EXPERIMENTAL STUDIES ON THE ETIOLOGY OF TYPHUS FEVER.

V. SURVIVAL OF THE VIRUS IN COLLODION SACS IMPLANTED INTRAABDOMINALLY IN GUINEA PIGS.

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In a previous paper 1 of this series, one of the writers showed that the typhus virus found in the blood of guinea pigs during the height of typical experimental typhus fever does not survive at 37°C. in anaerobic media for as long a period as in the same media under aerobic conditions. When oxygen is excluded, the viability period is 24 to 48 hours, whereas in the same media unprotected from atmospheric oxygen, the period is usually 5 days.

The virus as it exists in the tissues of guinea pigs with the experimental disease has been found to resist drying for 6 days. 2 Landsteiner and Hausmann 3 determined that frozen brain emulsions containing the virus remained active for 6 to 7 days. Nicolle and Blaizot 4 kept similar frozen material mixed with two parts of saline solution and one part of gelatin for 6 days without loss of virulence. At incubator temperature (37°C.), however, the activity of the material was lost after 2 days. Otto and Chou 5 and Otto and Papamarku 6 showed that virus in brain emulsions kept in the ice box (at +8°C.) retained its infectivity for 4 to 7 days, but the authors first mentioned found that when the material is added to normal guinea pig serum, the period of viability is lengthened to 10 days. However, at 37°C., the period is diminished to 6 days. Recently, Wolbach and his coworkers 7

4 Nicolle, C., and Blaizot, L., Compt. rend. Acad., 1915, clxi, 646.
employing tissue cultures of infected guinea pig brains, have found these cultures of the first generation to be infective up to 14 days. This is the longest period as yet recorded during which the virus has maintained its activity.

A review of the literature reveals that the typhus virus present in the brain of the guinea pigs with the experimental disease survives 6 to 7 days at freezing temperatures but when mixed with ordinary media, such as serum, under aerobic conditions, it survives for 10 days. At 37°C., the virus in the blood or brain can maintain its infectivity for 6 days; in ordinary media such as broth or serum, from 5 to 6 days; and in tissue cultures for periods up to 14 days.

In work previously reported we have employed the deductive method to study the incitant of typhus fever and have demonstrated the need of the virus for oxygen. Using the method once again we have now attempted to lengthen by experimental procedures its period of survival under artificial conditions.

We sought to employ a method which would keep the virus under constant oxygen tension and at the same time give a continuous supply of nutritive elements. It is known that there is a considerable oxygen tension in the peritoneal cavity. Haggard and Henderson estimated this tension at about 45 mm. Gates has shown that nutrient materials from the body of the guinea pig pass through collodion sacs with sufficient rapidity to promote a luxuriant growth of bacteria, even when they are in distilled water. By employing the collodion sacs described by Gates and implanting them intraperitoneally after inoculation with the typhus virus, both our requirements are met.

Method.

Collodion sacs for intraperitoneal or intraabdominal implantation were carefully prepared according to Gates' instructions. Caution was exercised to make the sacs permeable, yet not so thin as to cause breakage.

4 cc. of citrated blood were obtained, as described elsewhere, from guinea pigs in the 1st, 2nd, or 3rd day of the febrile period of the experimental disease. By means of a Pasteur pipette the blood was transferred to the sac, which was sealed and then implanted, under the strictest asepsis, in the peritoneal cavity of a nor-

9 All operations were performed under complete ether anesthesia.
real, healthy, 350 gm. guinea pig. In some experiments, two inoculated sacs were placed in the same guinea pig without any apparent inconvenience to the animal. After 28 days to 6 weeks the sacs were removed from the guinea pig and the contents poured into a Petri dish or the clotted blood with which, as a rule, the sacs were tensely distended was macerated and from 2 to 3 cc. of this material were injected intraperitoneally into normal guinea pigs to test for infectivity.

In duplicate experiments, instead of employing virulent blood, the infected cerebral or splenic tissue was inoculated into the sacs. In all these instances in which tissue was used, the sacs were found broken after 1 to 2 weeks, probably as result of osmosis brought about by tissue autolysis. This difficulty remains to be overcome.

Guinea pigs harboring intraperitoneally sacs inoculated with blood containing typhus virus showed in three different experiments no febrile or other reactions. When, on the other hand, the contents of sacs removed after 28 to 31 days were injected into normal guinea pigs, these developed typical experimental typhus fever, showing that the virus was still active. The criteria for determining the typical reaction were: (a) Transmissibility of the virus from guinea pig to guinea pig indefinitely. (b) Specific pathology of the affected animals during the height of the reaction. There were no apparent macroscopic changes in the organs except an enlarged spleen and a petechial rash in the skin. Histological examination revealed the typical vascular and nodular changes in the different organs, especially about the blood vessels of the brain. (c) Absence of concomitant or secondary infections with bacteria of the ordinary species, and absence of growth on blood culture in the usual media. (d) The presence of cross-immunity on test with known typhus virus. All these conditions were fulfilled in our inoculated animals, as required for the definition of the experimental disease, a point emphasized by Doerr and others.

Guinea pigs carrying sacs inoculated with blood containing typhus virus intraabdominally for 7 weeks were, like those observed for shorter periods, unaffected throughout this time; but the sac contents tested at the end of the period failed to be infectious.

Two experiments were made in which sacs with a typhus content were implanted intraabdominally in rabbits. After 2 weeks in the animals, the sac contents proved inactive for guinea pigs.

12 The skin lesion will be described in detail in a later paper.
By implanting intraperitoneally in guinea pigs Gates' permeable sacs inoculated with blood containing typhus virus, the period of viability of the virus under artificial conditions has been extended to 31 days. This we believe is the longest time the typhus virus has been so maintained. The oxygen tension in the peritoneal cavity fulfills a necessary requirement of the virus, and the ready osmosis from the body fluids of the guinea pig into the sac undoubtedly acts to prolong viability. In this latter relation it is significant that similar experiments with the rabbit have failed. It is difficult to state at present whether an active multiplication of the virus occurs within the sac, but the prolongation of the viability of the virus therein offers ground for the hope that the method may be useful in cultivation tests.

CONCLUSION.

The typhus virus contained in the blood of guinea pigs at the height of the experimental disease remains infective for 31 days in collodion sacs placed within the abdominal cavity of guinea pigs.