Experimental Infection of Macacus Rhesus with *Rickettsia quintana* (Trench Fever) (20464)

HERMAN MOOSER AND FRITZ WEYER, (Introduced by Peyton Rous)

From the Hygiene-Institut der Universität, Zürich, Switzerland, and Bernhard-Nocht-Institut, Hamburg, Germany.

Of all the known rickettsiae pathogenic for man *Rickettsia quintana* exhibits the strictest host specificity. No small laboratory animal has been found susceptible to the agent of trench fever. So far all attempts at cultivation of *R. quintana* in the chick embryo and in tissue cultures have failed. The same narrow range of susceptibility to *R. quintana* obtains with respect to blood sucking arthropods. The 2 species of lice parasitizing man, *P. humanus* and *Ph. pubis,* are apparently the sole arthropods in which *R. quintana* is able to survive and multiply(1). Codeleoncini reported the successful inoculation of 2 baboons, *Papio doquerae*(2). They reacted with intermittent paroxysms of fever similar to those occurring in trench fever of man. A small percentage of the lice (*Pediculus*), which were fed on one of the baboons, became infected with extracellular rickettsiae. Codeleoncini had accidentally recovered his strain of *R. quintana* from lice which had been reared on natives in Erythrea for the preparation of Weigl's antityphus vaccine. Several cases of trench fever had occurred among them before he experimented with baboons. In a laboratory, where work is done with *R. quintana,* a small percentage of lice may become infected by feeders who never show any morbid signs. Hence the possibility that some of Codeleoncini's "clean lice" had become infected before they were transferred to the baboon cannot be excluded with certainty. Constant control of the lice of the breeding stock is indispensable in order to be able to exclude an occasional infection of a small percentage of them.

Materials and methods. Seven rhesus monkeys were inoculated intravenously, each with a suspension in nutrient broth of the intestines of 9-12 heavily infected lice. Four strains of *R. quintana* were at our disposal. Four of the monkeys were inoculated, each with the intestines of a different batch of lice infected with strain Ossijek. The remaining 3 monkeys were inoculated, each separately, with one of the 3 other strains. Strain Ossijek was isolated in Jugoslavia from cases of trench fever among prisoners of war(3). Since its isolation this strain has caused trench fever in 12 persons. Nine of the cases were due to experimental inoculation. The remaining 3 occurred spontaneously in a bacteriologist and 2 members of his family. A thirteenth person, who was inoculated twice (a Swiss, who had never been outside the country), remained entirely well. Unfortunately the blood of that person was not examined for the presence of rickettsiae. The other 3 strains of *R. quintana* were isolated in Hamburg, Germany, between 1949 and 1953. Two of these strains were obtained from lice which had been reared and fed separately on 2 healthy laboratory aides with no previous history of trench fever. According to previous conceptions these 2 strains would have to be classified as *R. pediculi,* Rocha Lima, 1917. The third strain was recovered from a person who had acted as feeder of lice during several years. He fell ill on December 20, 1952, with intermittent fever and headaches. Lice fed on him in the hospital during a whole week remained free of rickettsiae. Nevertheless, trench fever was suspected and the patient received an intensive treatment with aureomycin which was followed by prompt and lasting result with respect to clinical symptoms. However, 100% of the lice fed on him after his discharge from the hospital in January 1953 showed *R. quintana.* At present, end of May, he still infects a high percentage of the lice. These Hamburg strains have been kept going exclusively by Weigl's method since their isolation.

The feeding of *Pediculus* on monkeys is a tedious and cumbersome procedure. We therefore had recourse to Weigl's method, *viz.*, the rectal inoculation of clean lice with

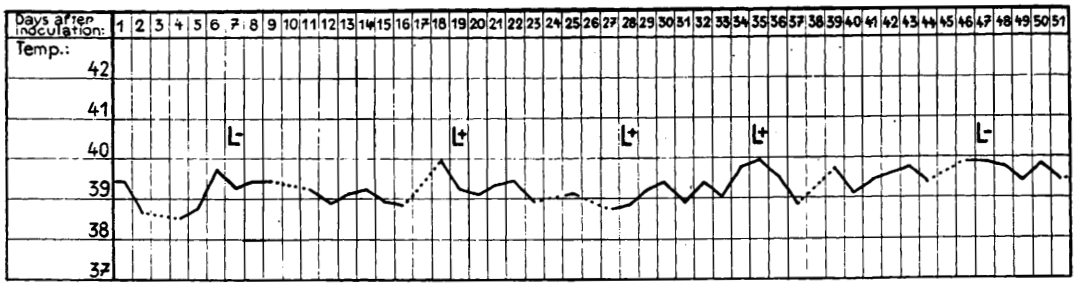

FIG. 1. Temperature chart of *M. rhesus* No. 1, inoculated on 9/29/1952, with strain Ossijek. L—, lice showed no rickettsiae. L+, lice showed rickettsiae.

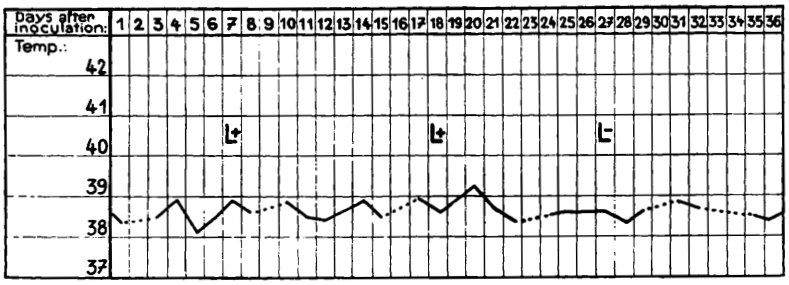

FIG. 2. Temperature chart of *M. rhesus* No. 2, inoculated on 11/21/1952, with strain Hagel I (Hamburg). L—, lice showed no rickettsiae. L+, lice showed rickettsiae.

a drop of blood drawn from the ears of the inoculated monkeys. It was diluted 1:5 with broth to prevent coagulation. Each louse received a single inoculation. In 3 instances lice were allowed a single feeding on a given monkey. In 7 louse tests performed with the monkeys' blood, the lice were inoculated through the vagina into the celom. Thereafter all lice were fed on a healthy person. The stocks of lice, from which batches were removed for the louse tests, were controlled by the daily examination of stained smears of the feces which had accumulated in the feeding boxes and by the examination of smears of the guts of frequent samples of lice taken from the stocks. The intracelomic inoculation served as an additional check of the origin of the rickettsiae which appeared in the lice which had fed on or which had been inoculated with the blood of a given monkey. *R. quintana* multiplies abundantly in the celom of the louse(4). The rickettsiae are easily demonstrated in a smear of the small drop of hemolymph which extrudes from the stump of an amputated leg. An admixture of intestinal contents with the lymph is impossible by this procedure. Rickettsiae found in the hemolymph of the lice intracelomically inoculated with the blood of one of the monkeys could therefore not derive from one of the human louse feeders. The first louse test on each monkey was performed 6-9 days after its inoculation, with one exception. The tests were repeated at intervals of about 12 days until the infectivity for lice of the blood of a given monkey had ceased. For each test 25 to 50 lice were used. All the lice which survived 5 days were examined for the presence of rickettsiae.

Course of infection in M. rhesus. None of the 7 monkeys showed definite signs of disease. Some of them seemed somewhat more restless and irritable than usual at the beginning of the blood invasion with rickettsiae, about 6-7 days after inoculation. The rectal temperature, taken every morning except Sundays, did not show any impressive alterations. In 3 monkeys there were slight peaks above the normal temperature range at irregular intervals, suggesting temperature curves of mild cases of the disease in man.

In the 4 remaining animals the temperatures remained entirely within normal limits during the whole course of observation, 42 to

100 days, respectively.

Rickettsemia. R. quintana appeared in the blood of all the 7 monkeys. The rickettsemia lasted from 10 to 82 days as estimated from the length of the interval between the first and the last positive louse test performed on each monkey. In 6 monkeys the first blood withdrawal for the louse test was performed 6, 7, 8, 8, 9, and 16 days, respectively, after the monkeys had been inoculated. All tests were positive. The blood of the 7th monkey was negative on the 7th day, positive on the 16th. The degree of the rickettsemia could be estimated approximately in each monkey from the percentage of lice which became positive in each consecutive test. In the lice which had been inoculated rectally, the proportion of positive lice reached from 39 to 100%, respectively. Materially the same percentages of positive lice were obtained with intracelomic inoculation. Of the 3 batches of lice which were fed, each on a different monkey, one batch consisted of freshly hatched larvae, the other 2 consisted of adult females. Ten per cent of the larvae became infected with *R. quintana,* whereas of the females 79 and 100%, respectively, became infected. The last positive louse test performed on each monkey scored 4 to 24% of infected lice. Considering the fact that the lice received either a single intrarectal or intracelomic injection of blood diluted 1:5, or were allowed only a single feeding, the high percentage of positive takes indicated a considerable degree of rickettsemia in all our monkeys. After a single feeding on a human case of trench fever, the percentage of lice showing rickettsiae, in our experience, may be much lower than that observed in these 7 *rhesus* monkeys.

Summary. Rhesus monkeys were successfully inoculated with one or another of 4 strains of *R. quintana.* Several of the animals showed a long-lasting rickettsemia. As is the case in trench fever of man, the infection in some of the monkeys remained subclinical. There was no correlation between clinical manifestations and the degree of rickettsemia. The *rhesus* monkey is a satisfactory animal for the experimental study of trench fever.

1. Weyer, F., *Z. Tropen. Parasitol.,* 1952, v3, 302.
2. Codeleoncini, E., *Boll. Soc. Ital. Med. Ig. Trop,* 1946, v6, 129.
3. Mooser, H., Leeman, A., Chao, S. H., and Gubler, H. U., *Schweiz, Z. Path. Bakt.,* 1948, v11, 513.
4. Weyer, F., *Z. Tropen. Parasitol.,* 1950, v2, 40.

Received June 26, 1953. P.S.E.B.M., 1953, v83.

CH Hemagglutinogen in Primates.* (20465)

DONALD C. A. BUTTS (Introduced by R. T. Hill)
(With the technical assistance of Doren E. Bergstresser)
From the Department of Tropical Disease Research, Division of Research and Industry, University of Miami and the Veterans Administration Hospital, Coral Gables, Fla.

Since the Ch factor was first reported(1), our efforts have been directed toward the development of high titer anti-Ch serum devoid of any heterospecific agglutinins and to extend our observations to individuals of all blood groups rather than limiting our studies to individuals of blood group O, as was previously done.

Preparation of anti-Ch serum. Prior to starting the immunization, the sera from young, male rabbits were tested for their normal heterospecific titer using OMN Rh-positive washed human erythrocytes for the determination. In no case were rabbits employed which showed the presence of species agglutinins in a titer exceeding 1-32. Following such screening, immunization with washed chimpanzee cells was started. Two male

* Reviewed in the Veterans Administration and published with the approval of the Chief Medical Director. The statements and conclusions published by the author are the results of his own study and do not necessarily reflect the opinion of the Veterans Administration.