

## EXPERIMENTAL EPIDEMIOLOGY OF TUBERCULOSIS.

### THE ELIMINATION OF TUBERCLE BACILLI IN THE FECES, BILE, AND URINE OF INFECTED GUINEA PIGS.

BY DAVID PERLA, M.D.

(From the Henry Phipps Institute, University of Pennsylvania, Philadelphia.)

(Received for publication, February 8, 1927.)

The study of an induced epidemic of spontaneous tuberculosis in guinea pigs, the results of which have been reported in a previous paper (1), has shown that the portal of entry of the infection is with few exceptions the intestinal tract, the mesenteric lymph nodes being secondarily involved. It has seemed desirable to determine whether the elimination of tubercle bacilli in the feces of experimentally infected guinea pigs is a source of infection and causes the spread of spontaneous tuberculosis.

It has been demonstrated that bacteria introduced into the circulation may be eliminated through the intestinal tract. Emmerich (2) in 1885 recovered the cholera vibrio from the intestinal tract of cats 6 hours after intravenous or subcutaneous inoculation. Hess (3) in 1910 found that *B. prodigiosus* introduced into the circulation is eliminated from the blood through the liver and the kidneys 1 hour after its injection and even after he tied off the duodenum just below the common bile duct and the canal of Wirsung he was able to recover the bacilli in the small intestine 2 hours after injection. Following the ligation of the common bile duct in guinea pigs, Ribadeau-Dumas and Harvier (4) were able to isolate *B. paratyphosus* from the intestinal tract 24 hours after intravenous injection. Breton, Bruyant and Mézie (5), repeating the experiments of Hess, recovered *B. prodigiosus* from the bile from  $\frac{1}{2}$  hour to 24 hours after intravenous injection of 1 mg. of a 24 hour culture. They cite an experiment in which they recovered tubercle bacilli from the bile at the end of 6 hours.

Though the literature<sup>1</sup> on the elimination of tubercle bacilli by tuberculous patients and cattle is extensive, there is no study which

<sup>1</sup> The literature on the elimination of tubercle bacilli in man and cattle is reviewed by Calmette in "Tubercle bacillus infection and tuberculosis in man and animals," translation by Soper, W. B., and Smith, G. H., Baltimore, 1923, p. 444.

demonstrates that tubercle bacilli are eliminated in the feces of patients or cattle when tuberculous lesions are not in open communication with the surface. When a definite communication exists between a caseous focus and the intestinal tract, it is obvious that tubercle bacilli will be eliminated in the feces.

*The Elimination of Tubercle Bacilli in the Feces of Experimentally Infected Guinea Pigs.*

An attempt was made to determine the significance of fecal contamination as a source of infection in the spread of spontaneous tuberculosis in guinea pigs.

*Experiment 1.*—Five guinea pigs were injected intraperitoneally with 0.001 mg. of a glycerol agar transplant 3 weeks old made from a virulent human strain of tubercle bacilli (P 15-B). One normal guinea pig received 0.01 mg. of the same culture subcutaneously. These animals were used in the epidemiological experiments described in a previous paper (1). The feces of these six guinea pigs were tested every week.

If the abdomen of a guinea pig is gently massaged 1 hour after feeding, fecal boluses are readily passed. These boluses, in the absence of an enterocolitis, are firm and uniform in size. Two boluses from each of the guinea pigs tested were collected in sterile Petri dishes. The feces of each animal were ground up in a test-tube with a small quantity of sterile saline solution and the emulsion was filtered through several layers of gauze. Twice the volume of 3 per cent sodium hydroxide was added to the tube and the whole was incubated during 1 hour at 37°C. in a water bath. The emulsion was then centrifuged and the supernatant liquid decanted. The sediment was neutralized against phenolphthalein solution (1 per cent) with 6 per cent hydrochloric acid. The volume was then brought to 5 cc. with sterile saline solution. 2 cc. of this emulsion was injected into the subcutaneous tissue of the thigh of each of two guinea pigs. Monthly intradermal tuberculin tests with 0.02 cc. of "old tuberculin" were made on all the test pigs. All test pigs were killed at the end of 3 months. The results of this experiment are given in Table I. All of the animals of which the feces were tested died of generalized tuberculosis.

Tubercle bacilli have passed through the tissues of the normal animal and are eliminated during the 1st week of infection. The development of immunity against the microorganism may later cause fixation of the bacilli within the tissue thus preventing their elimination. With dissemination of the infection, bacilli are again eliminated.

In the following experiment all animals were inoculated with the

TABLE I.

*Elimination of Tubercle Bacilli in the Feces of Experimentally Infected Guinea Pigs.*

	Tubercle bacilli in the feces tested at intervals indicated below:						
	1 wk.	2 wks.	3 wks.	4 wks.	5 wks.	6 wks.	
Guinea Pig 1, 0.001 mg. <i>B. tuberculosis</i> , intra-peritoneally	+	0	0	+	X	X	Died at end of 7 wks.
Guinea Pig 2, 0.001 mg. <i>B. tuberculosis</i> , intra-peritoneally	+	+	X	0	0	0	Died after 13 wks.
Guinea Pig 3, 0.001 mg. <i>B. tuberculosis</i> , intra-peritoneally	+	0	0	+	+	X	Died after 8 wks.
Guinea Pig 4, 0.001 mg. <i>B. tuberculosis</i> , intra-peritoneally	+	0	0	0	0	X	Died after 14 wks.
Guinea Pig 5, 0.001 mg. <i>B. tuberculosis</i> , intra-peritoneally	+	0	0	X	X	X	Died after 4 wks.
Guinea Pig 6, 0.01 mg. <i>B. tuberculosis</i> , subcutaneously	X	X	0	+	+	X	Died after 14 wks.

X = no test made on this date. + = tuberculosis present in the test animals.  
0 = no evidence of tuberculosis in the test animals.

TABLE II.

*Elimination of Tubercle Bacilli in the Feces of Experimentally Infected Guinea Pigs*

	Tubercle bacilli in the feces tested at intervals indicated below:							
	Before inoculation	1 day	2 days	4 days	1 wk.	3 wks.	5 wks.	
Guinea Pig 7, 0.001 mg. <i>B. tuberculosis</i> , intra-peritoneally	0	0	0	0	0	0	0	Died after 51 days of tuberculosis
Guinea Pig 8, 0.001 mg. <i>B. tuberculosis</i> , intra-peritoneally	0	0	0	0	0	0	0	Killed after 56 days of generalized tuberculosis
Guinea Pig 9, 0.001 mg. <i>B. tuberculosis</i> , intra-peritoneally	0	+	0	0	0	0	+	Died after 56 days of tuberculosis
Guinea Pig 10, 0.001 mg. <i>B. tuberculosis</i> , intra-peritoneally	0	0	0	0	0	0	+	Died after 52 days of tuberculosis

dose of tubercle bacilli used in Experiment 1 (Guinea Pig 6 excepted).

*Experiment 2.*—Four guinea pigs received 0.001 mg. of tubercle bacilli (P 15-B) injected into the peritoneal cavity. After intervals of 1, 2, 4, 7, 21 and 35 days the feces of each of these animals were collected and injected into two normal guinea pigs.

The foregoing experiments were not carried beyond the 6th week, and the following experiment was performed in order to collect more information concerning the elimination of tubercle bacilli.

*Experiment 3.*—Four guinea pigs with negative tuberculin reactions were inoculated with human tubercle bacilli of a glycerol agar growth (P 15-B) 3 weeks old, as follows:

Guinea Pig 11 received 0.001 mg. intraperitoneally and died during the 9th week.

Guinea Pig 12 received 0.00001 mg. intraperitoneally and died at the end of the 11th week.

Guinea Pig 13 received 0.01 mg. subcutaneously in the left thigh, and died at the end of the 7th week.

Guinea Pig 14 received 0.0001 mg. subcutaneously in the left thigh, and died at the end of the 13th week.

All of these animals died of disseminated tuberculosis. On the day when the feces were collected the animals were tested with 0.02 cc. of tuberculin. The procedure was that described in Experiment 1.

None of the guinea pigs inoculated with feces taken during the first 4 weeks of the infection developed tuberculosis. It is however noteworthy that the only animal in Experiment 3 comparable with those of Experiments 1 and 2 is Guinea Pig 11, but in this instance an early elimination of tubercle bacilli in the feces did not occur. Guinea Pig 12 received a very small intraperitoneal injection and in Guinea Pigs 13 and 14, injections were subcutaneous.

Tubercle bacilli were eliminated in the feces of the animals in Experiment 3 during the last few weeks of the infection. The elimination of tubercle bacilli occurred just before and during the interval when the tuberculin reaction was diminishing in intensity or was negative. The number of experiments, however, is not sufficient to establish definitely the relation of the tuberculin reaction to the terminal elimination of tubercle bacilli. The negative tuberculin reaction has been a manifestation of overwhelming infection and only after the disease is apparently well advanced and disseminated, are tubercle

bacilli eliminated in the feces in sufficient quantities to infect guinea pigs.

*The Elimination of Tubercle Bacilli in the Bile of Experimentally Infected Guinea Pigs.*

There have been numerous studies of the elimination of tubercle bacilli in the bile.

Calmette and Guérin (6) found that tubercle bacilli are eliminated in the bile of tuberculous rabbits. They injected a series of rabbits intravenously with 10 mg. of bovine tubercle bacilli and killed them at intervals of 24 and 48 hours, 3, 4, 5, 6 and 7 days. In each instance, the contents of the gall bladder were aspirated and injected into guinea pigs. Tubercle bacilli were recovered from the bile obtained on the 3rd and 7th days. By establishing a permanent biliary fistula in a heifer (7), they were able to make daily tests of the bile aspirated through the fistulous opening. Following intravenous injection of 3 mg. of virulent bovine tubercle bacilli, they found that tubercle bacilli were eliminated in the bile only after 19 days. The heifer died of miliary tuberculosis 26 days after inoculation. In a series of twenty-four guinea pigs with disseminated tuberculosis killed at a time when death was expected as a result of the infection, Remlinger (7) tested the contents of the gall bladder for tubercle bacilli by direct inoculation into guinea pigs and observed eighteen positive results. Sasano and Medlar (8) found that the appearance of tubercle bacilli in the bile of experimentally infected guinea pigs is indicative of tuberculosis of the liver and is usually evidence of an ulcerative tuberculous lesion of the biliary system. From the time the bile duct becomes involved until death, there is a continuous elimination of tubercle bacilli into the intestinal tract (9). The exact time of appearance of the tubercle bacilli in the bile is not stated in their work.

Tubercle bacilli have been found in the bile of patients dying of pulmonary tuberculosis by Fraenkel and Krause (10) and by Corper, Simmons and Freed (11). In patients suffering with tuberculosis tubercle bacilli have been discovered in the bile aspirated through a duodenal tube, by Carnot and Libert (12), Freed and Black (13), Olmer and Crémieux (14) and others. There is, however, no evidence recorded in the literature which conclusively demonstrates the elimination of tubercle bacilli through the biliary system in the absence of lesions of the liver or of the biliary tract.

To explain the spread of spontaneous tuberculosis it is desirable to determine when tubercle bacilli appear in the bile of infected guinea pigs and to compare the elimination in the bile with that in the feces. It may be that this is the main source of tubercle bacilli when the disease is disseminated and involves the liver. The attempt has been

TABLE III.  
Elimination of Tubercle Bacilli in the Feces of Experimentally Infected Guinea Pigs.

	Tubercle bacilli in the feces tested at intervals indicated below:																		
	24 hrs. before inoculation	24 hrs. after inoculation	2 days	4 days	6 days	11 days	2 wks.	3 wks.	4 wks.	5 wks.	6 wks.	7 wks.	8 wks.	9 wks.	10 wks.	11 wks.	12 wks.	13 wks.	
Guinea Pig 11 0.001 mg. <i>B. tuberculosis</i> , intraperitoneally Tuberculin reaction	0	0	0	0	0	0	0	0	0	0	+	+	+	+	+	+	+	+	+
Guinea Pig 12 0.00001 mg. <i>B. tuberculosis</i> , intraperitoneally Tuberculin reaction	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	1+	3+2+	4+	4+	4+	3+	1+	+	+	+	+	+	+
Guinea Pig 13 0.01 mg. <i>B. tuberculosis</i> , subcutaneously Tuberculin reaction	0	0	0	0	0	0	0	0	0	0	+	+	+	+	+	+	+	+	+
Guinea Pig 14 0.00001 mg. <i>B. tuberculosis</i> , subcutaneously Tuberculin reaction	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	1+	2+	2+3+	4+	4+	2+	+	0	0	0	0	0	0

The intensity of the tuberculin reaction is indicated by 1+, 2+, 3+ or 4+.

made to study the elimination of tubercle bacilli in the bile of infected guinea pigs throughout the course of the infection.

*Experiment 4.*—Nine normal guinea pigs of the same weight and age and from the same stock were inoculated subcutaneously with 0.001 mg. of the strain of virulent human tubercle bacilli (P 15-B) used in the previous experiments. These animals were killed at intervals of 24 and 48 hours, 4, 7, 14, 21, 28 and 42 days. The last animal died after 44 days. The animals were chloroformed and the ab-

TABLE IV.

	Interval between infection and death in days	Tubercle bacilli in bile	Tubercle bacilli in urine	Remarks
Guinea Pig 15 not infected		0	0	No tuberculosis
Guinea Pig 16	1	0	0	No tuberculosis
Guinea Pig 17	2	0	0	No tuberculosis
Guinea Pig 18	4	0	0	No tuberculosis
Guinea Pig 19	7	0	0	No tuberculosis
Guinea Pig 20	14	+	0	Tuberculous lesion at site of injection and in regional lymph nodes
Guinea Pig 21	21	+	0	Tuberculous lesion at site of injection and in inguinal, iliac and lumbar lymph nodes. Spleen enlarged but liver apparently normal
Guinea Pig 22	28	+	0	Same as Guinea Pig 17
Guinea Pig 23	42	+	0	Tuberculosis of spleen, liver and lungs
Guinea Pig 24	44	+	+	Tuberculosis of lymph nodes, liver, spleen and lungs

domen opened with aseptic conditions. The gall bladder was brought prominently into view, the surface thoroughly seared and the entire contents of the bladder aspirated. The organs of these animals were carefully examined and sections were made from the liver, kidney and site of inoculation.

The total volume was brought up to 4 cc. with sterile saline solution and injected subcutaneously into two guinea pigs. These test guinea pigs were killed as soon as the tuberculin reaction was strongly positive or at the end of 3 months. The results of this experiment are given in Table IV.

Tubercle bacilli were eliminated in the bile of the experimentally infected guinea pigs from the 14th day until death.

The following experiment was made to determine whether tubercle

bacilli were eliminated with the bile during the first few hours following inoculation.

*Experiment 5.*—Guinea pigs were injected into the peritoneal cavity with 0.01 mg. of a culture of *B. tuberculosis*, and after intervals of 4, 8, 12, 24 and 48 hours the bile of each of these pigs was collected and injected into two normal pigs. The results of this experiment are given in Table V.

After subcutaneous inoculation of tubercle bacilli with the dose used (0.01 mg.) tubercle bacilli have appeared in the bile only once within the first 2 days after injection.

TABLE V.

	Interval between infection and death in hrs.	Tubercle bacilli in bile
Guinea Pig 25	4	0
Guinea Pig 26	4	0
Guinea Pig 27	8	0
Guinea Pig 28	8	0
Guinea Pig 29	12	0
Guinea Pig 30	12	0
Guinea Pig 31	24	+
Guinea Pig 32	24	0
Guinea Pig 33	48	0
Guinea Pig 34	48	0

*Elimination of Tubercle Bacilli in the Urine of Experimentally Infected Guinea Pigs.*

Remlinger (7) in a series of twenty-four guinea pigs with disseminated tuberculosis killed at a time when death was expected found that the urine aspirated from the urinary bladder in half of the animals, contained virulent tubercle bacilli, demonstrated by animal inoculation.

Although there are numerous clinical studies demonstrating the presence of tubercle bacilli in the urine of individuals in whom there has been no evidence of renal tuberculosis, it is not improbable that transient bacteremia has caused minute focal lesions of renal glomeruli which have healed or have been overlooked at autopsy.

An attempt has been made to determine whether tubercle bacilli eliminated with the urine of tuberculous guinea pigs experimentally



infected for the elimination of tubercle bacilli in the urine, may facilitate the spread of spontaneous tuberculosis. In guinea pigs used in Experiment 4, the urinary bladder was seared and the urine was aspirated aseptically. The total volume from each animal was brought up to 4 cc. with sterile saline solution and injected subcutaneously into two guinea pigs. The results of this experiment are given in Table IV.

The urine aspirated from the bladder of infected guinea pigs did not contain tubercle bacilli until the terminal stage of the infection. Guinea Pig 24 died 44 days after inoculation and the urine aspirated from its bladder caused tuberculosis in guinea pigs.

#### DISCUSSION.

In the course of experimental infections in animals and in spontaneous disease in man, tubercle bacilli doubtless appear intermittently in the blood stream.

Calmette (15) in a review of the literature of bacteremia with tuberculosis maintains that with experimental tuberculous infection of animals bacteremia is present before a lesion appears at the site of inoculation and continues throughout the infection. A large quantity of blood may be necessary to show presence of tubercle bacilli. Calmette demonstrated tubercle bacilli in the blood stream by direct transfusion of 10 cc. of blood from a tuberculous into a normal guinea pig. This huge quantity of blood is approximately equivalent to a liter of human blood.

Bergeron (16) states that bacteremia in experimental tuberculosis of rabbits occurs soon after injection and is ephemeral.<sup>2</sup> Haga (18) tested the blood of guinea pigs infected subcutaneously with virulent human tubercle bacilli. He found by animal inoculation and by staining methods that tubercle bacilli were present in the blood stream during the first 10 days after inoculation in five of twelve experiments. The number of positive results diminished from the 10th to the 30th day, and almost wholly disappeared during the interval from the 30th to the 40th day. From the 40th day until death bacilli were again discovered in the blood stream in increasing numbers and were present in two-thirds of the animals examined. In 66 per cent of instances, they appeared between the 50th and the 70th day after inoculation.

In normal rabbits, Opie (19) has demonstrated that antigens such as horse serum or egg white are demonstrable in the blood during a period from approximately 7 to 9 days after injection. "With the progress of immunization injected antigen

---

<sup>2</sup> The literature of the subject is thoroughly reviewed by Löwenstein (17) and by Calmette (15).

exhibits a decreasing tendency to find its way into the circulating blood so that in a well immunized animal horse serum or egg white injected into the subcutaneous tissue is not demonstrable in the blood serum even if tests are made at intervals from 1 to 24 hours after injection." In a subsequent study it has been found that the antigen introduced into an immune animal is fixed at the site of injection (20).

In the studies of Krause (21) and Willis (22) on the spread of tubercle bacilli in normal and immune guinea pigs infected by subcutaneous inoculation, it has been demonstrated that tubercle bacilli inoculated intracutaneously or subcutaneously into normal guinea pigs are carried to the regional lymph node within an hour, and within 3 or 4 days they have been disseminated throughout the body and may be isolated from the lungs, hilum lymph nodes and other tissues. In immune animals their spread is greatly retarded. They remain fixed at or near the site of entry during about 7 days, do not reach the regional lymph nodes until 2 weeks after infection and are not disseminated throughout the body until 2 or 3 weeks after infection. "The immune state (that is, the allergic reaction) operates to retard the spread of the bacilli . . . rather than to inhibit . . . their activities or their multiplication" (21).

The observations described in this paper show that tubercle bacilli may be eliminated with the feces during the 1st week following inoculation. At a later period elimination ceases and does not begin again until the disease is far advanced. Elimination by way of the bile occasionally occurs immediately after inoculation, but is insignificant until tuberculosis is well established in the body. Elimination by way of the urine has occurred only when the disease has reached its terminal stage.

Tubercle bacilli appear in the blood stream of infected guinea pigs during the first 10 days following subcutaneous injection, but gradually disappear and reappear after about 40 days (Haga). Elimination of microorganisms in the bile of rabbits injected intravenously with large quantities of tubercle bacilli occurs within 3 days (Calmette and Guérin), and as early as 6 hours (Breton, Bruyant and Mézie). The evidence presented by these investigators and the observations reported in this paper show that tubercle bacilli introduced forcibly into the tissues of normal animals enter the blood stream and are eliminated in the feces before immunity against the microorganism is established. With the progress of immunization the bacilli become fixed in the tissues, are no longer capable of permeating intact mucous membranes and do not appear in the feces. When the infection is widely disseminated, resistance is overcome and the elimination of tubercle bacilli recurs.

## CONCLUSIONS.

1. After intraperitoneal inoculation of guinea pigs with tubercle bacilli, the microorganisms may appear in the feces and elimination may persist during 1 week. Subsequently during several weeks no tubercle bacilli can be recovered from the feces.

2. When tuberculosis is widely disseminated in the body of guinea pigs, tubercle bacilli are discharged with the feces.

3. Tubercle bacilli are occasionally eliminated in the bile of guinea pigs immediately after inoculation but are almost constantly found in the bile after several weeks when the disease is well established.

4. Tubercle bacilli are eliminated in the urine of experimentally infected guinea pigs only when infection is far advanced.

## BIBLIOGRAPHY.

1. Perla, D., *J. Exp. Med.*, 1927, xlv, 209.
2. Emmerich, R., *Arch. Hyg.*, 1885, iii, 291.
3. Hess, A. F., *Arch. Int. Med.*, 1910, vi, 522.
4. Ribadeau-Dumas, L., and Harvier, P., *Compt. rend. Soc. biol.*, 1910, lxi, 181.
5. Breton, M., Bruyant, L., and Mézie, A., *Compt. rend. Soc. biol.*, 1911, lxxi, 568; 1912, lxxiii, 58, 118.
6. Calmette, A., and Guérin, C., *Compt. rend. Acad.*, 1909, cxlviii, 601; *Ann. Inst. Pasteur*, 1913, xxvii, 162.
7. Remlinger, P., *Compt. rend. Soc. biol.*, 1923, lxxxviii, 409.
8. Sasano, K. T., and Medlar, E. M., *Am. Rev. Tuberc.*, 1924, x, 392.
9. Medlar, E. M., and Sasano, K. T., *Am. Rev. Tuberc.*, 1924, x, 351.
10. Fraenkel, E., and Krause, P., *Z. Hyg. u. Infektionskrankh.*, 1899, xxxii, 97.
11. Corper, H. J., Simmons, J. S., and Freed, H., *Am. Rev. Tuberc.*, 1923, viii, 260.
12. Carnot, P., and Libert, E., *Bull. et mém. Soc. méd. hôp. Paris*, 1921, xlv, 1101.
13. Freed, H., and Black, L. T., *Am. Rev. Tuberc.*, 1923, vii, 174.
14. Olmer, D., and Crémieux, A., *Rev. tuberc.*, 1924, v, 365.
15. Calmette, A., Tubercle bacillus infection and tuberculosis in man and animals, translation by Soper, W. B., and Smith, G. H., Baltimore, 1923, 238-249.
16. Bergeron, A., La présence du bacille de Koch dans le sang, Thesis, Paris, 1904.
17. Löwenstein, E., Vorlesungen ueber Bakteriologie Immunitaet, spezifische Diagnostik und Therapie der Tuberkulose, Jena, 1920, 146-164.
18. Haga, I., Mitteilungen der Robert Kochstiftung, ii, No. 1, cited by Löwenstein.
19. Opie, E. L., *J. Immunol.*, 1923, viii, 55.
20. Opie, E. L., *J. Exp. Med.*, 1924, xxxix, 659.
21. Krause, A. K., *Am. Rev. Tuberc.*, 1924, ix, 83; *Tubercle*, 1925, vii, 29.
22. Willis, H. S., *Am. Rev. Tuberc.*, 1925, xi, 427, 439.