SPIROCHÆTA ICTEROHÆMORRHAGIÆ IN AMERICAN WILD RATS AND ITS RELATION TO THE JAPANESE AND EUROPEAN STRAINS.

First Paper.

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Infectious jaundice has been known for a long time to occur among troops in barracks, among sewer workers, miners, and rice planters, and its entity has been recognized (Larrey, Ozanam, 1849, Monneret, 1859, Laverau, 1865, Lancereau, 1882, Landouzy, 1882, Mathieu, 1886, and Weil, 1886). Weil described four cases with typical symptoms, and the disease is very often called by his name. Weil's disease is characterized by sudden onset of malaise, often intense muscular pains, high fever for several days, followed by jaundice and the appearance of bile pigments, albumin, and casts in the urine; in severe cases epistaxis, subcutaneous hemorrhages, and lymphadenitis are observed.

A disease supposed to be identical with the Weil's disease of Europe is prevalent in Japan, and in 1914 Inada and Ido succeeded in transmitting to guinea pigs the typical experimental disease, accompanied by jaundice, hemorrhages, albuminuria, etc., by inoculating them with the blood of patients suffering from the Japanese form of infectious jaundice. In the blood and various organs of the animals they discovered a new spirochete, which they also found in the human specimens. *Spirochæta icterohæmorrhagiæ*, as it was designated by Inada and his associates,[1] has since been proved beyond doubt to be the causative agent of the disease in question. A year and a half later, Uhlenhuth and Fromme,[2] and also Hübener and Reiter,[3] showed that the Weil's disease prevalent among the German soldiers during the present war could also be transmitted to guinea pigs by injecting them with the blood of patients. It was not until 1916, however, that Hübener and Reiter[4] announced the finding of a spirochete in the experimental animal material (liver, blood, kidney, etc.), which they called *Spirochæta*

[1] Inada, R., Ido, Y., Hoki, R., Kaneko, R., and Ito, H., *J. Exp. Med.*, 1916, xxiii, 377.

[2] Uhlenhuth and Fromme, *Med. Klin.*, 1915, xi, 1202, 1264, 1296, 1375.

[3] Hübener and Reiter, *Deutsch. med. Woch.*, 1915, xli, 1275.

[4] Hübener and Reiter, *Deutsch. med. Woch.*, 1916, xlii, 1.

nodosa. Following the publication in America[1] of the article by Inada and his collaborators, Stokes and Ryle[5] succeeded in transmitting the disease to guinea pigs by inoculating them with the blood of British soldiers in Flanders who had Weil's disease, confirming at the same time the presence of a spirochete closely resembling the *Spirochæta icterohæmorrhagiæ* of the Japanese workers. Stokes, Ryle, and Tytler[6] left the question of the identity of the two strains (the Belgian and the Japanese) open for future discussion. In the meanwhile, Martin and Pettit[7, 8] Costa and Troisier,[9, 10] Garnier and Reilly,[11] Renaux,[12] Merklen and Lioust,[13] Ameuille,[14] Salomon and Neveu,[15] and others, working among the French soldiers on the western front, reported similar clinical and experimental findings. They also considered the spirochete isolated from the French specimens to be closely related to the Japanese strain. On the Italian front numerous cases of jaundice have been observed, and Monti[16] has demonstrated the spirochete in the experimental material. According to MacKenzie, there were at one time a considerable number of cases of infectious jaundice among the Canadian soldiers stationed at Salonica.[17] It is of interest to note that while the mortality among the Japanese is as high as 38 per cent, that of the European soldiers did not exceed 2 to 3 per cent. It seems reasonable to assume that the Japanese strain has already acquired a marked increase in virulence for human subjects, owing probably to a more frequent passage from man to man. Such transmission is more frequent in Japan, for example, among the rice planters and miners with imperfectly protected feet, than in Europe, where exposure to the infection was brought about only through the unsanitary conditions of war.

The entrance of this spirochete into the human body seems to be of comparatively recent occurrence. The discovery of the spirochete in apparently healthy wild rats caught near the infected districts

[5] Stokes, A., and Ryle, J. A., *J. Roy. Army Med. Corps*, 1916, xxvii, 286.

[6] Stokes, A., Ryle, J. A., and Tytler, W. H., *Lancet*, 1917, i, 142.

[7] Martin, L., and Pettit, A., *Presse méd.*, 1916, 569.

[8] Martin and Pettit, *Bull. Acad. méd.*, 1916, lxxvi, 247.

[9] Costa, S., and Troisier, J., *Compt. rend. Soc. biol.*, 1916, lxxix, 1038.

[10] Costa and Troisier, *Bull. et mém. Hôp. Paris*, 1916–17, xl, series 3, 1928.

[11] Garnier, M., and Reilly, J., *Bull. et mém. Hôp. Paris*, 1916–17, xl, series 3, 2249.

[12] Renaux, E., *Compt. rend. Soc. biol.*, 1916, lxxix, 947.

[13] Merklen, P., and Lioust, C., *Bull. et mém. Hôp. Paris*, 1916–17, xl, series 3, 1865.

[14] Ameuille, P., *Bull. et mém. Hôp. Paris*, 1916–17, xl, series 3, 2281.

[15] Salomon, M., and Neveu, R., *Compt. rend. Soc. biol.*, 1917, lxxx, 272.

[16] Monti, A., *Boll. Soc. med. chir. Pavia*, 1916, Nos. 3–4.

[17] Personal communication.

seems to support the theory that the disease was originally epizootic among certain rodents, particularly wild rats, and after a long sojourn in this species of hosts its virulence for these animals has been reduced to such an extent as to cause the latter no inconvenience, or, at least, a state of tolerance for the spirochete has developed.

Inada, Ido, Hoki, Ito, and Wani[18] found nearly 30 per cent of all the rats examined to be infected; that is, to carry the spirochete in their kidneys. Stokes Ryle, and Tytler,[6] as well as Martin and Pettit,[19] were able to demonstrate the spirochete in rats captured on the western front, not only in those taken in the immediate neighborhood of the infected zone, but also in rats captured in localities some distance from the trenches.

In America, especially the United States, there have been few epidemic or endemic cases of infectious jaundice reported from various quarters of the continent (Toronto, Middle Western and Southern United States) and from Cuba, but it was not known whether or not these cases corresponded with those found in Europe and Asia. The discovery of the specific pathogenic agent now enables us to answer this question experimentally.

Isolation of the Organism.

We have collected a large number of wild rats in this country and removed their kidneys for the purpose of ascertaining whether or not the organs contained the spirochete which causes the typical experimental lesions characteristic of the organism of infectious jaundice. Leaving the experimental details for a future communication, it may be well to state here briefly that by inoculating the emulsion made of the kidneys of forty-one wild rats into fifty-eight guinea pigs during the last 3 months, we have been able to produce in three groups of guinea pigs (four in each group) a typical icterohemorrhagic spirochetosis altogether identical with the findings in the guinea pigs which died of the injections of the Japanese and Belgian strains of *Spirochæta icterohæmorrhagiæ.*

Since it was our practice to make an emulsion from the kidneys (eight) of four wild rats and inject the same into four to six normal

[18] Inada, R., Ido, Y., Hoki, R., Ito, H., and Wani, H., *J. Exp. Med.*, 1916, xxiv, 485.

[19] Martin and Pettit, *Compt. rend. Soc. biol.*, 1917, lxxx, 10.

guinea pigs, it is difficult to say whether the icterohemorrhagic spirochetosis produced in some of the guinea pigs in each group was due to one or more of the rats employed. It would have been better if we had inoculated several guinea pigs with the emulsion of kidneys from each rat, but this would have involved a large number of guinea pigs, and hence our method of mixing four in each group. Since the successful isolation of the organisms from three of the pooled groups, we have resorted to individual tests, the results of which will be reported later.

The strain of spirochete isolated from the American wild rats caught in the vicinity of New York City produced death in guinea pigs within 9 to 12 days, attended by marked jaundice, cholemia, choluria, and extensive hemorrhages in various viscera. The temperature seldom exceeds 39°C.; that is, it does not show the fever which usually precedes the collapse and the appearance of jaundice in the guinea pigs inoculated with the Japanese or Belgian strains, which have at this time been passed through many guinea pigs. The spirochetes are found to be abundant in the internal organs, but are only moderately numerous in the blood. In the urine of some guinea pigs, which succumbed to the experimental icterohemorrhagic spirochetosis, varying numbers of the organisms were found in the urinary casts.

The morphology of the organism corresponds with that of the Japanese and Belgian strains, with which we were able to compare it.[20] Its elementary structure is that of a closely wound slender cylindrical thread with gradually tapering ends, averaging 9 by 0.25 μ. Individuals of 3 to 4 μ or 20, 30, and even 40 μ are met with in a culture. The number of coils is greater in a given length than that of any spirochete hitherto known. It is so closely wound that within 5 μ there are 10 to 12 coils. Near the extremities, the coils become closer. They are never very deep, and in general, the aspect of the whole body is that of a transversely barred chain of streptococci. The winding is rarely seen distinctly, although it can be brought out well by a carefully fixed stained preparation (osmic vapor fixation

[20] I am greatly indebted to Dr. Victor G. Heiser for the Japanese strain and to Dr. Alexis Carrel for the Belgian strain. The Japanese strain was kindly furnished by Dr. M. Miyajima, and the Belgian by Dr. Carl Browning, who obtained it from Dr. Adrian Stokes.

and Giemsa stain), or under powerful dark-field illumination. It should be noted that the description of the organism by most authors leaves this point unclear, and so far no satisfactory microphotograph has been reproduced. The movement and customary position of the organism in a free space are characteristic. Active specimens show a straight body with one or both ends curved in the form of a semicircle. The length of the hook at the end varies somewhat but is usually about 3 to 5 μ. While in motion, the organism, without relaxing its elementary minute windings, rotates around its axis, making about two to four turns per second, giving the impression of a drawn out figure eight. The movement is bipolar, and its direction alternates at short intervals. When passing through a semisolid medium, such as fibrin or soft agar, the body of the spirochete assumes a wavy spiral not unlike the *refringens* type. The number of waves may vary from a few broad ones to as many as five or six in some of the long individuals found in a culture. The movements are brusque and erratic, changing suddenly the direction of progression. At times, the organism moves about with one end taking various directions. The facility with which these spirochetes travel through the fibrin or semisolid agar medium is without a parallel among any known spirochetes. The body is absolutely flexible. There is a distinct halo around the organism, but no membrane has so far been demonstrated. The part of the body which forms the hook terminates in a fine point, but no minute flagellum-like projection could be demonstrated by staining (Loeffler, Pitfield, Casares-Gil, Fontana, etc.), or by dark-field illumination. It is devoid of a terminal filament such as is characteristic of a spironema or treponema, and is resistant to saponin (10 per cent), unlike all other spirochetes. It calls for a new genus, and on account of its fine and minute windings, the name *Leptospira* is suggested.

The hooked ends form one of the most characteristic poses of the organism while rotating on its axis in a free space, but as soon as it meets a solid or semisolid obstacle, it begins to penetrate into it. Its habitat seems to be a porous gelatinous mass of substance, the organisms swarming in and out of it. In a culture the majority of the organisms will be found in a semisolid piece of medium. After death it may retain its position at the moment of death; hooked or

contorted forms, resembling the letters c, s, l, and b, are most frequently met with among immobile or dead organisms. When destroyed by a strong acid or alkali, they lose their minute curves, swell up, and become indistinct and straight. The organism has the power of almost perpetual motion. A culture as old as 3 months still displays as much vigor as does a young one. None of the other varieties of spirochetes have this property in cultures. The organism passes the Berkefeld candle V.

Cultures.

The Japanese investigators[1] succeeded in obtaining a culture of their strains by using a medium similar to that previously introduced by the writer for the spirochete of relapsing fever.[21] Ito and Matsuzaki approved this technique and recommended also the use of blood gelatin or blood agar in various concentrations.[22] In Europe, Reiter[23] cultivated the German strain in the sera of various animals, sometimes diluted several times with isotonic salt solution. Recently, Martin, Pettit, and Vaudremer[24] obtained a culture of the French strain by the use of animal sera, particularly that of beef, diluted with Locke's solution. In all these instances, the surface of the culture medium was covered with a layer of sterile paraffin oil, as recommended by the writer for the cultivation of spirochetes in general. The Belgian strain of Stokes has not yet been cultivated, and Stokes, Ryle, and Tytler particularly laid stress on its resistance to artificial cultivation as a characteristic of the strain.

The writer has been interested in determining the cultural conditions which will give uniform results, and has found that the following methods give the most successful growth, not only with the Japanese, but also with the Belgian (Stokes) and American strains, which were obtained in permanent cultures for the first time by these means.

In our experience, it seemed best to make a distinction between the initial culture or the first generation and the subcultures, since there is a great difference in the readiness with which the first and subsequent generations of the spirochetes grow in culture. For example, all three strains, in our hands, failed to grow, or grew poorly

[21] Noguchi, H., *J. Exp. Med.*, 1912, xvi, 199.

[22] Ito, T., and Matsuzaki, H., *J. Exp. Med.*, 1916, xxiii, 557.

[23] Reiter, *Deutsch. med. Woch.*, 1916, xlii, 1282.

[24] Martin, L., Pettit, A., and Vaudremer, A., *Compt. rend. Soc. biol.*, 1917, lxxx, 197.

in the various media recommended by different authors. The best procedure, and one which has always been reliable for securing initial growth was to produce strands of loose fibrin in the fluid culture media by using a small quantity of citrate plasma in combination with the diluted or undiluted serum of a suitable animal. The beneficial effect of a loose fibrin upon the culture of the spirochetes of relapsing fever has already been mentioned by the writer.[21] The dilution of the serum may be made in any proportion above 1:10 by adding a sterile saline (0.9 per cent) Ringer solution, or even plain water. For obtaining the spirochetal material for inoculation, the citrate blood derived from the heart of a guinea pig having the disease is best, although an emulsion of the liver or kidney may also be used. When no secondary bacteria are present, a positive culture can be secured at the first attempt. By using graduated quantities of the infected guinea pig's blood (citrate), the writer secured a good growth in as high a dilution as 1:100,000.

Undoubtedly blood cultures for diagnostic purposes in human cases are feasible with a suitable medium. For this purpose I should recommend two different media, one being apparently as good as the other: (*a*) rabbit serum 1 part + Ringer or 0.9 per cent sodium chloride solution 3 parts + citrate rabbit plasma 0.5 part, covered with a thin layer of sterile paraffin oil; (*b*) the same, except for the use of 0.5 to 1.0 part of neutral or slightly alkaline agar (2 per cent), which should be added while in a liquid state and quite hot (60–65°C.) in order to get a uniform mixture of the agar. These culture media, because of the paraffin oil layer, can be preserved at room temperature for many months, though a cooler place is better. They may be inoculated with suspected blood by introducing a quantity which is regarded as adequate in each instance. In the case of an infected guinea pig, the detection of the spirochete in the blood can be made within 48 to 72 hours, if the culture tubes are placed at 30–37°C. The search for the organism should be made within the aerobic zone immediately below the surface (1.0 to 1.5 cm.), because, according to the experience of the writer, the organism is an obligatory aerobe, unable to grow in the absence of oxygen. This would explain the unsuitability of a solid medium, which prevents access of air.

The use of fresh tissue, as in the case of the relapsing fever spiro-

chete, is not required for the cultivation of this organism, and is perhaps seriously detrimental in a fluid medium. Growth takes place at any temperature from 10° to 37°C., being much more rapid at higher than at lower temperatures. The growth is almost invisible in a fluid medium, but appears as a somewhat distinct haze in semi-solid medium such as described above (*a* and *b*). The appearance is not unlike the hazy, diffuse growth of various treponemata in similar media, the only difference being that the haze in the case of the latter stops abruptly a few cc. below the surface, while the present organism is unable to grow beyond a few cc. below the surface. So far, no investigator has mentioned the visible growth of the spirochete, all describing it as invisible.

Virulence.

The American strains possess an average degree of virulence, having killed guinea pigs in 9 to 12 days in the first generation in this animal. The lesions, as well as the jaundice, were severe and typical in every respect. The second passage killed the guinea pigs in a much shorter period, 6 to 8 days, the lesions being similar to those of the first passage. Again there was no fever. They seem to be running a course similar to that of the Japanese strain in attaining a higher virulence by successive passages. The virulence of the Belgian strain appears to be less readily increased, as the guinea pigs survive 9 to 10 days. This distinction in virulence may be due to the fact that the Belgian strain was brought to us in infected rats, instead of in guinea pigs, in which the Japanese strain was received.

Immunity Relations.

Reserving the experimental details for a fuller report, it may be stated briefly that the guinea pigs which had been made immune to the Japanese or Belgian strain resisted the inoculation of the liver emulsion containing large numbers of the spirochetes of American origin, while control animals succumbed to the typical infection in 6 to 8 days. The blood sera from guinea pigs and rabbits immunized with the Japanese or Belgian strain agglutinated the American strain as strongly as they did their own strains. The immune sera exerted

germicidal or lytic action upon the three strains indiscriminately. Whether or not protection experiments in guinea pigs will show any distinctions between the Belgian and the Japanese strains on the one hand and the American on the other will shortly be determined. As far as the former are concerned, the active immunity developed in guinea pigs or rabbits and their immune sera are effective, the one against the other, in almost equal titers.

The finding of the causative organism of infectious jaundice among wild rats in America, and the identification of this strain with those found in Asia and Europe seem to be particularly important in revealing a latent danger to which we have been constantly exposed but from which we escape as long as sanitary conditions are not disturbed by untoward events.

SUMMARY.

The principal points brought out in the present article are the following.

1. Wild rats captured in this country carry in their kidneys a spirochete which possesses the morphological and pathogenic properties characteristic of *Spirochæta icterohæmorrhagiæ* discovered by Inada in the Japanese form of infectious jaundice.

2. Cultures of the American, Belgian, and Japanese strains of the spirochete were obtained by a special technique described, the first two strains having been cultivated artificially for the first time.

3. Animals actively immunized against the Japanese strain resist inoculation, not only of the same strain, but also of the Belgian and American strains. The Belgian strain produces immunity equally effective against all three strains. Experiments to ascertain whether the immunity afforded by the American strain also protects against the Japanese and Belgian strains are in progress.

4. These findings warrant the conclusion that the spirochetes designated here as the Japanese, Belgian, and American strains are probably identical.

5. On account of its distinctive features, a new genus, *Leptospira*, has been suggested as the designation of this organism.