unquestionably negative.

A guinea-pig which received part of a mixture of the sediment from the first five of the six bleedings was killed eleven weeks after inoculation and found to be normal.

The cow's temperature rose immediately after inoculation to 107.5, and in a few hours fell almost to normal. Then the temperature rose gradually to 107.8 and subsided almost to normal. The curve of this last rise and fall suggested the course of a severe tuberculin reaction. No corresponding rise and fall had occurred in the temperature of Steer 18. The cow died nine weeks after inoculation. Autopsy showed extensive chronic lesions and no acute miliary tuberculosis. The right submaxillary, bronchial, anterior mediastinal, portal and mesenteric lymph-nodes were found calcified. The left mediastinal and parotid lymph-nodes were caseous. The left submaxillary lymph-nodes had broken down and had formed a large abscess. Both lungs were extensively involved with old lesions, some connecting with the bronchi. The spleen showed a calcified nodule 2 cm. in diameter. The liver and kidneys were normal.

A third time we introduced tubercle bacilli into the circulation of the cattle and attempted to recover them from their blood. Cow 3 of Group A had advanced tuberculosis. Tubercle bacilli had been found in the pus of a large axillary abscess. The animal was put deeply under the influence of chloral and its right carotid artery was exposed. Fifteen c.c. of suspension of 180 mg. of live bovine tubercle bacilli, which had been washed, dried and ground in a revolving flask for one hour before the addition of normal salt solution, was introduced through a needle into the artery. Blood was withdrawn from the jugular vein of the same side, but from a point distant from the operation wound, twenty-five minutes, one and one-half hours, two days, and six days after the inoculation. The blood drawn twenty-five minutes after the introduction of the organisms showed tubercle bacilli as follows:

A search of seventy minutes in the specimen from the centrifugalized and laked blood showed 5 clumps, one group of 4, one group of 2, and one single bacillus.

A search of fifty-five minutes in the first preparation from the top layer (decolorized by rosolic solution) showed two clumps.

A search of sixty minutes in the second preparation from the top layer (decolorized by nitric acid) showed one clump.

Both smears from the bottom of the sediment (one decolorized by rosolic and one by nitric acid) showed no bacilli after sixty-six and seventy-two minutes' search.

No tubercle bacilli were found in specimens from the bleedings made from one and one-half hours to six days after inoculation.

Half of a mixture of the sediments drawn twenty-five minutes and an hour and a half after inoculation was inoculated intraperitoneally into a guinea-pig. Ten and one-half weeks later the guinea-pig was killed. Three tubercles 2 mm. in diameter were found in the spleen. No other lesions were discovered.

A guinea-pig was inoculated intraperitoneally with the combined sediments of the blood specimens drawn two and six days after inoculation. The animal died six weeks after receiving the blood. Autopsy showed the presence of three full-term fetuses, but no lesions of tuberculosis. The results of the guinea-pig inoculation in this case confirmed the microscopic findings. The natural conclusion would be that the abundant tubercle bacilli had been rapidly filtered from the blood.

The temperature of Cow 3 dropped for two hours after inoculation and then rose rapidly to 106. The temperature soon fell slightly and then slowly developed a marked daily variation. This animal had been running an irregular elevated temperature before the experiment. Death resulted five weeks after inoculation from hemorrhage from a ruptured aneurism at the point of inoculation in the carotid. Autopsy showed many hard tubercles averaging 0.5 cm. in diameter scattered throughout both lungs. One small tubercle was seen in the right kidney and one in the tissues near the aneurism. The mediastinal lymph-nodes were calcareous and the post-pharyngeal nodes were purulent. Most of the lesions appeared to have been of long duration, and autopsy gave no evidence of acute miliary tuberculosis.

The experiments just described show that artificially introduced tubercle bacilli are rapidly removed from the circulation of healthy and of tuberculous cattle. That the capillaries of the lungs are more efficient as filters than the capillaries of the peripheral circulation is suggested by the almost complete, if not entire, absence of tubercle bacilli from blood which had necessarily passed through the pulmonary circulation, and by the presence of bacilli in blood which needed to go only from a peripheral artery to the corresponding peripheral vein. Possibly this quality of the lung capillaries may be one of the causes of the great frequency of pulmonary involvement when tubercle bacilli have entered the body at points distant from the lungs.

It will be noticed that inoculation produced in a healthy young steer a rapid and fatal miliary tuberculosis, while in Cows 3 and 7 not more than an aggravation of the existing extensive chronic tuberculosis was brought about. It would appear that long-standing chronic tuberculosis produced a much higher power of resistance against newly introduced infection than did the intravenous injection of attenuated cultures.

For information regarding the relative value of the methods used in detecting tubercle bacilli each of two tubes of blood-mixture was inoculated with one drop of suspension of tubercle bacilli. The tubes were thoroughly shaken and contents allowed to settle. Search of the usual number of preparations from each tube gave results consistent with those obtained in the positive specimen of blood which had been experimentally inoculated within the artery of Cow 3. In that blood the figures already given showed that the one preparation from the concentrated sediment of the laked and centrifugalized blood showed more bacilli than did all four of the slides from the unlaked gravity sediment, and that the specimens from the top layers of the latter contained bacilli, while smears from the bottom contained none. Each of the specimens from the two inoculated tubes of blood was studied thirty minutes and the following results were obtained:

One preparation from each laked and centrifugalized sediment showed:

First specimen: 21 masses of tubercle bacilli.

Second specimen: 21 large clumps, 3 small clumps, and a few scattering bacilli.

Two preparations from the top of each of the unlaked gravity sediments showed:

First specimen: 1 large clump and 2 single bacilli in each of the two preparations.

Second specimen: no bacilli in either slide.

Two preparations from the bottom of each of the unlaked gravity sediments showed no bacilli in any of the four slides.

In the examinations of the positive blood from the inoculated Cow 3 and of the blood to which bacilli had been added, the method of searching the sediment after laking and centrifugalizing proved very much more effective than the study of unlaked gravity sediment. Apparently the tubercle bacilli settled more slowly than blood corpuscles, with the result that the few bacilli found in the gravity sediments were in the top layers. A series of test-tubes of laked blood and another of unlaked blood-mixture were inoculated. Spreads from their gravity sediments showed many more bacilli in the preparations from the laked specimens than in those from the unlaked. It was also observed that many more scattered bacilli and small clumps were found in the top of the unlaked sediment than in the bottom. No such difference was noticed in the number of very large clumps in the top and bottom. When one considers that laking reduces the amount of sediment to a mere film, while the unlaked sediment is usually over one-fifth of the total volume of the blood-citrate

mixture after twenty-four hours of sedimentation, it is not surprising that laking greatly increases the chance of finding organisms.

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BRIEF NOTES ON THE TUBERCULOUS ANIMALS OF GROUP A

Cow 1.—Ten years old. Poor condition. Discharging sinus in right temple. Tubercle bacilli not found in the discharge. Condemned after tuberculin test in 1908. Alive Dec. 1, 1909.

Cow 2.—Nine years old. Emaciated. Condemned on account of tuberculosis in 1908. Slaughtered Oct. 23, 1909. Posterior mediastinal lymph-node caseous and calcareous. Lesion in lung 5 cm. in diameter, caseous and calcareous.

Cow 3.—Eight years old. Poor condition. Large tuberculous abscess in left axilla. Crepitation and lameness in left hip joint. This animal was used in the inoculation experiments. Died Aug. 24, 1909. Autopsy (already described) showed extensive chronic lesions of tuberculosis in the lungs and lymph-nodes.

Cow 4.—Seven years old. Fair condition. Slaughtered Aug. 25, 1909. Old tuberculous lesions in apex of right lung.

Cow 5.—About 7 years old. Fair condition. Occasional cough. Slaughtered Aug. 25, 1909. Scattered tubercles in both lungs. Caseous focus in left lung. Mediastinal lymph-nodes involved. One tubercle 2 cm. in diameter in surface of liver.

Cow 6.—Six years old. Condemned on account of tuberculosis in 1908. Well nourished at time of blood examination. Coughed frequently. Rales in lungs. Lost weight during October. Slaughtered Oct. 28, 1909. Extensive tuberculous lesions of lungs and mediastinal lymph-nodes.

Cow 7.—Six years old. Very poor condition. Large tuberculous submaxillary abscesses on both sides interfering with breathing. Frequent cough. This animal was used in the inoculation experiments. Died Sept. 15, 1909. The autopsy (already described) showed very extensive involvement of lymph-nodes and lungs.

Cow 8.—About 7 years old. Fair condition. Slaughtered Aug. 25, 1909. Left bronchial lymph-node enlarged and caseous. One posterior mediastinal lymph-node enlarged with a tubercle in the center. Foul-smelling abscess of liver (probably not tuberculous). Middle lobe of right lung solidified (also probably not tuberculous). Several pieces of wire found imbedded in stomach wall.

Cow 9.—Five years old. Good condition. Slaughtered Aug. 25, 1909. Right and left posterior pharyngeal glands caseous.

POST-MORTEM EXAMINATION OF THE THREE-YEAR-OLD ANIMALS OF GROUP B

Steer 12.—Slaughtered with the other animals of group B on Aug. 25, 1909. Right precrucial lymph-node caseous.

Steer 16.—Fair condition. No definite lesions of tuberculosis found in rapid search. The animal had been tested with tuberculin a few days before being

killed. Hyperemic lymph-nodes. Minute hemorrhages in posterior pharyngeal lymph-node.

Cow 17.—Poor condition. Caseous lesions and many scattered tubercles in right lung. A few tubercles in the left. Mediastinal glands caseous and enlarged.

Steer 19.—Excellent condition. No tuberculous lesions found in rapid search.

Steer 23.—Good condition. Anterior mediastinal and left bronchial lymph-nodes caseous.

Steer 26.—Fair condition. Mesenteric lymph-nodes caseous.

Steer 27.—Good condition. Right submaxillary and the mediastinal lymph-nodes caseous and calcareous. One mesenteric and the hepatic lymph-nodes caseous. Few caseous lesions in lungs.

Steer 30.—Good condition. Had been tested with tuberculin a few days before slaughter. No unquestioned tuberculous lesions found. Minute hemorrhages in mesenteric and left bronchial lymph-nodes.

Steer 38.—Good condition. Posterior and anterior mediastinal lymph-nodes enlarged and caseous.

Steer 43.—Good condition. Few tubercles in lungs. Left bronchial and anterior and posterior mediastinal lymph-nodes caseous.

Steer 44.—Fair condition. (Had had frequent cough and râles in lungs.) Both lungs extensively involved. Mediastinal lymph-nodes calcareous. Crural lymph-nodes affected. Tubercles in liver. One tubercle 1 cm. in diameter in muscle of left ventricle of heart.

Steer 51.—Excellent condition. Anterior and posterior mediastinal and portal lymph-nodes caseous.

CONCLUSIONS

1. In 167 preparations made from the blood of 39 bleedings from 21 tuberculous cattle no tubercle bacilli were found in 110.9 hours' search; 133 of the specimens, made from gravity sediments of blood-citrate mixture, were searched 88.9 hours; the remaining 34 slides, made from laked and centrifugalized blood, were searched 22 hours.

2. In one instance tubercle bacilli were recovered by both methods of examination from blood drawn twenty-five minutes after inoculation of the arterial blood-stream.

3. Guinea-pig inoculations with the blood sediments of twenty tuberculous cattle failed to produce tuberculosis.

4. In experiments in which tubercle bacilli had been introduced into blood, either in the circulation or in test-tubes, the examination of laked and centrifugalized blood proved much more effective in the detection of the organisms than did the search of gravity sediments from unlaked mixtures of blood and citrate solution.

5. The upper portions of gravity sediment from mixtures of blood and citrate solution are much more apt to contain bacilli than the lower parts.

6. Tubercle bacilli which have been experimentally thrown into the blood-stream are rapidly removed from the circulation. Experiment suggests that the capillaries of the lungs are more efficient in arresting bacilli than the peripheral capillaries.

7. Chronic tuberculosis may produce a marked partial immunity against fresh inoculation with tubercle bacilli.

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EDITOR'S NOTE

In the paper by Drs. Marine and Lenhart on the "Relation of Iodin to the Structure of Human Thyroids," in *THE ARCHIVES* for November, 1909, on page 487, lines 17-16 from the bottom, occur the words, in parenthesis, "2 to 5 grams daily." This should read "2 to 5 grains three times daily."