SPIROCHAETOSIS OF MICE DUE TO SPIROCHAETA MURIS N.SP. IN THE BLOOD.

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(One Figure.)

WHILE studying the infection of *Trypanosoma dimorphon* in mice at the Pasteur Institute some five months ago, I noticed in one of the fresh blood preparations made from a brown mouse (*A*) infected with the trypanosomes, a small and very active spirochaete. At first I was of the opinion that its presence in the blood was due to contamination from the skin, but, as it was constantly present and was found in blood taken from any part of the body, it was evident that it was parasitic in the blood.

Two mice (*B, C*) were inoculated subcutaneously with blood taken from this mouse. Mouse *A* died shortly afterwards of its trypanosome infection. About three hours after its death, preparations were made from many of the internal organs and the spirochaete was found to be present therein. At the same time other mice (*D, D'*) were inoculated with the juice of the liver and spleen. Of these mice, those which had been inoculated from the liver developed a trypanosome and spirochaete infection which proved fatal, while those inoculated from the spleen developed only a spirochaete infection. This fact is interesting as showing that in the spleen the trypanosomes are more readily destroyed than the spirochaetes after the death of the animal.

Returning to the mice (*B, C*) which were inoculated with the fresh blood of the original mouse (*A*) before it had died, it was found that there developed first a spirochaete infection and that a few days later trypanosomes appeared in the blood, thus giving rise to a mixed infection. Advantage was taken of this observation when I subsequently proceeded to isolate the spirochaetes.

Blood from one of these mice (*B*, *C*) was taken before the trypanosomes had appeared but after the spirochaetes had been found in its blood, and was inoculated subcutaneously in another mouse (*E*). This mouse developed a pure spirochaete infection. It was thus possible to isolate the spirochaete in two different ways, in the one case by inoculations with material from the spleen, in the other by inoculations with blood prior to the appearance of trypanosomes therein. In other words a pure spirochaete infection was successfully induced.

Description of Spirochaeta muris.

In the fresh blood of the mice the spirochaete is found as a very active spiral. There is difficulty in seeing it clearly in the living condition owing to its active movements and small size. Moving along very rapidly, and turning on its long axis, it will suddenly come to rest and as suddenly dart off in an opposite direction. It seems a matter of indifference which end is directed forward. At times it spins rapidly about its central point, and at other times it rests one end against a red corpuscle or other body, while the free end vibrates rapidly to and fro. The spirals are permanent and the body fairly rigid though admitting of a certain amount of bending. Of course, owing to its small size, little of its structure could be made out in the fresh specimen, but all its movements seem to indicate the presence of flagella though these were never actually seen. There was no sign of an undulating membrane.

In blood preparations the spirochaetes remained active for hours after the trypanosomes had ceased to move. In stained preparations the spirochaete is seen as a uniformly staining spiral, the longer forms, however, show a clear unstained central spot (see Diagram, Nos. 8, 9). The ends are slightly tapering; there is no sign of a

Diagram 1. *Spirochaeta muris* n.sp. Different forms of the spirochaete. The circles represent the contours of red blood corpuscles. Giemsa stain. Drawn under Zeiss $\frac{1}{2}$ inch oil immersion.

nucleus or undulating membrane. As just mentioned, the larger forms have a clear spot at their centre, and in some of these the body

of the spiral tapers towards this spot. In some cases two small spirals are attached end to end by an unstained region. These forms are evidently stages of transverse division. No indications of longitudinal division were seen nor any mode of reproduction other than the one just mentioned.

The number of turns of the spiral varies from six in the longest forms to two in the shortest. The length of the spirochaetes varies from 6 or 7 μ to 3 or 4 μ . The width is about 2 μ ¹.

The spirochaete was always seen in the spiral form; no other forms were found at any time.

Inoculation experiments.

As mentioned above, it is possible to transmit the infection to mice by inoculating them with the blood of an infected animal. The smallest drop of blood is sufficient to give rise to a typical infection. There is always an incubation period of 5—6 days before the spirochaetes are found in the peripheral blood. At first very scanty, they increase in numbers during several days till about the tenth day, when they attain their maximum, and then their numbers begin to decline. At the height of the infection the numbers present are never very great, being at most five or six to a field (Zeiss D objective), but even this number is unusually large. The parasites disappear from the blood gradually in the course of two or three months, and, even when apparently absent, a prolonged search will generally reveal the presence of a few. I have a mouse now which was inoculated last March, over five months ago, and still at least one spirochaete may be found in each blood preparation. Judging from the results obtained by the inoculation of no less than 50 mice, these animals are all susceptible to infection by inoculation, the period of incubation not varying. I have never encountered a case of natural immunity.

The spirochaete infection does not appear to produce any abnormal condition in mice. In three very young mice which were inoculated the infection ran an ordinary course, and although two of the mice died after the intensity of the infection had commenced to decline, their death was probably due to other causes. The third mouse is still living (four months) and occasionally a spirochaete may be found in its blood.

¹ In specimens stained by Giemsa's method and examined in cedar oil without mounting in balsam. The spirochaete may be readily stained by any of the ordinary stains, Giemsa, fuchsin, methylene blue, etc.

One guinea-pig was inoculated with the whole of the blood of an infected mouse, but spirochaetes were never found in its blood.

An adult rat was also inoculated but it did not contract an infection. Three young rats were inoculated and two of these showed an infection after six days. The spirochaetes were never more numerous than in the blood of infected mice.

It appears therefore that the spirochaete produces in mice a definite infection having an incubation period of 5 to 6 days, reaching its maximum on about the tenth day and then gradually declining. Spirochaetes may be found even after 4 to 5 months.

The question of the origin of this spirochaete is one of interest. As stated above, it was first found in a brown mouse which had been infected with *Trypanosoma dimorphon*. Any relation with the trypanosome is of course disproved by the fact that it was quite easy to separate it and to obtain a pure infection. A parallel to this observation is that of Theiler who found spirochaetes and trypanosomes causing a mixed infection in cattle. Both my observation and that of Theiler give no support to the view that the two organisms are in any way connected with one another.

Mice fed on the blood of infected mice do not contract the infection.

Spirochaetes, identical with those found in the blood, were found in lice which infested the mice. These spirochaetes were few in number and showed no sign of active multiplication. Attempts at infecting mice by means of lice were not successful.

It has not been possible to cultivate the spirochaete on any of the ordinary media. Blood kept 5 days in the ice box and afterwards inoculated into mice gave rise to no infection.

The origin of the Spirochaeta, discussed.

A small spirochaete was seen by Borrel (1905) to occur in the juice of malignant growths of mice and he supposed it had migrated there from the intestine just as worms are liable to do in mice suffering from these growths. M. Borrel kindly permitted me to see some of his preparations and there appears to be no doubt that the two spirochaetes are identical. M. Mesnil, to whom I am much indebted for valuable advice and information, tells me that he has on several occasions noticed a small spirochaete in fresh blood preparations from mice, but he has not pursued the matter further.

In connection with my own observation it may be possible that the

spirochaete was brought over with the strain of *T. dimorphon*, but the observations of Borrel, and the fact that the strain of *T. dimorphon* did not originally contain the spirochaete, indicate clearly enough that it is a separate and distinct mouse parasite. This is amply proved by the experiments here recorded and the apparently harmless character of the spirochaete infection.

The intestine of mice is generally crowded with spirochaetes, though none of these exactly resemble the one under discussion. In order to test the suggestion of Borrel that the spirochaetes had come from the intestine, mice were inoculated subcutaneously with mucus from the intestine of a mouse in which there were many active spirochaetes. The result of the inoculation was negative. No abscess formed, and no infection followed. A mouse in whose blood spirochaetes were present was killed and a small quantity of the contents of its intestine, which contained active spirochaetes, was inoculated subcutaneously into two mice. Here again no abscess formed and no infection followed. A control mouse inoculated with the blood of this mouse developed an infection. There is thus no evidence that the spirochaetes have originated from the intestine.

Regarding other Spirochaetae.

In 1887, Vandyke Carter published a note on the occurrence of a minute *Spirillum* (*Sp. minor*) in the blood of the Indian rat (*Mus decumanus*). The parasite measures 5—9 μ in length, is very active, has no flagella, etc., possesses four to eight spiral turns and is slender and slightly pointed. There were at most only two to four spirilla visible in a field. The presence of the parasite in the blood did not apparently produce any pathological effects. Blood inoculated into a healthy rat and a monkey (*Macacus*) produced no infection. This spirochaete bears a close resemblance to the spirochaete of the mouse, but it is impossible to state whether the two are identical.

Lingard also describes a spirochaete ("an exceedingly minute *Spirillum*") as being present during the rainy season in the blood of Bandicoots (*Nesocia bandicota*). Infection may be conveyed by inoculation to rabbits and guinea-pigs. In rabbits the incubation period lasted about 20 days and the infection was paroxysmal, the animals dying in 16—37 days. In guinea-pigs the incubation period lasted about 24 days and the animals died on the day the spirochaetes appeared in the blood. The spirochaete is probably distinct from that of the mouse,

but it is more difficult to form an opinion regarding Carter's spirochaete as he did not try inoculation into mice, and, in fact, was unsuccessful in all attempts at transmission.

Balfour (1906) has described a small *Spirillum* as occurring in the intestinal ulcers of cases of trypanosomiasis in dogs and monkeys in the Soudan. This spirochaete, judging from Balfour's figure, bears some resemblance morphologically to the present one but it is not known to occur in the blood.

Since the morphological characters of spirochaetes are not sufficient to establish the identity of any form it is necessary to rely on other characters, notably upon their behaviour in various hosts and their pathogenic or other action. As nothing is known of Carter's *Spirillum* of the rat, apart from its morphology, the spirochaete of the mouse must be considered to be new to science.

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