Notes on Results of a Test for Tuberculosis in Rhesus Monkeys (*Macaca mulatta*)[1]

By C. R. CARPENTER *and* C. A. KRAKOWER[2]

From the Free Range Primate Colony, Santiago Island, The School of Tropical Medicine, San Juan, P.R.

THIS is a preliminary report dealing with the general results of applying by an intrapalpebral injection a specially prepared dosage of tuberculin (.025 mg. *Tablets Tuberculin* in .10 cc. of a sterile lactose diluent) to test Rhesus monkeys for tuberculosis.

Tablets Tuberculin, P.P.D. (Purified Protein Derivative) is a product obtained from "standard B.A.I. synthetic tuberculin" by the method of Dr. Florence B. Seibert.[3] Whereas the dosage for human subjects is divided into a First Test Strength of .00002 mg. and a Second Test Strength of .005 mg., it was decided, because of previous work by Schroeder,[4] to increase the strength for the monkeys to 1250 times the human First Test Strength or five times the human Second Test Strength, i.e., .025 mg., and to give the entire test in one injection.

In a previous paper Schroeder[4] has described the experimental basis for concluding that this test might detect successfully a high percentage of Rhesus monkeys having tuberculosis. Penrose, White, Brown and Pearson in 1923 adapted to monkeys the test employed by the Bureau of Animal Industry for detecting tuberculosis in cattle (Fox[5]). A later development in technique made by White and Fox[6] was the use of the temperature response as an indicator of positive or negative reactions. This method, though highly efficient, was impractical for testing large numbers of animals since it required a long routine for establishing individual temperature norms and

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2. C. R. Carpenter, now of the Pennsylvania State College; C. A. Krakower, of the Department of Pathology, School of Tropical Medicine, San Juan, P.R. Aided by a grant to Columbia University from the John and Mary Markle Foundation.

3. F. B. Seibert, J. D. Aronson, J. Reichel, L. T. Clark, and E. R. Long, a series of papers on Purified Protein Derivitive, a standardized tuberculin for uniformity in diagnosis and epidemiology, *Amer. Rev. Tuberc.*, 30:713–768. 1934.

4. C. R. Schroeder, "A Diagnostic Test for the Recognition of Tuberculosis in Primates. A Preliminary Report." *Zoötogica*, 23:298–400. 1938.

5. H. Fox, "Diseases in Captive Wild Mammals and Birds." Lippincott, Philadelphia. pp. 519. 1923.

6. C. Y. White and H. Fox, "Results of Tuberculin Tests in Monkeys at the Philadelphia Zoölogical Garden," *Arch. Int. Med.*, 4:517. 1909.

subsequent plotting of variations following the test injection. For this reason it was not used for the testing of the Santiago Rhesus colony.

Schroeder[7] described a modification of the intradermal test established by the Bureau of Animal Industry. This modification involved the Tablets Tuberculin solution in the eyelid (the intrapalpebral injection) rather than subcutaneously at some other site. The positive reaction typically shows a swelling of the eyelid, redness, and sometimes an area of necrosis. There is, however, a wide range of reactions from individual to individual, but the clinical significance of these variations is not yet known. The reaction can be easily and reliably observed from 24 to 96 hours following the injection, but most reactions occur within 48 hours.

When a breeding stock of monkeys was to be collected in India and taken to Santiago Island off Puerto Rico, the dangers of tuberculosis were already clearly vizualized; a danger which had been described by Schroeder.[8] Apparently, when wild primates are captured and come into contact with the human type tuberculosis, the type usually found in monkeys, a large percentage of the animals develop the disease shortly afterwards, as shipping records indicate, and many eventually die.[9] In order to prevent, as far as possible, the spread of the disease during shipment on a 14,000 mile voyage around the Cape of Good Hope, the monkeys were, in the main, caged individually, but it was also considered desirable to test the animals in India and eliminate positive reactors.[10] In India, however, it was found that time did not permit the testing of all animals due to the complicated operations of trapping, selecting, and caging over 500 animals for shipment. In addition, Hindu caretakers refused to catch and restrain the adults for testing purposes. Therefore, only a sampling of juveniles could be tested in Calcutta. These juveniles were shipped in group cages (15 to 20 animals in each cage) under the usual shipping conditions where the disease might be expected to spread.

All of the animals were trapped and caged in twelve districts in the region around Lucknow and shipped about 400 miles to Calcutta.

7. C. R. Schroeder, *loc. cit.*

8. C. R. Schroeder, "Acquired Tuberculosis in the Primates in Laboratories and Zoölogical Collections," *Amer. J. Pub. Health*, 28:469. 1938.

9. C. R. Schroeder, *loc. cit.*

10. For this purpose and for subsequent testing, Dr. John Reichel and Mr. Paul S. Pittenger of Sharp and Dohme Company made available to us 160 vials, each containing 10 tablets of .025 mg. Tuberculin P.P.D. and 160 vials, each containing 1.0 cc. of a sterile lactose diluent.

On September 24, 1938, in Calcutta, where they were being prepared for shipment to America, 70 specimens of the 6 to 8 and 8 to 10 pound classifications were tested. The single injection .025 mg. dosage was used. On the following day, about 24 hours after the injection, two of this group gave clear-cut, positive reactions. One of these showed marked swelling, redness, and an area of necrosis, while the other animal showed only a slight swelling. These animals were segregated in a single cage and prepared for shipment in order that we might learn whether or not they would survive the voyage, and if they did, they were to be re-tested and then autopsied in Puerto Rico.

On September 25, another group of 50 juveniles of the 6 to 8 pound class was tested. None of these gave positive reactions during three days of regular inspection. Therefore, in the total of 120 juveniles, the age usually handled by animal dealers, only two gave positive reactions. One of these died on shipboard shortly after leaving Capetown, and autopsy (Carpenter) showed extensive spread of tubercular nodules throughout the lungs, liver, spleen and intestines. The second animal, No. 381 in the autopsy report, survived the voyage, but after a second test in Puerto Rico gave a negative reaction. A third test was given to this animal in January, 1939, and resulted in a positive reaction; the autopsy reports proved the monkey to be tubercular:

> January 25, 1939, E.P. No. 548, Santiago Island No. 381. *Positive reactor-positive findings:* primary complex right lower lobe 1.5 x 0.7 cm.; caseous enlarged right peribronchial lymph node, also caseous tracheobronchial lymph nodes—no dissemination in other lobes of lungs; large conglomerate tubercules of spleen; tubercules in liver; tuberculosis of peripancreatic lymph node.

Through the contribution of Mr. A. W. Chater, 70 of the 118 juveniles tested in Calcutta which showed negative reactions were brought along with the shipment of adults for further tests after arrival in Puerto Rico. Some of these animals died during shipment, and autopsies showed negative findings for tuberculosis as far as could be determined, considering the inadequate facilities to be found on shipboard. When the remaining juveniles were tested in Puerto Rico, *none gave a positive reaction*. It is interesting to speculate on how many of these juveniles would have become tubercular had the two diseased animals been put with them in the

crowded group-cages during the voyage. The regrettable conditions found in these group-cages are customary for shipments of Rhesus monkeys from India.

The total number of animals tested for the Colony at the time of their release on Santiago Island consisted of 16 juvenile males, 30 juvenile females, 100 young adults or late juvenile females, and 174 adult females, making a total of 320 specimens tested. Twenty-seven adult males, for whom we did not have equipment or trained assistants for handling, were not tested. The 85 infants, still with their mothers, with the exception of three infants with positive mothers (see page 9) were not tested.

From that portion of the Colony's breeding stock tested while they were still at the School of Tropical Medicine in Puerto Rico, nine showed positive reactions. These varied in degree and extent of redness, swelling, and necrosis.

The nine animals were brought to necropsies within a few days after they were detected by the test described above. The autopsy records relating to tuberculosis in these monkeys are summarized in the following notes:

November 30, 1938. E.P. No. 451. *Positive reactor—positive findings:* mesenteric glandular tuberculosis with massive conglomerate tuberculosis of spleen and disseminated tubercules in liver. Tuberculous nephritis.

December 5, 1938. E.P. No. 459. *Strongly positive reactor—positive findings:* large primary tuberculous lesion left middle lobe with involvement of left peribronchial, tracheobronchial and paratracheal lymph nodes; and myriads in omentum, spleen, tail of pancreas (large tuberculous mass), liver, and kidney; caseous mesenteric lymph nodes.

December 5, 1938. E.P. No. 460. *Positive reactor—positive findings:* Tuberculoma of spleen; many tubercules in liver and kidney, mesenteric caseous adenitis and juxtaurinary vesicular tuberculous mass with serosal tubercules.

December 7, 1938. E.P. No. 463. *Strongly positive reactor—positive findings:* extensive abdominal lymph nodal tuberculosis (mesenteric, aortic, head of pancreas); numerous tubercules in all lobes of lungs and tracheobronchial lymph nodes; conglomerate tubercules in spleen, liver, and kidney.

December 8, 1938. E.P. No. 466. Santiago Island No. 261. *Strongly positive reactor—positive findings:* tuberculous pneumonia left upper lobe with cavitation; conglomerate tubercules in other lobes of lung. Massive caseous involvement tracheobronchial nodes with esophageal sinus and large caseous intrathoracic paratracheal lymph node; few small tubercules in spleen, liver; rare tubercules in kidney; caseous right submaxillary and right supraclavicular and tuberculous involvement of axillary lymph nodes.

December 9, 1938. E.P. No. 467. Santiago Island No. 221. *Mildly positive reactor—negative findings:* no trace of reaction in eyelid. Negative for tuberculosis.

December 12, 1938. E.P. No. 469. Santiago Island No. 250. *Mildly positive reactor—positive findings:* primary tuberculous complex right middle lobe (.8 cm. in diam.) with some softening. Early involvement of moderately enlarged tracheobronchial lymph nodes. No other lesions of tuberculosis.

December 14, 1938. E.P. No. 495. *Strongly positive reactor—positive findings:* tuberculosis of skin of face with massive caseous tuberculosis of submaxillary lymph nodes, also superior cervical lymph nodes; caseous left supraclavicular, right peribronchial and several abdominal lymph nodes; primary lesion right middle lobe (.5 to 1 cm. in diam.); scattered pulmonary conglomerate tubercules in liver; few tubercules in kidney.

December 16, 1938. E.P. No. 526. Santiago Island No. 271. *Positive reactor—positive findings:* left lower lobe large tuberculous pulmonic area, primary type, with cavitation; involvement of left peribronchial lymph node; few discrete tuberculous nodules in spleen; cirrhotic liver changes with tubercules microscopically; single tubercle in kidney and tubercules in one enlarged abdominal aortic lymph node.

After a period of about two weeks at the School of Tropical Medicine, the Colony, still in shipping cages, was moved to Santiago Island where the testing programme was continued. Twelve additional positive reactors were detected and isolated for further checks and retests.

On January 1, 1939 (three weeks after they had given the first positive reactions), the dose used in human tests was employed on the 12 specimens, using the First Test Strength of the human dosage, .00002 mg. None gave positive reactions. After the Second Test Strength, human dosage (.005 mg.), given five days later, eight of the tubercular animals showed marked positive reactions.

Two of the four monkeys who did not react to this test became very thin and ill, and after death were embalmed and sent to the School for detailed autopsies. The other two non-reactors were held until January 14, when they were given a third test of the specially prepared dosage of .025 mg. On the fifteenth of January, these two specimens, nos. 412 and 474, showed positive eyelid reactions.

Summaries of autopsy records for the 12 animals reacting positively from among those tested on Santiago Island follow:

January 19, 1939. E.P. No. 537. Santiago Island No. 395. *Positive reactor* (twice)—*positive findings:* extensive pneumonia tuberculosis right upper lobe, right middle lobe, left upper lobe, left lower lobe; extensive and marked caseous tuberculosis of mediastinal lymph nodes compressing left bronchus and displacing arch and thoracic position of aorta; large caseous tuberculomata of spleen; numerous conglomerate tubercules in liver; single tubercule in kidney; two small caseous mesenteric lymph nodes and three enlarged caseous peripancreatic lymph nodes. Subject embalmed.

January 19, 1939. E.P. No. 538. Santiago Island No. 460. *Positive reactor* (twice)—*positive findings:* single large caseous mesenteric lymph node; tubercules in spleen and liver. Subject embalmed.

January 21, 1939. E.P. No. 538. Santiago Island No. 481. *Positive reactor—positive findings:* primary complex upper lobe (.8 cm. in diameter). Caseous mediastinal and left peribronchial lymph nodes; single conglomerate tubercule in liver.

January 21, 1939. E.P. No. 542. Santiago Island No. 382. *Positive reactor—positive findings:* confluent caseous tuberculosis right upper lobe; frequent conglomerate tubercules in other lobes of both lungs; large caseous mass right peribronchial lymph node, tuberculosis of other mediastinal lymph nodes (tracheobronchial and right paratracheal); caseous conglomerate tubercules of spleen; myriads of tubercules in liver; few conglomerate tubercules in kidneys; caseous peripancreatic lymph nodes.

January 23, 1939. E.P. No. 543. Santiago Island No. 290. *Positive reactor—positive findings:* tuberculous bronchopneumonia left middle lobe and right lower lobe; miliary tubercules in mediastinal lymph nodes. No evidence of tuberculosis elsewhere.

January 23, 1939. E.P. No. 544. Santiago Island No. 275. *Positive reactor—positive findings:* caseous mesenteric and mesocolic lymph nodes, also abdominal aortic and peripancreatic lymph nodes; miliary peritoneal tubercles; tubercules in spleen and liver; single small tubercule in one kidney.

January 24, 1939. E.P. No. 545. Santiago Island No. 489. *Positive reactor—positive findings:* left peribronchial lymph node with thick fibrous capsule and several foci of caseation but no calcification, and adjoining smaller lymph node of same character; single tubercule in liver with microscopic tubercular lesion in lung.

January 24, 1939. E.P. No. 546. Santiago Island No. 383. *Positive reactor—positive findings:* primary complex right lower lobe 2.5 x 1.5 cm. Elsewhere conglomerate tubercules in lung; caseous right peribronchial lymph node; large conglomerate tubercules of spleen, liver; tubercules in kidney; tuberculosis of abdominal aortic lymph nodes.

January 25, 1939. E.P. No. 547. Santiago Island No. 385. *Positive reactor—positive findings:* caseous tuberculosis of right axillary, right inguinal and right iliac lymph nodes; otherwise no evidence of tuberculosis.

January 25, 1939. E.P. No. 548. Santiago Island No. 381. *Positive reactor—positive findings:* primary complex right lower lobe 1.5 x 0.7 cm.; caseous enlarged right peribronchial lymph node, also caseous tracheobronchial lymph nodes—no dissemination in other lobes of lungs; large conglomerate tubercules of spleen; tubercules in liver; tuberculosis of peripancreatic lymph node.

January 25, 1939. E.P. No. 550. Santiago Island No. 474. *Positive reactor—positive findings:* in left lower lobe of lung, single small lesion scarce .3 cm. in diam.; three large conglomerate tubercules in liver.

January 26, 1939. E.P. No. 551. Santiago Island No. 412. *Positive reactor—positive findings:* diffuse bronchopneumonic type of tuberculosis left upper lobe; small conglomerate tubercules in other lobes; marked caseous mediastinal lymphadenopathy with tracheal extension and sinus formation; tubercules in spleen, liver, and bone marrow.

Three of the Colony females who were found to be tubercular carried infants who had been born in India during July or August, 1938, and had been shipped with their mothers. On January 1, 1939, these infants were tested with the .025 dosage, but showed no positive eyelid reactions.

We are now ready to answer one question relative to the effectiveness of the test under consideration in detecting tuberculosis in Rhesus monkeys, namely, how reliable is the test in terms of the percentage of specimens showing the positive reactions which are confirmed by detailed autopsies? Of the 21 specimens which were selected as being tubercular on the basis of the eyelid reaction, 20 were found to have tuberculosis ranging from very limited to very extensive infection, as indicated by pathological data. Of those selected by the test, 95.2 per cent were found to be tubercular, as verified by autopsy observations.

A second question is more difficult to answer, namely, how effective is the test when we consider the number of animals which did not show the positive reaction and yet were tubercular? To answer this question conclusively it would have been necessary to have autopsied all tested monkeys. This was obviously impossible, but it is worth while collecting all available evidence bearing on this question.

One unhealthy specimen died and two more were brought to autopsy shortly after the testing programme was completed. Tissue samples from the first specimen were sent to the Department of Pathology; for the other two, the entire specimens were available for autopsy. None of these animals had given observed positive

reactions to the test but, upon being examined after death, all showed unquestionable tuberculous infections as summarized below:

March 1, 1939. E.P. No. 585. Santiago Island No. 314. *Negative reactor—positive findings:* large confluent primary tuberculous mass in one lung; numerous conglomerate tubercules elsewhere in both lungs; massive tuberculosis of spleen; discrete tubercules in liver and kidney.

March 11, 1939. Santiago Island No. 362. *Negative reactor—positive findings:* massive caseous mediastinal mass about apex of left lung; left peribronchial caseous and calcified lymph node; conglomerate tubercules in lung, spleen and liver; miliary tuberculosis of peritoneum (chiefly mesentery and omentum).

March 15, 1939. E.P. No. 592. *Negative reactor—positive findings:* extensive bilateral caseous pneumonia with cavitation; caseous enlarged mediastinal lymph nodes; tubercules in spleen, liver, kidney, muscular fasciae, peritoneum; caseous peripancreatic lymph node.

Though the autopsy records show advanced tuberculosis, there are no means of knowing whether or not these particular specimens had the disease in December, when they were tested. It is known that tuberculosis runs a rapid course in captive Rhesus monkeys and the keen competition among the animals with consequent lowered nutritional status may have hastened the course of the disease. Hence, it is entirely possible that they contracted the disease after the systematic testing program of December.

There is another possibility—the tuberculin test may hasten the progress of the disease. We have noted that some positive reactors rapidly approach the terminal course of tuberculosis and death.

Throughout the year there was an occasional animal (sometimes while it was still free, but usually after it had been caught and isolated) which proved upon autopsy to have tuberculosis. No. 377 died on April 2, and after being autopsied (Carpenter) showed extensive tubercules with nodules thickly bunched in liver and spleen. An old, weak male, who had been held in captivity for about six weeks because he could not compete with normal Colony males, died, and the extensive tubercular nodules throughout the lungs, spleen, liver, and small intestines, indicated that this disease had been the cause of his death. At the same time three juveniles were autopsied and found to have died of tuberculosis. Another old male who had long been held captive in the isolation cage died on April 30, and autopsy showed widespread tuberculosis.

On June 25, five underweight animals were caught and removed from the Colony. Upon being tested for tuberculosis, two reacted positively and were sent to the Pathology Department to await autopsies.

In addition, No. 325 died November 4, 1939, and our Island records list his death as being caused by tuberculosis.

Repeatedly we have had the opportunity to study animals in free ranging groups which have become sick or weak, and who are therefore strongly dominated by their fellows. These repeated attacks and the consequent deprivation of food accelerated the weakening process, the progress of the disease, and eventual death. Oftentimes, after these sick animals are weakened by disease, they are killed by the dominant, healthy animals of the same or other groups. These social antagonistic responses to sick animals, those with tuberculosis as well as other diseases, function as a means of eliminating the unhealthy specimens.

The fact that the Santiago Colony lives under free range conditions made it impossible to get records, especially autopsy records, on all specimens who died or who were killed in fights on the Island. We can only report that at least 12 individuals, and perhaps more, had tuberculosis in more or less advanced stages between the first tests in November and December, 1938, and the last two months of 1939.

Mr. M. I. Tomilin and Mr. L. M. González[11] retested the entire Colony during January and February, 1940. Using the same testing procedures and the same specially prepared tuberculin as was used by Carpenter the previous year, seven animals were found to give positive reactions. Since most of these monkeys were pregnant females which were being held for other experimental work, autopsy records are not available.

It seems important to record that Rhesus monkeys with advanced tuberculosis show a characteristic behaviour syndrome which is apparently highly indicative of the disease. When an untamed monkey is approached, it may try to escape by withdrawing or it may show evidence of attacking or bluffing. We have observed that in advanced cases of tuberculosis the sick animal is relatively inactive and listless; hence its behaviour tendencies are not expressed by normal withdrawal or attack; instead, the monkey cringes, bares its teeth and shows other evidencies of weakness or fright. In addi-

11. Thanks are extended to Mr. M. I. Tomilin and to Mr. Luis M. González for their effective assistance.

tion, poor hair coats and subnormal weight indicate probability of tuberculosis. These qualities of behavior and physique indicate that further checks should be made by X-ray, and the animals should be isolated until proved to be negative for tuberculosis.

Our records show that 7.5 per cent of the animals tested in Puerto Rico after their long voyage from India gave the positive eyelid reaction to the Sharp and Dohme test and dosage. Of our entire group, 7.19 per cent were shown to have tuberculosis by autopsy methods. Our records show that approximately 3 per cent of the total number of animals released on the Island died of tuberculosis during the first year, and that at the end of that time about 2.2 per cent of the total remaining monkeys gave positive reactions to the tuberculin test. It is significant to note that the Colony has lost at least 40 monkeys, predominantly adult females, either because they were eliminated as positive reactors, or because they died with the disease. We believe that more than this number, approximately 60, died of dysentery during the voyage from India and during the first year on the Island. Another factor, fighting, which caused death or fatal wounds, would rank equally with tuberculosis and dysentery as a cause of the heavy mortality rate of the first year.

This preliminary work on the programme of tuberculosis control in the Santiago Island Colony of Rhesus monkeys may be summarized as follows:

1. The number of juveniles giving the positive reaction to P.P.D. .025 mg. dosage injected into the eyelid, was found to be 1.66 per cent for 120 young Rhesus monkeys tested in India. This contrasts markedly with the findings of Schroeder[12] that some shipments of these monkeys newly arrived in America for medical laboratories have been found to show as high as 50 per cent positive tubercular reactions. The young Rhesus monkeys brought to Puerto Rico in group cages after positive tubercular reactors had been eliminated, were 100 per cent negative to the test. Though this result may have been partially due to the masking effects of the first tests, nevertheless, these facts strongly suggest that tuberculosis control in Rhesus specimens for research purposes should begin in India. All specimens should be tested and positive reactors eliminated before shipment to America.

2. The P.P.D. test as used in the work reported in this paper is found to be about 95.2 per cent reliable for specimens which showed the positive eyelid reaction and were later autopsied.

12. C. R. Schroeder, *loc. cit.* 8.

3. Three infants of tubercular mothers were found to react negatively to the .025 mg. dosage of P.P.D.

4. Data have been given that show that after about two months following testing, in which they reacted negatively, three specimens died of *advanced* tuberculosis. It is suggested that the test as administered may not accurately detect all stages of tuberculosis in monkeys. A minimum of nine other specimens are believed to have died of the disease during the following year in the free ranging Colony.

5. The percentage of positive reactors in the Colony seemingly decreased during the period from December, 1938 to February, 1940, from 7.5 per cent to about 2.2 per cent. These findings begin to show the efficacy of the tuberculosis control programme, although further systematic testing needs to be completed at more frequent intervals than a year in order to eliminate the disease from the Colony.

6. Tuberculosis, dysentery and fighting have been the main causes of the high mortality rate in the Colony during its first year on Santiago Island.