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## ETIOLOGY OF OROYA FEVER.

### V. THE EXPERIMENTAL TRANSMISSION OF *BARTONELLA BACILLIFORMIS* BY TICKS (*DERMACENTOR ANDERSONI*).

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The geographical distribution of Oroya fever, which occurs within certain limited areas of Peru, has suggested to many students of disease that infection is carried by some biting insect or arthropod, and Castellani and Chalmers<sup>1</sup> note particularly the analogous localization of the tick-borne disease, Rocky Mountain spotted fever. Entomological investigation in relation to Oroya fever has not so far implicated any particular insect, with the possible exception of *Phlebotomus verrucarum* Townsend,<sup>2</sup> and experimental work has been hampered by the lack of animals susceptible to infection with *Bartonella bacilliformis* and methods of cultivating the organism. These obstacles have recently been overcome,<sup>3,4</sup> however, and the presence of *Bartonella bacilliformis* in insects can now be definitely determined both by cultural methods and by infection experiments. There is considerable evidence,<sup>3-5</sup> moreover, that verruga peruviana is also caused by *Bartonella bacilliformis*, as had long been suspected because of the similar geographical distribution of the two conditions and their frequent association in the same individuals.

It was not possible at the moment to undertake an investigation of insects from the infected districts, but an indirect method of approach to the problem suggested itself, that of determining whether

<sup>1</sup> Castellani, A., and Chalmers, A. J., A manual of tropical medicine, London, 3rd edition, 1919.

<sup>2</sup> Townsend, C. H. T., *J. Am. Med. Assn.*, 1913, lxi, 1717.

<sup>3</sup> Noguchi, H., and Battistini, T. S., *J. Exp. Med.*, 1926, xliii, 851.

<sup>4</sup> Noguchi, H., *J. Exp. Med.*, 1926, xliv, 697.

<sup>5</sup> Noguchi, H., and Herculles, O., *Science*, 1926, lxiv, 121.

some well known carrier of infectious agents—such, for example, as the tick which transmits Rocky Mountain spotted fever,<sup>6</sup> tularemia,<sup>7</sup> and a filterable virus pathogenic for guinea pigs<sup>8</sup>—could also act as a vector of *Bartonella bacilliformis* under experimental conditions. Success in transmitting the parasite under such circumstances, while not solving the problem of the actual vector, would nevertheless furnish a starting point for the investigation of the question.

For the specimens of ticks (*Dermacentor andersoni*) used in these experiments, I am indebted to Dr. R. R. Parker, of the United States Public Health Service, Hamilton, Montana. The ticks were first allowed to feed on normal guinea pigs to determine whether or not they were free from the spotted fever virus. If the guinea pigs escaped infection, the ticks were then placed on the shaved abdominal skin of monkeys (*Macacus rhesus*) in various stages of infection with *Bartonella bacilliformis* and allowed to feed for periods varying from 24 hours to 6 days. Tests of infectiveness were made by feeding the ticks on normal *rhesus* monkeys either immediately following their removal from the infected animal or after an interval of 12 to 14 days.

Two series of experiments were made. In the first, the periods of tick feeding were relatively short—24 hours to 4 days, and several days were allowed to elapse between the removal of the ticks from the infected animals and the test feedings. Infection not being obtained under these conditions, in the second series the period of infective feeding was lengthened to 5 days, the ticks were transferred to normal animals immediately after their removal from the infected ones, and 6 days were allowed for the test feeding. Under these circumstances the ticks proved infective.

#### *Experiment 1 (Negative).*

Feb. 1, 1926. Thirty ticks were placed on three infected monkeys (Nos. 7, 12, and 14), ten on each animal, and allowed to remain attached for 1 to 4 days.

<sup>6</sup> Ricketts, H. T., *J. Infect. Dis.*, 1907, iv, 141.

<sup>7</sup> Francis, E., *J. Am. Med. Assn.*, 1925, lxxxiv, 1243.

<sup>8</sup> Noguchi, H., *J. Exp. Med.*, 1926, xlv, 1.

*M. rhesus* 7<sup>a</sup> had been inoculated intravenously and intradermally on Dec. 21, 1925 (41 days previously), with blood and suspensions of nodular tissue from Monkeys 2, 3, and 4. *Bartonella bacilliformis* was present in the blood 4 days after injection and on several occasions during the following month. The local lesions were well developed by Jan. 19 and were excised\* on that date. From Jan. 27 to Feb. 1 fever had been continuous, fluctuating between 104° and 105°F. The ticks were left attached for 24 hours (Feb. 1 to 2). Fever continued until Feb. 9. The blood had yielded cultures of *Bartonella bacilliformis* previously in dilutions as high as 1:10,000,000, but it developed subsequently that the titer had fallen to 1:10 at the time of the tick feeding.

*M. rhesus* 12 had been inoculated intravenously and intradermally on Jan. 19, 1926, with first generation cultures, derived from the blood of Monkeys 4, 6, and 7, and grown for 14 days on leptospira medium. The blood was positive by culture on Jan. 25 and on Feb. 3 (6 and 15 days after inoculation). The intradermal injections gave rise to small nodules, which, however, never progressed to typical lesions but gradually receded within a month to mere traces of induration. The temperature was continuously high (104°) from Jan. 30 to Feb. 5. The ticks were allowed to feed 72 hours (Feb. 1 to 4).

*M. rhesus* 14<sup>a</sup> had been inoculated on Jan. 29, 1926, with a suspension of nodular tissue from *M. rhesus* 3 and with cultures of *Bartonella bacilliformis* derived from human and monkey blood, the material having been introduced intradermally, subcutaneously, and by scarification. The temperature rose after 24 hours, and the animal remained febrile (104–104.6°F.) for 4 days. The ticks were allowed to feed on the animal Feb. 1 and 2. Blood taken on Feb. 8 yielded cultures of *Bartonella bacilliformis* in a dilution of 1:10 only. The local and general symptoms progressed steadily until on Feb. 23 the blood yielded cultures of *Bartonella bacilliformis* in a dilution of 1:10,000, the nodules on the eyebrows were very large and protruding, and those on the abdomen measured 1.5 × 3 cm. It is evident that the ticks were placed on the animal too early in the course of the infection.

Feb. 16, 1926. Three ticks of each lot of ten were placed on the shaved abdominal skin of *M. rhesus* 19, and two of each lot were dissected and suspensions of the viscera inoculated intradermally into the shaved skin of the eyebrows and abdomen of *M. rhesus* 20. The suspensions were tested also by culture. No infection could be demonstrated in the ticks, and the cultures were negative for *Bartonella bacilliformis*.

*M. rhesus* 19. Nine ticks, of which three had been fed on *M. rhesus* 7, three on *M. rhesus* 12, and three on *M. rhesus* 14, were placed on the shaved skin of the

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\* All operations were performed under ether anesthesia.

abdomen on Feb. 16, 1925, and allowed to remain 4 days. All became attached. The animal showed no signs of infection—no enlargement of the lymph nodes, fever, or skin lesions. Because of prolapse of the rectum, the animal was killed by etherization on Mar. 6, 18 days from the time of tick feeding. Nothing abnormal was found at autopsy, and cultures of heart blood, bone marrow, spleen, lymph nodes, liver, kidneys, testis, and lungs were negative for *Bartonella bacilliformis*.

*M. rhesus* 20. Inoculated Feb. 16, 1926, intradermally into the shaved skin of eyebrows and abdomen with the mixed suspensions of the viscera of ticks fed on Monkeys 7, 12, and 14. There were small indurations at the sites of intradermal inoculation on the eyebrows after 10 days, but they disappeared within 3 weeks. The abdominal skin showed no reaction to the injections. The temperature rose to 104° or higher (104.6°) on several occasions (Feb. 19, 21, and 24, Mar. 7 and 11). No swelling of the lymph nodes was noted during 42 days of observation.

#### *Experiment 2 (Positive).*

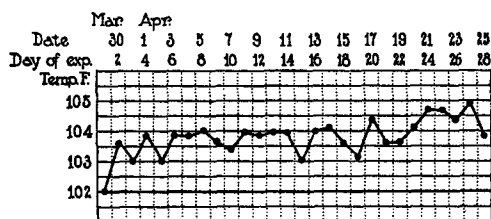
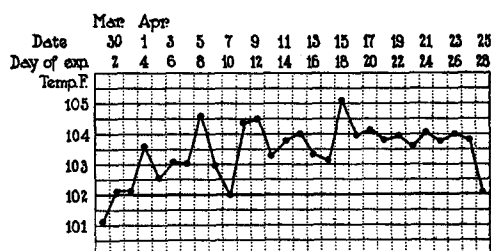
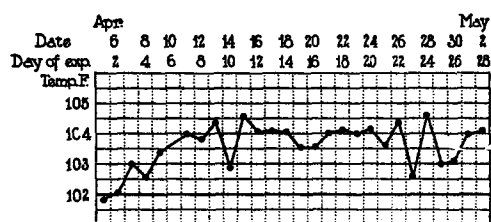
Four ticks were allowed to feed on each of two infected monkeys (*M. rhesus* 18 and *M. rhesus* 23) for 5 days, Mar. 24 to 29, 1926.

*M. rhesus* 18<sup>a</sup> had been inoculated on Feb. 15, 1926, intradermally and by scarification on the shaved eyebrow with a suspension of nodular tissue from *M. rhesus* 5. On Mar. 5 and 18 the blood yielded cultures of *Bartonella bacilliformis* in a dilution of 1:100,000. The nodules on the eyebrows became extremely large. During the time of tick feeding (Mar. 24 to 29) the temperature fluctuated daily between 104° and 105°. On Mar. 27 the blood was positive in a dilution of 1:10.

*M. rhesus* 23 had been inoculated Mar. 9, 1926, intravenously and by scarification with fourth generation cultures derived from *M. rhesus* 7, and intradermally with a suspension of the nodule excised from *M. rhesus* 18 on that date. The local inoculations induced in time very striking nodules and indurations, and blood taken on Mar. 22 yielded growth of *Bartonella bacilliformis* in a dilution of 1:100,000. The temperature was 104°F. on Mar. 24, when the ticks were placed on the abdominal skin. All were moderately engorged when removed on Mar. 29. The course of infection in this animal subsequently became very severe both locally and systemically. There was a prolonged period of fever, and 2 weeks later spontaneous miliary eruptions appeared on the face. The animal was sacrificed May 1, 42 days after inoculation.

On removal from the infected animals, each lot of ticks was immediately placed on a normal *rhesus* monkey and allowed to feed for a period of 6 days. After the feeding the ticks were dissected, and mixed suspensions of the viscera were inoculated into another normal

monkey. Definite, though mild, infection was induced in all the animals, and *Bartonella bacilliformis* was recovered in culture from the blood and lymph nodes. The suspensions of the tick viscera also yielded cultures of the organism.

TEXT-FIG. 1. *M. rhesus* 26.TEXT-FIG. 2. *M. rhesus* 27.TEXT-FIG. 3. *M. rhesus* 28.

*M. rhesus* 26. Mar. 29, 1926. Four ticks which had been allowed to feed for 5 days on *M. rhesus* 18 were placed on the shaved skin of the abdomen and left there for 6 full days. On removal, two of the four were found dead, the other two were well engorged. The animal began to show irregular mild fever (Text-fig. 1) 11 days from the time the feeding began, and the lymph glands in

the inguinal and axillary regions on both sides were somewhat swollen. On Apr. 14 one of the inguinal lymph nodes was excised for examination. A suspension of the tissue yielded cultures of *Bartonella bacilliformis*. The temperature was 104–105°F. from Apr. 21 to 25, and blood taken on Apr. 24 yielded cultures of *Bartonella bacilliformis* in a dilution of 1:10. The enlargement of the lymph glands has steadily progressed up to the time of writing (May 30, 1925.) No skin lesions have developed.

*M. rhesus* 27. Mar. 29, 1926. Four ticks which had been allowed to feed for 5 days on *M. rhesus* 23 were placed on the shaved skin of the abdomen and allowed to feed for 6 full days. All the ticks became attached and were moderately engorged at the time of removal. The reaction in this animal was much the same as that in the foregoing monkey. Cultures made with a suspension of one of the inguinal lymph nodes excised on Apr. 14 and with blood withdrawn on Apr. 24 were positive in dilutions of 1:10. The animal has shown irregular febrile reactions (Text-fig. 2), but no skin lesions have developed up to the time of writing.

*M. rhesus* 28 (Text-fig. 3). Apr. 5, 1926, inoculated intradermally on the left eyebrow with a suspension in 1 cc. saline of two ticks fed on *M. rhesus* 26 and on the right eyebrow with a suspension in 2 cc. saline of four ticks fed on *M. rhesus* 27. Six-tenths of a mixture of the two suspensions was injected intravenously. The course of events in this animal was very similar to that in Monkeys 26 and 27. Small nodules appeared at the sites of intradermal injection on the eyebrows about 8 days after inoculation, but they did not progress further. The lymph node removed from the inguinal region on Apr. 14 and blood withdrawn on Apr. 24 both yielded cultures of *Bartonella bacilliformis*. A few papular eruptions had appeared on May 19. The skin became yellowish in color about a month after inoculation and has remained so up to the time of writing.

#### SUMMARY.

Experiments are reported in which *Bartonella bacilliformis* was transmitted from infected to normal *rhesus* monkeys by the bite of the tick, *Dermacentor andersoni*. A long period of feeding, both on the infected animal and on the normal animal subjected to infection, was required in order to secure positive results. The infection transmitted by the ticks was mild, but definite, as shown by the recovery of *Bartonella bacilliformis* from the lymph nodes and blood.