EXPERIMENTAL STUDIES ON THE ETIOLOGY OF TYPHUS FEVER.

III. Filtration Experiments.

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In a previous paper[1] we presented a plan of studying the problem of the inciting agent of typhus fever by the deductive method; that is, by applying to the typhus virus a variety of known tests in order to determine the nature of the particular incitant which may reside therein. From the experiments performed, it was concluded that the virus, in inducing experimental typhus fever in guinea pigs, invites invasion of the body of the animals by a variety of bacteria, which complicate the infection but have no etiological relationship to typhus,[2] and that the virus itself survives for a longer period in aerobic than in anaerobic culture media.[1]

In this article, the deductive method is continued and additional experiments on the typhus virus are presented relating to the filter ability of the virus as it exists in the organs of guinea pigs during the height of the reaction to inoculation.

HISTORICAL.

Heretofore, most of the experiments on the filterability of typhus virus have been made with the blood taken from guinea pigs or monkeys at the height of the experimental typhus infection. Goldberger and Anderson,[3] after reviewing all previous experiments, state that there have been eight attempts, by different workers, to pass the virus of typhus through a Berkefeld filter. Of these, six were negative; in one of the other two (Wilder, 1911[4]), the monkey that had been

[1] Olitsky, P. K., *J. Exp. Med.*, 1921, xxxiv, 115.

[2] Olitsky, P. K., *J. Exp. Med.*, 1921, xxxiv, 525.

[3] Goldberger, J., and Anderson, J. F., *Bull. Hyg. Lab., U. S. P. H., No. 86*, 1912, 62.

[4] Wilder, R. M., *J. Infect. Dis.*, 1911, ix, 9.

inoculated with the filtrate, without giving any evidence of infection, was later found to be resistant to an immunity test with active blood; in the other (Nicolle, Conor, and Conseil, 1910[5]), one of two monkeys inoculated with filtrate is described as having presented a doubtful reaction, and later was found to be resistant to an inoculation with active blood.[6] Goldberger and Anderson then described their own experiments, which convinced them that the virus, as it exists in the blood, is not filterable.

Later, in 1914, Nicolle, Blanc, and Conseil[7] inoculated into monkeys filtrates prepared from lice harboring the virus, and while no typical reaction resulted, it was found that on reinjection with active blood only a delayed and mild reaction occurred, which these workers regarded as evidence of a partial immunity.

In 1917, the writer's[8] efforts to filter the virus in the blood of experimentally infected guinea pigs during the reaction were fruitless. He concluded that the virus was not filterable.

The foregoing review shows that as yet no conclusive experimental basis has been offered to prove that in typhus blood the virus is filterable, although Nicolle and his associates hold that opinion.

In 1911, Nicolle, Conor, and Conseil[9] assumed that the virus of typhus fever is an intracellular parasite. Later observers, especially those who have linked the *Rickettsia prowazeki* with the incitant of typhus fever (da Rocha-Lima,[10] Wolbach and Todd,[11] and others) have taken the same stand.

The experiments to be described relate to two points: first, the supposed intracellular nature of the inciting agent, and second, the filterability of the virus. In order to approach more nearly ideal conditions of experiment, we employed organs derived from the infected animals instead of the blood.

Methods.

The spleen and brain of typhus-infected guinea pigs at the height of their reaction were chosen. Two strains of virus from human

[5] Nicolle, C., Conor, A., and Conseil, E., *Compt. rend. Acad.*, 1910, cli, 685.

[6] In a later communication, we shall present evidence to show that the production of immunity in isolated instances after the injection of filtrates may possibly be dependent on other factors than a living multiplying agent.

[7] Nicolle, C., Blanc, G., and Conseil, E., *Arch. Inst. Pasteur Tunis*, 1914, ix, 84.

[8] Olitsky, P. K., *J. Infect. Dis.*, 1917, xx, 349.

[9] Nicolle, C., Conor, A., and Conseil, E., *Compt. rend. Acad.*, 1911, cliii, 578.

[10] da Rocha-Lima, H., *Arch. Schiffs- u. Tropen-Hyg.*, 1916, xx, 17.

[11] Wolbach, S. B., and Todd, J. L., *Ann. Inst. Pasteur*, 1920, xxxiv, 153.

sources were used: one originating at Warsaw,[12] the other obtained from a Czecho-Slovakian immigrant at the Port of New York. Both strains have been described in a previous report.[13] That the spleen and brain are capable of inducing the experimental disease in guinea pigs has been shown by Nicolle and Blaizot[14] as well as by later workers. Indeed, Landsteiner and Hausmann[15] have induced experimental typhus fever in guinea pigs by inoculating as small a dose as 0.005 gm. of the infected brain tissue.

The first experiments refer to the problem of the supposed intracellular location of the virus and to an attempt to free it through cellular disintegration.

Repeated Freezing and Thawing.—An infected guinea pig, at the height of its reaction, was exsanguinated.[16] The spleen was removed under sterile conditions; one-fifth was kept for a control test and the remainder finely pulped with scissors. The pulp was placed in a test-tube containing 20 cc. of Ringer's solution, and frozen solid by means of calcium chloride in cracked ice at −26°C. The frozen mass was then thawed out to the original fluid condition and immediately refrozen. A small portion of the pulp was removed and tested for growth *in vitro* with negative results.[17] In the meanwhile, the remainder of the pulp in suspension, with the exception of 2 cc. employed in the control test, was filtered through a tested Berkefeld V or N candle and the filtrate inoculated in the manner to be described.

Freezing and Desiccating.—In this test the spleen was frozen and then desiccated to a flaky powder according to the method of Swift.[18] As in the case of the previous method, controls of tissue before desiccation were employed. The remaining four-fifths of the desiccated splenic tissue was suspended in Ringer's solution and filtered after 4 cc. had been removed for control purposes.

Crushing in a Mechanical Tissue Crusher.—The same conditions existed in this test as in the above, except that the spleen was placed in a mechanical tissue crusher and the tissue juice and very fine homogeneous semifluid pulp resulting were suspended in Ringer's solution and filtered, the 2 cc. of the original unfiltered material being withheld for control purposes.

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[12] We owe this strain to the kindness of Dr. S. B. Wolbach.

[13] Olitsky, P. K., *J. Exp. Med.*, 1921, xxxiv, 365.

[14] Nicolle, C., and Blaizot, L., *Arch. Inst. Pasteur Tunis*, 1916, ix, 127.

[15] Landsteiner, K., and Hausmann, W., *Med. Klin.*, 1918, xiv, 515.

[16] All operations were performed under deep chloroform anesthesia.

[17] This test was performed by Dr. Alexis Carrel.

[18] Swift, H. F., *J. Exp. Med.*, 1921, xxxiii, 69.

Grinding with Sand.—In the same way, the cut up fragments of spleen were ground with sterile fine white sand in a mortar, with the addition, from time to time, of 5 cc. of Ringer's solution until the required amount (20 cc.) was obtained. All but 2 cc. of the thin, even, homogeneous suspension was then filtered.

Precisely similar procedures were carried out with brain tissue from infected guinea pigs.

Of the four methods employed in treating the cellular elements of the tissues, those of repeated freezing and thawing at $-26°C.$, and freezing and desiccating are known to kill the cells completely.[19] Crushing or grinding tissues destroys the cells, but it is presumed that an occasional living cell may escape. However, the following experiments demonstrate that the virus in the tissues prepared by any of these methods retains an undiminished infecting power. Hence one method serves to control the others.

There were thus available for testing (*a*) typhus-infected tissues of guinea pig spleen and brain, (*b*) the same tissues, either frozen, desiccated, crushed, or ground, and suspended in Ringer's solution, and (*c*) filtrates of these suspensions which were freed from the tissue cells and detritus.

Procedure.—Each experiment comprised six animals. Guinea Pig A was injected intraperitoneally with 2 cc. of the suspension of infected tissue. This animal served as a control to test the infecting power of the virus in the tissue.

Guinea Pig B was injected intraperitoneally with 2 cc. of the suspension of the unfiltered suspensions of infected tissue, either frozen, desiccated, crushed, or ground, in the order of the tests made. This animal served as a control to test the effect on the virus of the methods used in disintegrating the tissue cells.

Guinea Pigs C and D were injected with 5 cc. of the filtrate of the particular suspension of the tissue (either frozen, desiccated, crushed, or ground) to be tested. Of these animals, Guinea Pig C was allowed to run its course so as to detect any delayed reaction and, in certain instances, was reinjected, after a suitable interval, with active typhus virus in order to test its immunity. Guinea Pig D was killed from 4 to 7 days after injection,[20] and 3 cc. of its defibrinated blood were inoculated into each of two normal animals, E and F. In this way the possibility of transmission of a living, multiplying agent might be detected and the pathological changes in the body of Guinea Pig D studied.

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[19] For the literature on this subject, see Wells, H. G., Chemical pathology, Philadelphia, 4th edition, 1920, 373.

[20] During this period the blood of guinea pigs experimentally infected contains active virus.[2]

RESULTS.

In all, fourteen experiments, as detailed above, were made with infected spleen and brain derived from guinea pigs at the height of the experimental infection: Three were made with the frozen infected tissue; two with the desiccated tissue; three with the crushed tissue, and six with the ground tissue. The results of the different experiments were practically identical.

Guinea pigs of Series A, injected with the infected tissue which was not disintegrated, showed, after an incubation of from 5 to 10 days, typical experimental typhus fever. On transmission by means of the blood obtained during the height of the reaction to normal animals, the latter, in turn, exhibited the typical fever. This was characterized by the specific histopathological picture, immunity to subsequent injections of active virus, freedom from concurrent or secondary infections, and transmissibility of the fever to normal animals—thus agreeing with the definition of the experimental disease described elsewhere.[13] Hence the materials employed for the filtration tests were proved to have specific infecting power.

Guinea pigs of Series B, injected with the infected tissue which had been either frozen, desiccated, crushed, or ground, exhibited the typical experimental typhus fever paralleling that shown by the animals of Series A.[21] Therefore, the tissue cells killed by repeated freezing and thawing, or by freezing and desiccating, or crushed into detritus by mechanical means, or ground into a homogeneous fine pulp, have had no effect on the infecting power of the typhus virus.

Guinea pigs of Series C and D, inoculated with the filtrates of suspensions of the frozen, desiccated, crushed, or ground infected tissue, failed to show the typical experimental disease. In five experiments, the animals of Series C were reinjected with active typhus virus; all failed to show any immunity. In ten experiments, the blood of guinea pigs of Series D, obtained 4 to 7 days after injection with the filtrates, was inoculated into normal animals (Series E and F). In none of these was there induced either typical experimental typhus

[21] The unfiltered desiccated tissues were injected 6 days after drying; in the instances in which the tissues were disintegrated by the other methods, the injections were made immediately after disintegration.

fever or immunity. Hence the filtrates failed both to cause the typical disease and to produce immunity.

To summarize the results of these experiments, we find that fourteen attempts to filter the active typhus virus present in the brain or spleen of guinea pigs at the height of reaction have ended in failure.

SUMMARY AND CONCLUSIONS.

We have presented experiments to show that the typhus virus in the tissues of the guinea pig during the height of reaction to the experimental disease does not lose its infecting power when the cells of the brain or of the spleen are disintegrated by repeated freezing and thawing, or by freezing and desiccating, or by crushing by mechanical means, or by grinding into a homogeneous pulp with sand. The virus after such treatment is as actively infective as in the same tissue not subjected to the disintegrating influences. The possibility exists, therefore, of an extracellular condition of the typhus virus.

Fourteen attempts to filter through Berkefeld V and N candles the virus contained in the disintegrated tissue have all resulted in failure.