

ETIOLOGY OF OROYA FEVER.

VIII. EXPERIMENTS ON CROSS-IMMUNITY BETWEEN OROYA FEVER AND VERRUGA PERUANA.

By HIDEYO NOGUCHI, M.D.

(From the Laboratories of The Rockefeller Institute for Medical Research.)

(Received for publication, December 30, 1926.)

A common etiologic origin of Oroya fever and verruga peruana was first indicated experimentally by the production of verrucous lesions in monkeys (*Macacus rhesus*) by inoculation of cultures of *Bartonella bacilliformis* isolated from the blood in a fatal case of Oroya fever.¹ Subsequent experiments with the same strain^{2,3} gave evidence that the type of disease induced by the parasite was dependent upon two factors, the virulence of the strain, and the degree of susceptibility of the individual animal, various grades of infection being induced in the experimental animals, from a moderate or extensive local skin affection to a severe systemic disease terminating in fatal anemia. More recently,⁴ there has been isolated from the skin lesion of verruga peruana a microorganism morphologically and serologically identical with *Bartonella bacilliformis* and having similar pathogenic properties. It remained to be proven that animals which had recovered from infection with the Oroya strain of *Bartonella bacilliformis* would be able to resist infection with the verruga strain.

The disease induced by *Bartonella bacilliformis* in *M. rhesus* is chronic in nature, but complete recovery may take place and is evident from (1) the subsidence of the febrile reaction, (2) the disappearance of the skin lesions, (3) the sterility of the blood and lymphatic glands, as shown by cultivation experiments, and finally (4) immunity to the homologous strain. All of the ten monkeys

¹ Noguchi, H., and Battistini, T. S., *J. Exp. Med.*, 1926, xliii, 851.

² Noguchi, H., *J. Exp. Med.*, 1926, xliv, 697.

³ Noguchi, H., *J. Exp. Med.*, 1926, xliv, 715.

⁴ Noguchi H., *J. Exp. Med.*, 1927, xlv, 175.

(nine *Macacus rhesus* and one chimpanzee) selected for the present investigation had been shown by cultivation of blood and lymphatic gland tissue to be free from organisms, and five of them had been proved immune to the homologous (Oroya) strain by one or more previous immunity tests. The final test of immunity against both strains was made with suspensions of nodular tissue from *M. rhesus* 41 (verruca strain, second passage) and *M. rhesus* 36 (Oroya strain, fifth passage), the Oroya strain being inoculated on the right eyebrow and abdominal skin, intradermally and by scarification, the verruca strain similarly on the left side. Both suspensions induced well developed lesions in a control monkey, the verruca strain being decidedly the more virulent locally, as shown in the protocol of this animal. In the ten animals which had previously passed through a course of infection with the Oroya strain no signs of infection could be induced.

M. rhesus 1 had been inoculated on Nov. 16, 1925, intradermally and intravenously, with the first generation cultures of the blood of Case S. A. 15.¹ *Bartonella bacilliformis* was cultivated from the blood several times during the course of disease, and nodules arose at the site of intradermal inoculation. The lesions had disappeared within 2 months, and on Feb. 22, 1926, cultures of blood and lymphatic gland tissue were negative. Mar. 9, 1926, the animal received intravenously and intradermally a mixture of cultures and a suspension of nodular tissue from *M. rhesus* 18.² Blood taken on Mar. 31, and Apr. 13 proved sterile. On Apr. 29 the animal was inoculated again, intradermally and by scarification, with a mixture of cultures with a suspension of nodular tissue from *M. rhesus* 25.² On May 19, minute lesions appeared to be developing on the eyebrows, but the slight indurations disappeared, and blood and lymphatic gland tissue taken on June 1 yielded no cultures. The final test with both Oroya and verruca strains was made on June 3, the inoculation being made intradermally and by scarification. No lesions developed.

M. rhesus 2 was first inoculated Dec. 8, 1925, and had passed through a protracted course of infection with the Oroya strain.¹ The blood had yielded cultures on several occasions, and the nodules at the sites of intradermal injection on the eyebrows were still present on Mar. 27. They had disappeared by Apr. 27, and the blood and lymphatic gland tissue taken on that date were negative by culture test. On Apr. 29 the animal was reinoculated with a mixture of cultures with a suspension of nodular tissue from *M. rhesus* 25.² No signs of local or systemic infection appeared, and blood and lymphatic gland tissue proved sterile on June 1. On June 3 the animal was inoculated intradermally and by scarification with both the Oroya and the verruca strains. Blood and lymph node cultures made June 22 and July 13 proved sterile. No local lesions developed.

M. rhesus 3 had been inoculated with the same material as *M. rhesus* 2 and had passed through a similar course of disease. Only traces of the local lesions were visible on Mar. 8, 1926. On Mar. 9 the animal was inoculated intradermally and by scarification with a suspension of nodular tissue from *M. rhesus* 18.² Blood taken on Mar. 22 yielded cultures of *Bartonella bacilliformis*, and small but definite lesions developed at the sites of local inoculation. These had disappeared by Apr. 28, however, and the blood was sterile. On Apr. 29 a suspension of nodular tissue from *M. rhesus* 25² and cultures were introduced intradermally and by scarification. No local reactions resulted, and on May 19 and June 1 blood and lymphatic gland tissue were sterile. On June 3 the animal received the inoculation of both the Oroya and the verruga strains, intradermally and by scarification. The blood remained sterile, and no local lesions developed.

M. rhesus 12⁵ had been inoculated on Jan. 19, 1926, intravenously, subcutaneously, and by scarification with cultures derived from Monkeys 4, 6, and 7. The blood was positive by culture on two occasions, and there was high fever for 1 week, but the local lesions were never large, and they disappeared within a month. On Mar. 25 blood and lymphatic gland tissue were sterile. On Apr. 22 the animal was inoculated with a suspension of nodular tissue from *M. rhesus* 18,² cultures derived from Monkeys 7 and 20, and citrated blood of *M. rhesus* 25.² The blood was negative by culture on May 27, and lymphatic gland tissue was negative on June 1. No lesions developed. On June 3 the final test with both the Oroya and the verruga strains was made. The blood and lymphatic tissue were sterile when tested on June 22, and no lesions developed.

M. rhesus 16 had received two injections (Feb. 10 and Feb. 15, 1926) of killed cultures of *Bartonella bacilliformis*. No reaction resulted. On Mar. 6 active suspensions of the skin lesions from *M. rhesus* 5² and *M. rhesus* 18 were injected intradermally and by scarification. By Apr. 5 a large nodule had developed on the abdomen. On Apr. 14 it was larger and deep cherry red. By Apr. 28 the lesion had practically healed. On Apr. 29 the animal received cultures and a suspension of nodular tissue of *M. rhesus* 25² intradermally. Small papules appeared at the sites of injection on May 22, but they disappeared, and on June 1 no lesions were apparent. Blood and lymphatic gland tissue taken on this date were sterile. The final test with both the Oroya and the verruga strains was made on June 3. No lesions developed. The blood was sterile when tested on June 21.

M. rhesus 18² had been inoculated on Feb. 15, 1926, intradermally and by scarification with a suspension of nodular tissue from *M. rhesus* 5. Extraordinarily extensive lesions had developed by Mar. 17, and blood taken on Mar. 18 yielded cultures of *Bartonella bacilliformis* in dilutions as high as 1:100,000. The lesions persisted for many weeks but had become very small, pale, and fibrous by the middle of June. Blood and lymphatic gland tissue taken on May 15 yielded no cultures. No reaction followed the inoculation of the Oroya and verruga strains on June 3, and on June 22 the blood and lymphatic tissue were sterile by culture test.

M. rhesus 22, inoculated Mar. 5, 1926, intradermally and by scarification on

⁵ Noguchi, H., *J. Exp. Med.*, 1926, xliv, 729.

eyebrows and abdomen with nodular tissue of *M. rhesus* 5. Apr. 26, blood diluted 1:1,000 yielded cultures of *Bartonella bacilliformis*. A nodule had developed on the right eyebrow, and by May 6 it was very large and pedunculated. The lesion had disappeared by June 1, and blood and lymphatic gland tissue taken on that date were sterile by culture test. The final test with the Oroya and verruga strains was made on June 3. No lesions developed during 4 weeks of observation, and the blood was sterile by culture test on June 22.

M. rhesus 26⁶ had been infected by the bites of four ticks previously fed on *M. rhesus* 18, the ticks having been transferred from Monkey 18 to Monkey 26 on Mar. 29, 1926. Lymphatic gland tissue (inguinal) taken on Apr. 14 yielded cultures of *Bartonella bacilliformis*, as did also blood taken on Apr. 24. On May 28 blood and lymphatic tissue were both sterile. The animal was inoculated on June 3 with both the Oroya and the verruga strains. No lesions developed during 6 weeks of observation. The blood was negative by culture test on June 22.

M. rhesus 31 was inoculated intradermally Apr. 5, 1926, with a suspension of testicular tissue of Rabbit 1744⁶ and nodular tissue of Dog 1.⁶ Blood taken on Apr. 28 yielded cultures in a dilution as high as 1:10,000. The nodules were well developed at this time. May 22, the nodules were reduced in size. May 28, blood culture sterile. June 1, lesions had disappeared, blood and lymphatic tissue failed to yield cultures. June 3, final test with Oroya and verruga strains. No reaction. Blood sterile on June 22.

Chimpanzee (*Pan leucoprymnus*), had been inoculated Jan. 29, 1926, intradermally, subcutaneously, and by scarification, with mixture of cultures and suspension of nodule of *M. rhesus* 3. Blood positive in dilution of 1:10,000 Feb. 23, 1:100 Mar. 12. Large nodules developed on eyebrows and abdomen. The animal had recovered completely by May 1, and neither blood nor lymphatic gland yielded cultures of *Bartonella bacilliformis*. On June 3 the animal was tested for immunity against the Oroya and verruga strains in the same way as the nine *rhesus* monkeys. No lesions developed, and blood culture was negative on June 28.

The following protocol illustrates the effects produced in a non-immune control by inoculation of the materials used on June 3 for the final test against the Oroya and verruga strains of *Bartonella bacilliformis*.

M. rhesus 45 was inoculated June 3, 1926, with the same materials and in the same manner as the recovered monkeys, the Oroya strain being inoculated on the right eyebrow and abdominal skin, the verruga strain at corresponding sites on the left side. Several minute nodules were recognizable at the sites of inoculation on

⁶ Both the testicular tissue of the rabbit and the nodule of the dog yielded cultures of *Bartonella bacilliformis*; the report of these and other experiments will be published later.

the left side within 10 days and had attained considerable size (8 × 20 mm.) 32 days after the inoculation. The reactions at the sites of inoculation with the Oroya strain did not become noticeable until 3 weeks after inoculation. The nodules reached their maximum size (4 × 8 mm.) by the middle of July, never attaining the extent of the lesions on the left side. All the lesions were similar in character, however, being of the usual subcutaneous, *mular* type. Cultures made with blood withdrawn on June 28, 1926, 25 days after inoculation, yielded growth of *Bartonella bacilliformis* in a dilution of 1:100,000. All the lesions had healed by the middle of August.

The experiments demonstrate that *rhesus* monkeys which have recovered from infection with the Oroya strain of *Bartonella bacilliformis* are completely immune to the verruga strain. The results warrant the conclusion that the strains from Oroya fever and verruga peruana are identical, and that both conditions are manifestations of infection with *Bartonella bacilliformis*.

The duration of the infection in the present series of animals varied from 2 to 5 months. The cultural test of lymphatic gland tissue appears to be extremely important in determining whether or not the animal is free from infection, as has already been shown in another series of experiments,⁷ in which it was found that two animals of a group of four which had apparently recovered still harbored *Bartonella bacilliformis* in certain organs 48 and 58 days, respectively, after inoculation. In this earlier series of animals convalescence was established as early as 30 days in some instances, while in others the infection was still active 68 days after inoculation. The duration of a fatal infection varies from 25 to 57 days.⁷

SUMMARY.

Nine monkeys (*Macacus rhesus*) and a chimpanzee which had recently recovered from an infection with the Oroya strain of *Bartonella bacilliformis* were tested for immunity against the verruga strain of *Bartonella bacilliformis* as well as against the homologous strain. Complete immunity to both strains was demonstrated. The result establishes the identity of the strains and is in agreement with the result of comparative serological study.

⁷ Noguchi, H., *J. Exp. Med.*, 1927, xlv, 437.

The criteria of recovery include not only the subsidence of febrile reactions and local lesions but also a negative result of cultural tests of blood and lymphatic gland tissue.

Recovery may occur as early as 1 month after inoculation, but in most instances a period of 2 to 5 months is required for the completion of convalescence.