THE ETIOLOGY, MODE OF INFECTION, AND SPECIFIC THERAPY OF WEIL'S DISEASE (SPIROCHÆTOSIS ICTEROHÆMORRHAGICA).

By RYOKICHI INADA, M.D., YUTAKA IDO, M.D., ROKURO HOKI, M.D., RENJIRO KANEKO, M.D., and HIROSHI ITO, M.D.

(*From the First Medical Clinic of the Imperial University in Kyushu, Fukuoka.*)

Plates 56 to 62.

(Received for publication, December 1, 1915.)

INTRODUCTION.

In this communication we have summarized and in some details extended the publications on this subject which have appeared in the Japanese literature.[1] In the course of our investigations of that endemic disease of portions of Japan, which agrees clinically with Weil's disease, so called, we discovered a spirochætal microorganism which is now believed to be the cause of the disease. In the experiments recorded in this paper, Drs. Inada, Ido, Hoki, and Ito chiefly conducted the experiments on animals, and Dr. Kaneko the pathological and anatomical observations.

In the western part of Japan there prevails an epidemic and endemic disease characterized by conjunctival congestion, muscular pain, fever, jaundice, hemorrhagic diathesis, and albuminuria, which is known as Weil's disease or febrile jaundice. A similar disease is also present in Shikoku and is called *Odan-eki;* that is, icteric pestilence. At the end of last year (1914) the same disease was observed in Chiba, in the eastern part of Japan, near Tokyo, where the patients numbered 178. Thus this disease appears to be prevalent in various parts of Japan, although to a small extent.

We have been interested in Weil's disease in Kyushu for many years, and at the end of November, 1914, we detected a spirochæta in the liver of a guinea pig injected with the blood of a patient suffering from Weil's disease. We came to the conclusion, in January, 1915, that this spirochæta is the pathogenic cause of Weil's disease, and we named it *Spirochæta icterohæmorrhagiæ.* At Chiba, a distance of more than 700 miles from Kyushu, we detected in the blood of five out of

[1] A full bibliography is appended.

six patients who suffered from this disease in 1914 a substance which has a spirochæticidal power over this spirochæta. Nishida, a physician in Kusunoki Hospital in Kochi, Shikoku, a former pupil of Inada, also detected by our method, a spirochæta in a patient suffering from *Odan-eki*, and his spirochæta was morphologically identical with ours.

From these facts we were able to identify the pathogenic cause of Weil's disease prevailing in three different parts of Japan, that is, in Kyushu and Chiba, and *Odan-eki* in Kochi, Shikoku, as *Spirochæta icterohæmorrhagiæ*. We can now say that it is an independent disease, caused by a specific microorganism, and we therefore propose to substitute the name of spirochætosis icterohæmorrhagica for the various names already in use. This name, although somewhat inconvenient, expresses both the symptoms and cause of the disease. Thus, some patients suffering from Weil's disease exhibit rarely only slight jaundice or do not show hemorrhage, and for these cases the name may seem inappropriate; but the occurrence of such light and atypical cases is a common experience among infectious diseases. We might shorten the name to spirochætosis icterica, as the jaundice is the most striking and constant symptom, but there is danger of confusing it with recurrent fever accompanied by jaundice; while, moreover, the name spirochætosis japonica does not differentiate it from other diseases caused by various spirochætæ.

Opinions differ as to whether Weil's disease in Europe and Japan are identical. The symptoms are similar, but there are some variations. The mortality of Weil's disease is 32 per cent in our clinic; splenic enlargement is rare (10 per cent in our clinic). Spirochætosis icterohæmorrhagica is present endemically in various localities, but rarely causes a sudden epidemic, as is the case with the European Weil's disease. On the other hand, the European Weil's disease appears either as a transient epidemic or endemically, and in the latter case the state of its outbreak and symptoms resembles the Japanese disease closely. Thus the above question can only be solved by the detection of the presence or absence of the spirochæta in the European Weil's disease.

Bilious typhoid fever resembles the Japanese Weil's disease. According to Griesinger and others, this disease is a form of recurrent fever accompanied by jaundice; but Kartulis, Diamantopulos, Goldhorn, and Rabinowitsch believe that it has no connection with recurrent fever. Goldhorn states that Weil's disease is a light form of bilious typhoid, and Weil himself called attention to the similarity of the symptoms in the two conditions. Fiedler reports that pathological anatomical changes in Weil's disease are the same as those in bilious

typhoid. The mortality of bilious typhoid, according to Griesinger, is 60 to 70 per cent; compared with that of our cases of so called Weil's disease this is high. A few years ago Nishi identified Weil's disease with yellow fever, but he was opposed by Ohno. We have also emphasized the differences from the epidemiological point of view between yellow fever and Weil's disease, although the symptoms are very much alike. The poison in Weil's disease is sometimes capable of filtration through a Berkefeld filter, as is that of yellow fever, and the microbic causes of both diseases are present in the blood only in the early stage of the illness. Both diseases are prevalent in the lowlands. Yellow fever, however, is communicated to man by the *Stegomyia* mosquito, while there is at present no proof that Weil's disease is communicated by the bite of an insect.

Typical Japanese Weil's disease can be easily differentiated from catarrhal jaundice. On the other hand, very light cases or cases in which the conjunctival congestion has already passed or which have passed beyond the initial stages and show no hemorrhages may be difficult to diagnose. Clinically one never sees in catarrhal jaundice such marked congestion of the conjunctiva as is present in the Japanese Weil's disease, and the general condition of the patients shows great differences in the two diseases. In Weil's disease the patient is weak and cannot stand up even on the 1st or 2nd day of the illness, while in catarrhal jaundice one does not meet with such symptoms. The state of the circulatory system and other symptoms make further differentiation possible. However, the two diseases may not be differentiated through the presence or absence of bactericidal substances in the blood of the patients.

Spirochæta icterohæmorrhagiæ.[2]

In February, 1915, we announced the discovery of the spirochæta of Weil's disease. It may be interesting, therefore, to review briefly the reasons for the failure to detect the spirochæta at an earlier period: (1) All investigators searched for bacteria. (2) The spirochæta resides in the blood only during the early stages of the disease and is present there in small numbers. (3) At the period at which fatal cases come to autopsy the liver is either devoid of spirochætæ or they are so few or modified in form as to be difficult of discovery or recognition.

In our early studies we searched for bacteria in the blood, urine, and feces. Having failed in this quest, we inoculated the monkey, rabbit, rat, and guinea pig with the blood of patients, and in July, 1913, we observed that the guinea pig developed albuminuria, conjunctival congestion, jaundice, and hemorrhages when the blood was

[2] This part of the investigation was conducted by Drs. Inada and Ido.

injected during the first 7 days of the illness. We ascertained later that the number of spirochætæ in the blood is so small as to make their detection under the microscope almost impossible. In the guinea pig they multiply freely and are found in large numbers in the liver. We first detected the spirochætæ in this manner in November, 1914. Inasmuch as the injection of the blood of healthy persons or even of patients suffering from other diseases including catarrhal jaundice does not cause pathologic changes in the guinea pig or lead to the occurrence of spirochætæ in the liver, we concluded that the spirochætæ were derived from the blood of the cases of Weil's disease. Our deductions were confirmed when we discovered the same spirochætæ microscopically in six specimens of patients' blood, in the intestinal wall of one case, and in the adrenal glands of another among eleven autopsied cases. The autopsies were performed on cases which had succumbed on the 8th to the 14th day of illness, the most fatal period of the disease. At this period the spirochætæ have already diminished greatly in numbers. At this time we also detected a bactericidal substance for the spirochætæ in the blood of patients, and we assumed that the disappearance of the organisms from the body was connected with the formation of this substance. Probably in this way is explained either the entire absence or the occurrence merely of atypical forms in small numbers in the organs. On the other hand, we detected later in the liver of two patients who succumbed on the 6th day of illness spirochætæ as numerous as in the liver of the guinea pig. These facts led us to assert that the spirochæta is the pathogenic cause of Weil's disease.

EXPERIMENTAL.[3]

Hecker and Otto injected defibrinated blood of soldiers suffering from Weil's disease in Europe into the skin, peritoneum, and veins of rats, guinea pigs, rabbits, and monkeys, with negative effects. Fiedler and Schittenhelm report similar results. We have injected blood of seventeen cases of spirochætosis icterohæmorrhagica into the peritoneum of the guinea pig. Thirteen positive results with typical symptoms,—conjunctival congestion, jaundice, hemorrhage, and albu-

[3] These experiments were carried out by Drs. Inada, Ido, and Hoki.

minuria,—were obtained. The blood of all the patients in the 4th to the 5th day of the illness gave positive results, while that taken on the 7th day in one instance gave positive and in another negative results. The blood in one case, on the 9th day of illness, also gave a positive result; but in no instance from the 12th day on was a result obtained, although blood from three such cases was tested. Thus, in order to obtain positive results one must inject the blood within the first 7 days of the illness. The symptoms following the injection are similar to those after inoculation from one guinea pig to another, except that the incubation period is longer. The incubation period may be reduced by making the inoculation from a patient suffering from severe illness in its early stage.

The time required after the inoculation for the appearance of jaundice is usually 7 to 8 days. The shortest time was 6 days, and the longest 13 days. The infection can be transferred from an infected guinea pig to others through many generations, and the oldest of our six strains of spirochæta has now reached the fifty-first generation (July 15, 1915). The inoculation succeeds by intraperitoneal, subcutaneous, or oral injection of 2 cc. of the heart's blood or of an emulsion of the liver. The former is the most certain. The method of inoculation also affects the incubation period. The animals succumb in from 5 to 8 days after intraperitoneal, and in from 9 to 10 days after inoculation through the uninjured skin or the alimentary canal; after inoculation into the injured skin death follows in 7 to 8 days. The spirochæta occurred in the animals experimented upon practically constantly. Of the large number of experiments made, in only two were they discovered in the liver, although jaundice and hemorrhage were present.

The symptoms produced in the guinea pig by inoculation are similar to those occurring in man. The animals show loss of appetite, conjunctival congestion, anemia, jaundice, hemorrhagic diathesis, and albuminuria. The fever comes on suddenly, reaching nearly 40°C. on the 4th or 5th day after intraperitoneal inoculation, and on the 5th or 6th day after the inoculation through the injured skin. The spirochæta appears in the blood about this time and the animals generally succumb within 24 hours after the appearance of the jaundice. Just before death the temperature falls rapidly, as in collapse.

Rabbits fail to become infected even when large quantities of the blood capable of producing the disease in guinea pigs are injected. In our experiments,

death never followed the intraperitoneal or intravenous inoculation of 20 to 40 cc. of blood or liver emulsion of the guinea pig containing the spirochæta, the fatal dose for the guinea pig being 4 to 5 cc. However, eleven of the thirteen rabbits showed a rise of temperature on the 4th or 5th day. Conjunctival congestion was present in six, and a slight jaundice in four animals. All the animals became excited. One which showed marked jaundice showed no hemorrhages or spirochætæ in the liver.

Fourteen mice were inoculated with the liver emulsion or blood of the infected guinea pig, of which four showed jaundice and slight hemorrhage and succumbed. One was examined, and no spirochætæ were found in the liver.

Two white rats were similarly inoculated. One developed jaundice and slight hemorrhage and died on the 8th day; the other remained healthy.

From the above experiments the rabbit appears to be the most insusceptible, the mouse and white rat coming next. The guinea pig is the most susceptible; it appears to be even more susceptible than man.

Pathological Changes in the Guinea Pig.[4]

The typical pathological changes consist of marked general jaundice, hemorrhages into the various parts of the body, and parenchymatous changes of the organs. The usual sites of hemorrhage are the lungs, intestinal walls, retroperitoneal tissues, and the fatty tissue of the inguinal region. When the changes are atypical there are either very slight or no hemorrhages, although the jaundice is marked, or there is no jaundice although hemorrhage is present. The latter condition is rare and generally when met with the spirochætæ are few in number or absent. After the injection of salvarsan this condition is found.

The liver shows cloudy swelling of the parenchyma, while the color varies according to the degree of the jaundice and the quantity of blood present. Microscopically the precipitation of the bile is not marked in spite of the presence of jaundice, and there is no congestion of the bile in the biliary duct.

The kidney shows an acute parenchymatous nephritis. The spleen is more or less enlarged, congested, and hemorrhagic. There is always some precipitation of the iron-containing pigment and the phagocytic cells contain red blood corpuscles. The lymphatic glands do not undergo enlargement, except those near the site of inoculation.

[4] This study was made by Drs. Inada and Kaneko.

The lungs present small and large hemorrhagic spots, like the wing of a mottled butterfly. This change is one of the most important in the diagnosis of the disease.

The intestines show hemorrhages in the walls. The muscles show a certain amount of hemorrhage and parenchymatous changes of the muscular fibers.

Distribution of the Spirochætæ.

The spirochæta lives in the blood outside the cellular elements and in various organs and tissues. When present in the cells it is within phagocytic cells. It is often seen in the epithelial cells. The liver contains the largest number of spirochætæ. They can be demonstrated most clearly by the older Levaditi method of silver impregnation. They occur usually in the spaces between the cells, and when present in large numbers are arranged about the individual cells like a garland. Next to the liver the adrenal glands and the kidneys contain the largest number of spirochætæ. In the kidneys the spirochæta occurs inside the interstitial tissues and also in the walls and lumen of the uriniferous tubules.

There are very few spirochætæ in the spleen, bone marrow, and lymph glands, although they are rich in blood; and the spirochætæ are extremely rare in the splenic follicles, as well as in the lymph glands and other lymphoid tissues. Diffuse hemorrhages in the lung and intestinal walls may contain only a small number.

With a given strain of spirochæta, the animals that show the most marked pathological changes have the most spirochætæ. Among the six strains of spirochætæ, two, which were obtained from severe cases, produced severe pathological changes associated with more numerous spirochætæ than followed the inoculation of the other strains. The pathological changes produced by the above two strains have, however, become gradually reduced as successive transfers have been made.

Comparison of the pathological changes in the guinea pig with those of man brings out certain differences: (1) The hemorrhage is severer and more wide-spread in the lungs of the guinea pig than in man. (2) Spirochætæ can always be found in the guinea pig when the inoculation gives a typical result, which is not the case in man. This

is the most important difference between the human disease and the infection in guinea pigs. (3) The spirochætæ are present within the liver cells in man, and lie between the liver cells in the guinea pig. This difference may be accounted for by the fact that no immunizing substance is formed in the guinea pig. In man a bactericidal substance injurious to the spirochæta appears in the blood on the 14th or 15th day of illness. Moreover, man is more subject to intercurrent mixed infections than the guinea pig.

Characteristics of the Spirochætæ.[5]

Spirochæta icterohæmorrhagiæ remains always outside the blood cells. It resides in the interstitial tissues of the organs and rarely in epithelial or phagocytic cells. Like that of recurrent fever it probably belongs to the blood spirochætæ. This view is supported by the acute nature of the disease and by the fact that the spirochætæ of chronic diseases like syphilis and frambesia belong to the group of tissue parasites. Although it is impossible to measure the length of the spirochæta exactly, because of its irregular wavy figure, the specimens seen in blood taken from a case of Weil's disease varied from half the diameter to the full diameter of the red corpuscles. The measurements are the same in the blood of the guinea pig. The commonest length is between 6 to 9 μ, the greatest reaching 12 to 13 μ. In the liver the short forms measure from 4 to 5 μ; but the common forms are longer and measure 8 to 9 μ. The longest individuals average 20 μ, although we have seen one measuring 25 μ.

The thickness varies according to the staining and fixing methods and the strength of the stain. The thickness is probably 0.25 μ. The ends are sharp and in most cases hooked. In some specimens one end only is hooked, and in others both ends are bent toward the same side, resembling the letter C; or the ends are turned opposite to each other forming the letter S. The undulations are not so regular as in *Treponema pallidum* and are usually composed of two or three large irregular, or four or five smaller waves.

Flagella cannot be demonstrated by Loeffler's method, and it is probable, therefore, that a membrane is absent. This point is not established.

[5] This study was made by Drs. Inada and Ido.

The methods employed in staining are as follows: Liver and blood specimens are fixed with absolute alcohol, methyl alcohol, or osmic acid (Weidenreich's method), and the staining is done with Giemsa's solution. The specimen fixed with methyl alcohol is stained for 2 hours or over with a mixture of about 2 cc. of water and three drops of Giemsa's solution. The spirochæta is usually well stained when the granules of the white blood corpuscles are deeply stained. The color of the spirochæta varies according to the degree of the staining, and is either red or red with a purplish tinge. For vital staining 50 per cent borax methylene blue is employed.

The spirochætæ stained in this manner look uniform, but under high magnification some individuals show folds and present the granular appearance seen under dark-field illumination. Moreover, some of the spirochætæ show the granular appearance distinctly when stained by Loeffler's method.

The spirochætæ in the blood usually show the typical and rarely the atypical form. But when they are very numerous, the atypical forms—ring form or two twined about one another—are more frequent.

The forms present in the liver are as variable as are the differences in length. One sees round or oblong granules, sometimes three or four in number, stained deep purple with Giemsa's solution in the body of the spirochæta. These granules appear to be collections of chromatin. In addition still larger granules sometimes project from the body of the organism forming the so called bud of the spirochæta. As in the case of the other spirochætæ, the significance of this bud is not apparent. Some of the spirochætæ resemble a platinum loop at one end or appear as rings, in which case the ends are not visible. The degenerative form is thick and straight, devoid of waves, and blunt at the ends.

The spirochætæ are not visible in the unstained condition under the microscope, even when very numerous. Under dark-field illumination they are readily found and present a characteristic appearance. The light is refracted unevenly, and portions showing strong light alternate with portions showing no light. The latter are narrower than the former, giving to the organism the appearance of a rosary. According to the length of the spirochæta the refractive

granules number 25 to 30 to 40. There are fore and aft movements besides movements of the ends to right and left. The anterior third of the spirochæta makes brisk movements to the right and left, while the posterior part makes only slow movements with the end turned either to the right or left. There is also a twisting motion about the long axis, which makes the spirochæta look like a figure 8, and there are spiral or snake-like movements, besides the formation of waves along the long axis, as if the waves of muscular contraction were transmitted to the spirochæta.

When a liver emulsion is left at room temperature, the spirochæta keeps its movements for 2 days, although they are not brisk, at the end of which period the majority are precipitated to the bottom of the culture tube.

The spirochæta immediately ceases to move when put in 50 per cent glycerine, 5 per cent sodium chloride, or 0.5 per cent acetic acid solution; it looks as if it were hardened and shows various degenerative changes, such as a bending small ring formation at one or both ends. 0.5 per cent sodium hydrate solution immediately dissolves the spirochætæ, but they continue to move for a comparatively long time in distilled water.

Filterability of the Spirochætæ.

The results of experiments conducted with five Berkefeld V, one N, and three W candles were variable. The filters had previously been tested with *Bacterium coli commune*, not with *Bacillus prodigiosus*, and were found to be impervious to the former. Some of the filtrates when injected produced infection in a guinea pig, but spirochætæ were not detected under dark-field illumination.

Of twenty-eight experiments made with liver emulsion of a particular strain, fifteen positive results were obtained. As a rule, the spirochæta appears to be more easily filterable when present in large numbers.

It is difficult to determine the mode of multiplication of the spirochæta, and various opinions might be expressed, as with the mode of the multiplication of other spirochætæ. We examined many fresh specimens under dark-field illumination daily for over 6 months. On one occasion Hoki saw a spirochæta suggesting transverse seg-

mentation, and Ito and Inada saw one suggesting longitudinal segmentation. We think, however, that multiplication probably occurs by transverse segmentation, since one sees numerous short forms at times when the multiplication of the spirochæta is going on rapidly, as is described below.

Cultivation of the Spirochætæ.[6]

It was not until April, 1915, that we succeeded in keeping the spirochæta alive from 13 to 17 days. In May we observed its multiplication and were able to transmit it from generation to generation. At that time we cultivated it through five generations, and the spirochæta lived in the culture medium for 2 months after it left the body of the animal. The method employed was that of Noguchi, by means of which various spirochætæ of recurrent fever have been cultivated. We employed guinea pig instead of rabbit kidney, and always used liquid paraffin. The most important fact, however, is that the temperature of 37°C. is not suitable for its development. When a well grown culture is kept at 37°C. the movements of the spirochæta become sluggish within 2 to 3 days, and almost all the spirochætæ undergo degeneration and finally disappear from the culture medium. Temperatures below 15°C. are also unsuitable, the best results being secured at temperatures of 22 to 25°C.

The culture does not yield any odor, and the ascitic fluid remains uncoagulated. Moreover, the fluid remains clear; and even slight cloudiness indicates contamination with some coccus or bacillus. The spirochætæ are distributed almost uniformly throughout the culture medium. The life of the culture is variable at 22–25°C. The first generation lives mostly from 3 to 6 weeks, the longest period observed being 55 days and the shortest 17 days. The life of the second and third generations is somewhat shorter than that of the first generation. The conditions which conduce to long life are at present not established. When extraneous bacteria are present in considerable numbers in the culture the spirochætæ die, but they can survive if the number of foreign bacteria is small, although they will not grow when transplanted. Thus protection from contamina-

[6] This work was done by Drs. Inada and Ido.

tion is an important factor in the cultivation. The multiplication of the spirochætæ in the culture starts at different periods, sometimes after 2 to 3 days and at other times only after 1 week or, rarely, after 2 weeks. In the transfer of the culture from one tube to another the addition of a small quantity of blood keeps up the development, while the best time for the transfer is when multiplication is going on rapidly, as is indicated by an examination of the culture every 2 or 3 days.

In character the cultivated spirochæta does not differ from that obtained directly from the animal body. When multiplication is at its height the spirochæta is very short and has brisk movements. In the young culture over half the spirochætæ may be composed of the short forms which, however, gradually become longer. Sometimes two spirochætæ are connected, and again one sees 8 to 9 to 15 spirochætæ collected about some granular substance in a manner resembling a rosette, with very brisk movements. Rarely very long spirochætæ (two or three times the normal length) composed of a single organism may occur. After the height of development the movements gradually become sluggish, the spirochæta takes on a hardened and bent appearance, assumes various degenerative forms, and finally dies. The second and third generations of the pure culture are capable of producing on inoculation infection in the guinea pig; whether later generations do so has yet to be determined. In the absence of a simpler method, we have successfully employed Noguchi's method of cultivation.

Mode of Infection.[7]

Weil believed that in the European Weil's disease infection occurred through the alimentary canal. Fiedler held the same view, but Hecker and Otto believed that infection might be communicated by the bite of a mosquito. As regards Weil's disease in Japan, Inada thinks that the infection occurs probably through the alimentary canal. It may, however, sometimes enter through the skin, as at times the disease begins with local swelling of the lymph glands. The throat is probably not the place of invasion, although it may show congestion. Oguro in Saga, on the other hand, has never observed

[7] This study was made by Drs. Ido and Hoki.

any circumstance which suggests the cutaneous mode of infection, but regards the alimentary tract as the portal of infection. Thus the mode of infection cannot be considered as established from clinical observation. On the other hand, since the discovery of the pathogenic cause of the disease, we have been able to determine it by experiments on animals.

The abdominal wall of the guinea pig is shaved without injuring the skin, washed with soap and then with alcohol, and dried. 1 cc. of the liver emulsion containing the spirochæta is dropped on its surface with or without abrading it. Thirty guinea pigs were so treated after incision of the abdominal skin to the extent of causing slight bleeding, and thirteen animals without injuring the skin. 10 of the latter 13 animals (77 per cent) and 26 of the first 30 (86 per cent) contracted the disease. Thus one can see that the spirochæta is able to penetrate through a macroscopically healthy skin and cause the disease in the guinea pig. The invasion occurs more easily and certainly where an obvious lesion exists. The incubation period, moreover, is 9 to 10 days in the former, and 7 to 8 days in the latter instances. Crushed liver acts as well as the emulsion for the purpose. The time required by the spirochæta to penetrate through the skin is short, and even after the skin is washed with alcohol or sublimate solution, the animals acquire the disease 5 minutes after the application of the emulsion to the skin. Whether penetration takes place in less than 5 minutes has not been determined. The spirochæta can also be made to invade the body through the alimentary canal. Feeding 2 gm. of liver emulsion, or giving an enema of 2 gm. of liver emulsion containing the spirochæta, produces the disease in the guinea pig. Thus, the spirochæta is able to invade animals through the mucous membrane of the alimentary canal.

Among fifty-five cases which were admitted to our clinic, only a few indicated cutaneous origin. The following facts, however, suggest this mode of infection: (1) When the disease occurs in coal mines the patients are numerous among the miners who work at a certain part of the mines. Numerous cases do not arise in the same barracks, even when the disease originates there. (2) The clerks working outside the mines do not contract the disease. (3) There are many cases in wet mines and few in dry mines. (4) It was noted that many cases occurred among the miners who worked in a particular part of the mine, but no case occurred when the accumulated water, as suggested by Inada, was pumped out. As mentioned above, the spirochæta is able to penetrate a healthy skin. Thus anyone who is working in an infected locality can easily contract the dis-

ease, even when the skin is uninjured, and the infection will take place still more easily if the skin is injured. Coal miners, who are liable to abrasion of the skin, and also to a skin lesion caused by working with the feet in water, can easily contract the disease.

We thought that infection through the skin was probably on account of the presence of enlarged lymph glands, but, later, after observing numerous cases, we considered the enlargement of the glands as a result of the general infection and not the result of the direct invasion of the spirochæta into the glands. However, from the animal experiments described above, we believe that the enlargement is partly due to the result of local infection of the glands in the affected part and partly to the general infection. The enlargement of the glands is not a symptom which is present in every case; it was present in about 60 per cent of fifty-five cases admitted to our clinic. The enlarged glands usually reach the size of a horse-bean or less. We have only once seen the gland as large as the end of the thumb. Besides skin infection, infection through the alimentary canal possibly occurs also. Cases arise in which two or three persons in a family are affected at the same time or at an interval of 1 or 2 days. However, we do not yet know how the infection in these instances occurs.

Since the origin of the infection through the skin is established, it is necessary to consider the question of whether the mosquito or flea may play a part in causing it. Among our clinical cases indication of direct infection from man to man is rare; while the relation of the seasons and the fact that the disease is common in Hakata and Chiyomachi and rare in Fukuoka on the other side of the river, make us think that the mosquito and flea do not play a part in the propagation of the disease.

The spirochæta of the Japanese Weil's disease is not present in such large numbers in the blood as is the spirochæta of recurrent fever; the number diminishes rapidly and they disappear comparatively soon from the blood. Moreover, the virulence in man is less than in the guinea pig, and wide-spread epidemics, as in recurrent fever, do not occur.

Mode of Excretion of the Spirochæta.[8]

In order to detect the mode of excretion of the spirochæta, it is necessary first to examine the excretions with dark-field illumination and to inoculate guinea pigs with them. This double procedure is important, for even when the spirochæta cannot be recognized with the ultramicroscope, the animal experiments may prove positive. Ida, of the Second Medical Clinic of Kyushu University, inoculated urine from a case of Weil's disease into a guinea pig and applied some of the urine to the skin of the animal, after injuring the surface. He obtained a positive result. Later we visited several coal mines and examined the excretions under the dark-field microscope. We conducted experiments with animals at various stages of the disease with twenty-four cases.

In twelve cases we examined the urine with the dark-field microscope within the first 10 days of illness, and recognized a small number of spirochætæ, in one case on the 6th and in another on the 10th day. The others gave negative results.

It is noteworthy that numerous spirochætæ are found in the urine at a time when the immune body appears in the blood; that is, about the 13th to the 15th day of illness. In seven cases from the 10th to the 30th day of illness, we found numerous spirochætæ in the urinary sediments of the five patients in which a negative result was obtained during the first 10 days. In four of these five patients the microscopic field contained countless spirochætæ. The spirochætæ were chiefly present in the cylindroids and nubeculæ, and in small numbers in the cylinders. We studied the spirochætæ in two of these five cases and found that they begin to degenerate and finally disappear from the urine altogether before the 40th day of illness. Later we studied the urine from ten cases after the 30th day of illness and detected a small number of degenerative spirochætæ in two on the 32nd and 38th days, respectively. The remaining eight cases gave negative results.

Hence the urine contains the spirochæta, beginning at an early stage of the disease, but in such small numbers as to make detection by the dark-field microscope difficult. However, from the 13th to

[8] This work was done by Drs. Ido, Hoki, and Ito.

the 15th day of illness, at the time that the immune substance appears in the blood, they become numerous and are easily discovered. After the 24th to the 25th day the spirochætæ begin to diminish in number and to assume a degenerative form, while, in our experience, they disappear completely from the urine before the 40th day of illness. However, these points require further investigation before a definite conclusion can be reached.

The results of the inoculation of urine into guinea pigs are given in Table I.

TABLE I.

Material used for inoculation.	No. of inoculations.	Positive result.
Urine from 5 patients before the 10th day of illness.....	9	3
Urine from 4 patients between the 11th and 20th days of illness.....	15	5
Urine from 9 patients after the 31st day of illness.....	12	0

The inoculation of the urine taken during the first 10 days of illness gave results in one-third of the cases, even though it was difficult to detect the spirochæta in the urine; and, conversely, although the urine contained many spirochætæ after the 13th to the 15th day of illness, the inoculations gave only the same percentage of positive results. Possibly the immune substance, which begins to appear in the urine about the 24th or 25th day, may bear upon that phenomenon.

Feces from eight patients were inoculated into guinea pigs on ten different occasions with one positive result, from a patient on the 7th day of illness.

Sputum from two patients was injected into guinea pigs on three occasions. A positive result was obtained once in a case in which the sputum was bloody. Two inoculations with vomitus gave no result.

Manner of Excretion in the Experimental Disease.

On examination of the urine, feces, and the contents of the gall-bladder in twenty-five infected guinea pigs, a small number of spirochætæ was detected in the urine by the dark-field apparatus in eight

of the thirty-two examinations. The examinations of the contents of the gall-bladder were all negative; while those of the feces and intestinal contents gave microscopically a positive result in one of twenty examinations.

The inoculation test with the materials mentioned gave a wholly different result: the urine produced infection in 7 of 9 tests, the feces and the intestinal contents in 7 of 11 tests, and the contents of the gall-bladder in 2 of 3 tests. Thus, the spirochæta is excreted in the urine, feces, and bile in the experimental disease of the guinea pig up to the time of death, although the number excreted in the urine is small.

The following experiments were made in order to determine whether the excretion of numerous spirochætæ in the patient's urine in the convalescent stage of the disease is related to the appearance of the immune substance in the blood.

Experiment 1.—The distribution of the spirochætæ in the various organs was studied after immune goat serum was injected into the guinea pig at the time of the appearance of jaundice.

Experiment 2.—The urine from the guinea pigs which contracted the disease from cutaneous inoculation and which had recovered without developing jaundice under treatment with immune goat serum, was examined daily at the time when the spirochætæ were present in the blood. The animals were killed about 20 days after the injection of the immune serum, and the distribution of the spirochætæ in the body was studied.

The first experiment was limited to two animals, and no spirochætæ could be discovered in the liver under the dark-field microscope, although the kidney showed a small number.

The second experiment was performed with four animals. In two that passed the 10th and 13th days respectively after the injection of the immune serum, the spirochæta was recognized in the urine. All four animals were killed on the 20th day after the serum treatment, and the blood, liver, kidneys, and urine were examined under dark-field illumination. The blood and liver showed no spirochætæ, while the kidneys and urine in the bladder showed typical spirochætæ with active movements.

In the experimentally infected guinea pigs the number of the spirochætæ excreted in the urine is usually small, and this is probably due to the circumstance that the animals die before the immune substance is formed in the body. This is indicated by the fact that the spirochætæ appear in the urine in the animals which are treated with the immune serum. The serum treatment probably confers an immunity upon the animals.

Whether the formation of the immune substances which appear to limit the development of the spirochætæ in the body and cause their collection in the kidneys and hence excretion into the urine is responsible for the appearance of the large numbers in the urine in human cases at the time of convalescence, is not clear. Perhaps the spirochætæ are merely able to multiply in the kidney for a time, after which they are excreted. The autopsies indicate that these phenomena occur. The immune substances, moreover, are present in the urine as well as in the blood for a certain period after the 24th or 25th day of illness. When the immune bodies begin to appear in the urine the spirochætæ gradually degenerate and disappear.

Immunity Phenomena.[9]

In the spirochætal disease under consideration a substance which has bactericidal and bacteriolytic action over the spirochætæ appears in the blood. The discovery of this substance was of great importance in the detection of the spirochætæ. The existence of the immune body can be demonstrated by Pfeiffer's method, as follows:

1 to 2 cc. of liver emulsion, containing spirochætæ, are injected into the peritoneal cavity of guinea pigs. In one series of animals the patient's serum is also injected, while in the control animal salt solution or the serum of a healthy person is employed for the injection. After ½ to 2 hours peritoneal fluid is withdrawn and examined microscopically. The fluid from the control animals shows numerous spirochætæ with active movements, although in somewhat diminished numbers after 2 hours, while after half an hour the fluid from the animal treated with the patient's serum will be free of spirochætæ.

Moreover, the subsequent condition of the animals confirms the presence of the immune body. Those in which the spirochætæ disappear from the peritoneal fluid after half an hour usually do not develop the infection, while those which still show after 2 hours a small number of spirochætæ in the peritoneal fluid develop jaundice and hemorrhage and finally die. These phenomena occur both with active and inactive serum.

The immune body exerts its action when injected along with the spirochætæ and also when the spirochætæ are already in the tissues. When the serum of a convalescent patient is injected into a guinea pig which has already developed jaundice and hemorrhage, the spiro-

[9] This work was carried out by Drs. Hoki and Ito.

chætæ disappear from the blood after half an hour, and after $3\frac{1}{2}$ hours only a small number of the degenerative type of the organism can be detected in sections of the liver by Levaditi's method of staining. At the end of 8 hours no spirochætæ are seen, but in these instances we were able to find a few spirochætæ in the other organs, especially the kidneys and suprarenals, by using the silver impregnation method. Identical results were obtained with each of the six strains of spirochætæ that we now possess.

In order to determine the time of appearance of the immune substance, sixteen cases of Weil's disease were examined within the first 30 days of illness. The majority of the sera examined had been preserved for 2 years. Pfeiffer's reaction proved positive in one case on the 14th day, in two cases on the 15th day, in one case on the 22nd day, and in three cases between the 24th and 28th days of illness.

These results show clearly that the immune substance is fully formed about the 14th to the 15th day of the illness. On the other hand, examination of eight cases from the 4th to the 11th day all proved negative, while one case was negative on the 9th day and positive on the 20th day, and another was negative on the 7th day and positive on the 15th day of illness.

The immunity endures for a long period of time. Of thirteen cases examined later than a month since the illness, immune bodies were detected at the expiration of over $5\frac{1}{2}$ years (two cases), 3 years and 2 months (one case), 2 years and 4 months (two cases), and 1 year and 10 months (one case). This immune substance is specific in Weil's disease and is not present in the serum of healthy persons who have not had Weil's disease or in persons with jaundice of other nature.

Distribution of the Spirochætæ in the Human Body.[10]

Postmortem examinations were performed on twelve cases which died between the 9th and 16th days of illness. Tissues from eight cases were fixed in formalin solution, and tissues from one case which died on the 8th day of illness had been preserved in Orth's solution for 8 years. The examination showed that while the kidneys contain the spirochætæ in the largest number, yet in every case spirochætæ were detected.

[10] This work was done by Dr. Kaneko.

Spirochætæ in the kidneys were mostly within the uriniferous tubules, and the majority were contained in the substance of urinary cylinders (casts). However, a small number occurred within the detritus in the uriniferous tubules or was attached to the epithelial cells. In tissues from one case which succumbed on the 10th day localized accumulations of the spirochætæ in the act of invading the tubules were seen in the interstitial tissues. The spirochætæ in the urinary cylinders were most numerous in a case succumbing on the 9th day, and within the lumen of the uriniferous tubules in a case succumbing on the 14th day.

The liver showed spirochætæ in smaller numbers, of which many were degenerated. No spirochætæ were found in the specimen of liver preserved in Orth's solution. Within the liver the spirochætæ were chiefly present within the hepatic cells; they occurred frequently also at a comparatively early stage of the disease in the interstitial tissues and often also in the stellate cells of Kupffer. In cases dying about the 9th day the spirochætæ were typical in form and occurred within the veins and on the outer surface of the cells, while in cases from the 14th to the 16th day of illness they were present only within the hepatic cells. The distribution of the spirochætæ in the suprarenal gland is similar to that in the liver.

The lymph glands and spleen contained a small number of spirochætæ, mostly in a degenerated condition. They were chiefly inside of phagocytic cells, while comparatively well preserved organisms were seen in masses of coagulated blood. This condition of the lymph glands is to be distinguished from what occurs in locally infected glands; in the latter in six cases examined typical spirochætæ existed, although in small number, while in the autopsied cases typical organisms rarely were met with.

As regards the other organs, comparatively well preserved organisms were found in the cardiac muscle, voluntary muscle, testicle, and arterial walls of the autopsied cases. The cardiac muscle especially exhibited well preserved, typical spirochætæ within the cell bodies and in the interstitial tissues. The spirochætæ were detected at times in the lung, pancreas, intestines, gall-bladder, genitals, nervous system, and skin, but they were mostly degenerated.

Thus the distribution of the spirochætæ in the human body differs from that in the guinea pig in that the number present is smaller, the degenerative forms are more abundant, they are more within cells, the kidney contains more, and the liver and adrenal glands contain fewer; the cardiac muscles, voluntary muscles, and arterial wall contain typical organisms.

The above differences can probably be explained by the action of the immune body, which appears in the course of the disease. This immune body destroys and dissolves the spirochætæ, and consequently one finds a smaller number of them in man. The greater occurrence of intracellular organisms is probably due to the fact that the spirochætæ invade cells in order to escape from the action of the immune body; while the presence of the spirochætæ in the epithelial cells of the glandular diseases perhaps indicates its way of escape from the body. The fact that the number of spirochætæ found in the autopsied cases is small may be explained partly by imperfect preservation of tissues and mixed infection; and we believe, therefore, greater numbers will be detected by means of a perfected method of study in the early stages of cases of pure infection. This view has recently been supported by finding the liver in two cases, which died on the 6th day, as heavily infected with spirochætæ as in the guinea pig.

Prophylaxis.

The mode of infection of the disease and the excretion of the spirochætæ indicate the means and the necessity of prophylaxis. As already mentioned, the disease occurs frequently among coal miners who work in that part of a mine which is inundated with water got rid of by pumping. It is, therefore, necessary to remove the water and then to disinfect the ground, when it is found that the disease occurs among the workmen who work at certain definite places in the mines. Lime is effective for the disinfection of the ground. It is also necessary to avoid infection through the healthy skin and the alimentary canal. Thus, when the infected locality is large so that it cannot be disinfected thoroughly, recourse must be had to the method of active immunization. Our study of this question is still under way, so that results can only partially be mentioned here.

Guinea pigs were immunized with repeated injections of liver emulsion of the infected animal and later with a pure culture of the spirochætæ which had been killed by carbolic acid. The animals thus immunized did not develop the disease on the injection of the spirochætæ, which, it was known, would produce the disease in healthy animals. Hence this method seems promising for the prevention of the disease in man. Our conclusion is that the flea and mosquito have no share in the infection.

Moreover, it is necessary to disinfect the urine for at least 40 days from the beginning of the illness, in view of the fact that the spirochætæ are incessantly excreted in the urine during the course of the disease, and even as late as the 40th day. Although the spirochætæ appear to be in small numbers only in stools, they should be disinfected, as should also bloody sputum.

The spirochætæ seem to live in water and mud and to invade the human body from there; hence it is important to study the conditions necessary for their existence and especially the question of temperature, and to discover the existence of the spirochæta carrier.

Experimental Therapeutics.

Treatment with Salvarsan.—Guinea pigs were used for this purpose. The method and time of the inoculation with the spirochætæ were different in various cases. We could not discover the spirochæta in the liver under dark-field illumination in fourteen out of nineteen guinea pigs which underwent treatment with salvarsan (0.1 gm. of salvarsan per kilo of body weight), but found jaundice and hemorrhage in six instances after inoculation of an emulsion made from livers in which spirochætæ were not found.

Of twenty-one guinea pigs which were treated with salvarsan (0.05 gm. of salvarsan per kilo of body weight), dark-field illumination showed spirochætæ in nine instances, and inoculation from the nine cases gave positive results in seven instances.

Examination of the blood for spirochætæ before and after the salvarsan injection was done in seven animals. Three cases proved negative after the injection, although they showed spirochætæ in the blood before the injection. In one animal spirochætæ were absent before the injection and were present for a day after the injection.

The other three animals proved negative both before and after the injection. From these results it may be concluded that the spirochætæ are removed from the blood by salvarsan.

The action of salvarsan against the spirochætæ in the liver and other organs is still under investigation and will be reported later. The animals often died from 4 to 8 days after salvarsan injection. Professor Hata informs us that this is due to the fact that the resistance of the guinea pig against arsenical compounds is low. This weak resistance to arsenic is unfortunate, since the guinea pig is the only animal possessing strong susceptibilities for the spirochætæ.

Treatment with Immune Serum.—At first we used serum taken from a convalescent patient, and later serum from an immunized goat. The treatment with both sera gave better results than did the salvarsan treatment.

1 to 2 cc. of the serum from the convalescent patient were injected, before the appearance of jaundice, into six guinea pigs. Five of them developed no typical symptoms and no spirochætæ were found in the liver.

One of the animals developed hemorrhage and jaundice, but upon examination of the liver no spirochætæ were found. Six animals which received the serum injection, after the appearance of jaundice, showed postmortem typical changes, but spirochætæ were present in the liver in two instances only.

Immune goat serum was injected into twelve animals before the appearance of jaundice and at the time when the fever developed and spirochætæ were present in the blood. The spirochætæ disappeared from the blood half an hour after the serum injection and all the animals remained alive, except one which died on the 3rd day. This animal showed typical postmortem changes, but spirochætæ were not found in the liver, and the inoculation test with the liver emulsion also proved negative. Two of the remaining eleven animals developed slight jaundice, but recovered.

The injection of immune goat serum is ineffective after jaundice has appeared, and the guinea pig dies after the treatment. We did not find spirochætæ in the liver, and the inoculation test was negative, as was the microscopical examination of the organ. These results indicate a great difference between salvarsan and immune serum in treating the experimental disease.

The temperature of the animals which received the immune serum before the appearance of jaundice does not fall as in collapse, but falls at first and then rises again slightly. This phenomenon resembles that of the fever following the crisis in Weil's disease.

The strength of our immune goat serum is the same as that of the patient in the convalescent stage of the disease. Whether the strength can be increased has not yet been determined.

The serum treatment of the experimentally infected animals has, so far, given satisfactory results and affords a good basis for the clinical use of the serum treatment on man. The serum treatment is not suitable for cases which suffer relapses, but may be considered hopeful for cases which have already developed an immune body in the course of the disease, with lasting immunity.

We have also immunized horses for the purpose of obtaining an immune serum and we have had at least a partial success with horse serum. In a case in which it was administered subcutaneously the spirochætæ disappeared from the blood within 24 hours.

BIBLIOGRAPHY.

Inada and Ido, A Report on the Discovery of the Causal Organism (a New Species of Spirochæta) of Weil's Disease, *Tokyo Ijishinshi*, 1915, No. 1908.

Inada and Ido, A Report on the Cause of Weil's Disease, *Tokyo Ijishinshi*, 1915, No. 1920–1921.

Inada and Ido, Weil's Disease in Chiba, *Tokyo Ijishinshi*, 1915, No. 1920.

Nishida, *Odan-eki* and Its Cause, a Spirochæta, *Tokyo Ijishinshi*, 1915, No. 1920.

Inada and Kaneko, A Report on the Pathology and Anatomy of Animal Experiments with Spirochætosis Icterohæmorrhagica, *Chugai Ijishinpo*, 1915, No. 849; *Nippon Naika Gakkai Zasshi*, iii, No. 3.

Inada, Hoki, and Ido, Animal Experiments on Spirochætosis Icterohæmorrhagica, *Chugai Ijishinpo*, 1915, No. 852; *Nippon Naika Gakkai Zasshi*, iii, No. 3.

Ido, Hoki, and Ito, The Mode of Excretion of *Spirochæta icterohæmorrhagiæ*, *Iji Shinbun*, 1915, No. 931.

Ido and Hoki, The Mode of Invasion of *Spirochæta icterohæmorrhagiæ*, *Nippon Naika Gakkai Zasshi*, iii, No. 3; *Iji Shinbun*, 1915, No. 932.

Inada and Ido, The Pure Cultivation of *Spirochæta icterohæmorrhagiæ*, *Saikingaku Zasshi*, 1915, No. 239.

Hoki and Ito, Immunity in Spirochætosis Icterohæmorrhagica, *Igaku Chuo Zasshi*, 1915, No. 113.

Kaneko, The Distribution of *Spirochæta icterohæmorrhagiæ* in the Human Body, *Tokyo Ijishinshi*, 1915, No. 1932–1936.

Kaneko and Okuda, The Pathological and Anatomical State of the Lymph Glands in the Early Stage, and the Distribution of the Spirochæta in Them, *Tokyo Ijishinshi*, 1915, No. 1940–1945.

Inada, Ido, Kaneko, Hoki, and Ito, Spirochætosis Icterohæmorrhagica. The Etiology, Morbid Anatomy, Pathology, Symptoms, Diagnosis, Prophylaxis, and Treatment, *Nisshin Igaku*, 1915, v, No. 1.

EXPLANATION OF PLATES.

PLATE 56.

FIGS. 1 to 5. Microphotographs of *Spirochæta icterohæmorrhagiæ* in human blood. Giemsa stain. × 750.

FIG. 1. H. K. 6th day of illness.

FIG. 2. I. H. 4th day.

FIG. 3. I. Y. 11th day.

FIG. 4. I. Y. 11th day.

FIG. 5. Y. T. 8th day.

FIGS. 6 to 9. Spirochætæ in the blood of a guinea pig.

FIG. 6. Giemsa stain. × 750.

FIGS. 7 and 8. Silver impregnation. × 1,000.

PLATE 57.

FIG. 9. Two spirochætæ intertwined.

FIG. 10. Cover-glass specimen of the liver of a guinea pig. Giemsa stain. × 2,500.

FIG. 11. Spirochætæ in the liver of a guinea pig which developed the disease after an injection of *Spirochæta icterohæmorrhagiæ*.

FIG. 12. Spirochætæ intertwined.

FIG. 13. A degenerative type.

FIG. 14. Spirochætæ ending in a cluster.

PLATE 58.

FIG. 15. A spirochæta in the skin of a guinea pig 10 hours after infection. The spirochæta is in the corium.

FIGS. 16, 17, and 18. Spirochætæ excreted in the urine of patients.

FIG. 16. Numerous normal spirochætæ in a cylindroid from a patient (S.) on the 16th day of the disease. Dark-field illumination.

FIG. 17. Numerous degenerative types in a cylindroid from a patient (I.) on the 23rd day of the disease.

FIG. 18. Microphotograph of spirochætæ in the urinary sediment from the same patient as Fig. 16.

PLATE 59.

FIGS. 19, 20, 21, 22, and 23. Spirochætæ in the organs in spirochætosis icterohæmorrhagica.

FIG. 19. Degenerative type of spirochætæ in the liver (intracellular) of a patient autopsied on the 13th day of the disease.

FIG. 20. Spirochætæ in the kidney of a patient autopsied on the 10th day of the disease.

FIG. 21. Spirochætæ in the cylinder of the kidney of a patient autopsied on the 8th day.

FIG. 22. Spirochætæ in the liver of a patient autopsied on the 6th day. × 350.

PLATE 60.

FIG. 23. Drawing of Fig. 22.

FIG. 24. Microphotograph of spirochætæ in the uriniferous tubules of a guinea pig, which recovered after treatment with immune serum. Killed after 21 days.

PLATE 61.

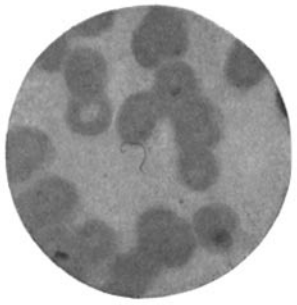
FIG. 25. Forms of *Spirochæta icterohæmorrhagiæ*.

1 to 65. Giemsa stain. 66 to 87. Silver-impregnated spirochætæ from tissues. 1 to 25 and 66 to 68. Various forms of the spirochæta. 26. A spirochæta 25 μ in length. 27 to 32, 73 to 74, and 77 to 87. Various forms of the degenerative type. 33 to 41, and 43. A mass of chromatin (?) and the so called bud of the spirochæta. 42, 69, and 70. A spirochæta ending in a cluster. 44 to 47, 75 and 76. The so called bud of a short spirochæta. 48 to 51. A spirochæta which resembles a platinum loop at one end. 52 to 59, 71 and 72. A spirochæta forming a ring. 60 to 63. A spirochæta with a small ring. 64 and 65. Forms which are considered to be in the stage of multiplication.

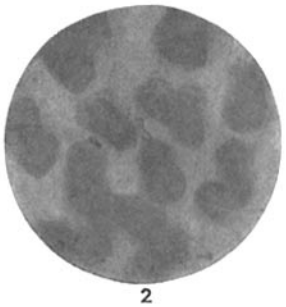
PLATE 62.

FIG. 26. Forms and movements of *Spirochæta icterohæmorrhagiæ*. The pure culture is observed under dark-field illumination.

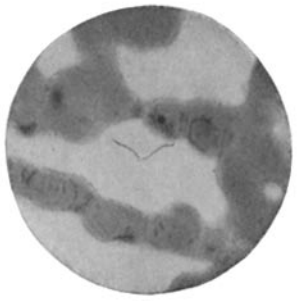
1. Forward motion. 2 to 4. Rotating motion. 5. Rotating motion of a less active spirochæta. 6. A snake-like movement. 7 and 8. Young, short forms. 9. Grouping in the form of a rosette. 10 to 12. A group of spirochætæ. 13. Pair-form. 14 and 15. A mass of chromatin (?). 16. A spirochæta ending in a cluster. 17 and 18. Long forms (two and three times the length of the usual spirochætæ). 19. A straight form. 20. A thick form. 21 to 29. Various degenerative forms (crooked form). 30 to 34. Other degenerative forms. 35. A transition form between the above and the normal. 36. Spiral movement.



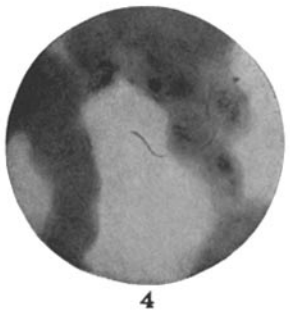
1



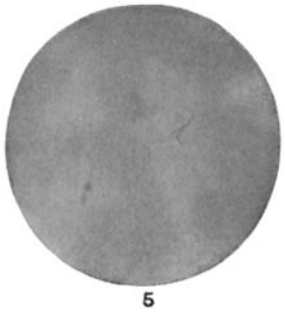
2



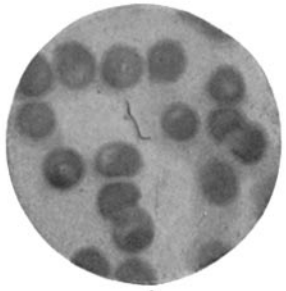
3



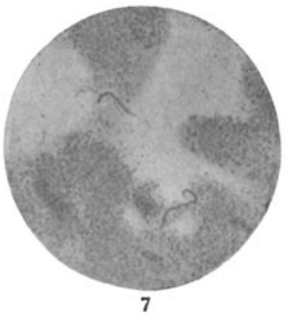
4



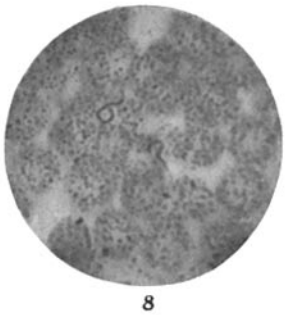
5



6

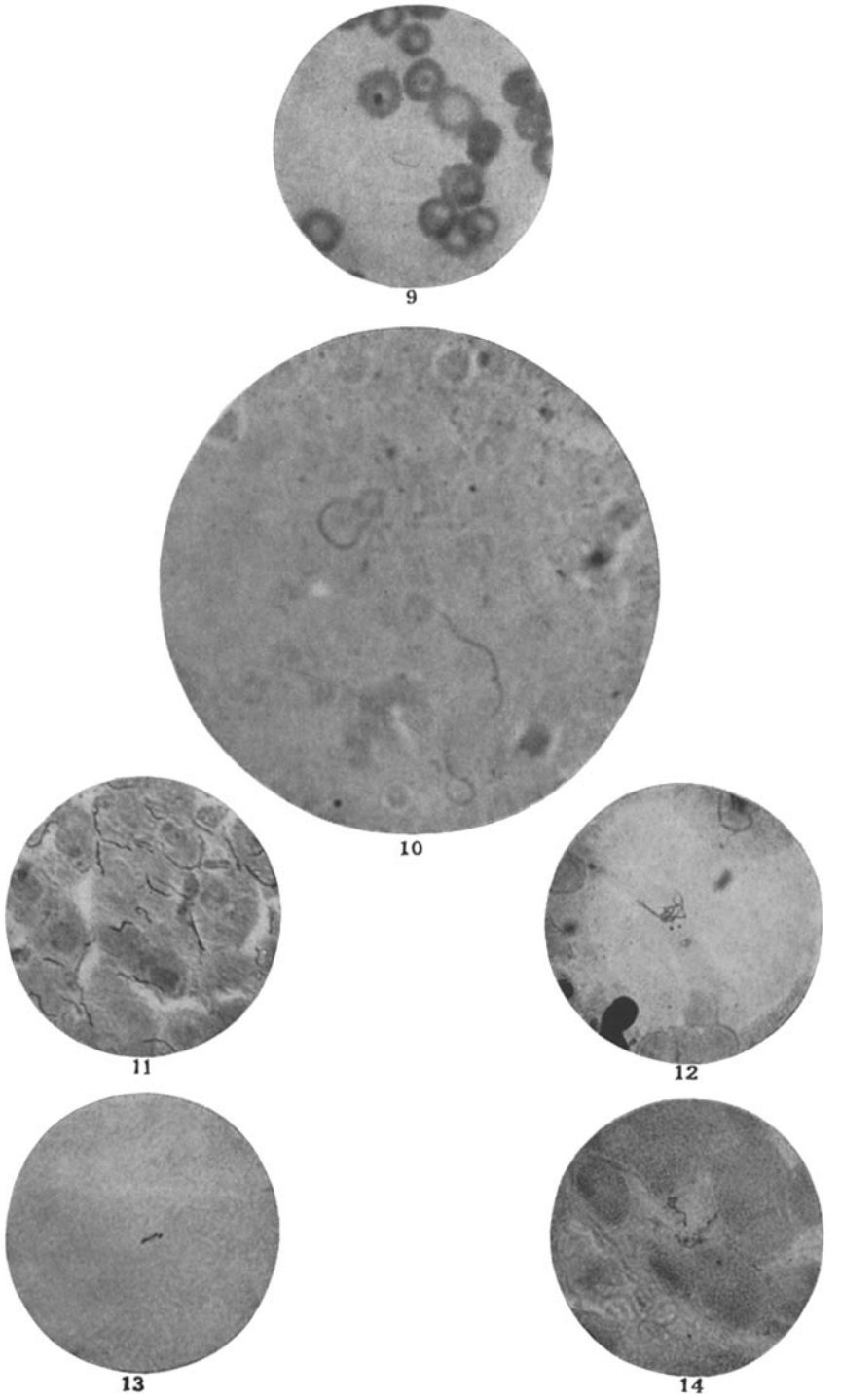


7

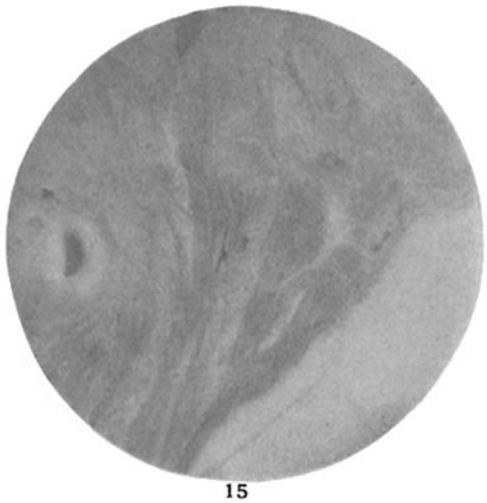


8

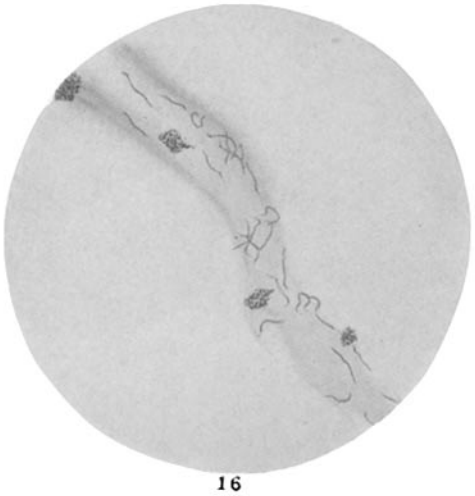
(Inada, Ido, Hoki, Kaneko and Ito: Weil's Disease.)



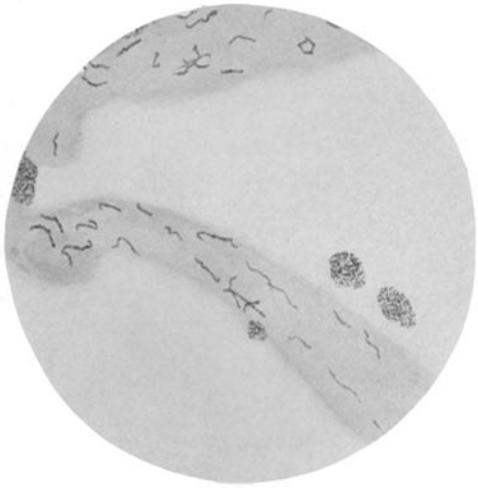
(Inada, Ido, Hoki, Kaneko and Ito: Weil's Disease.)



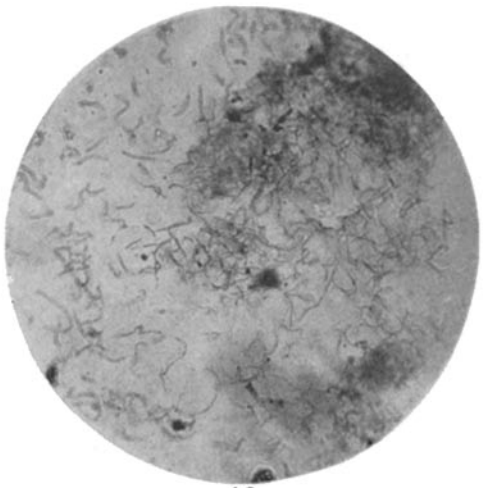
15



16

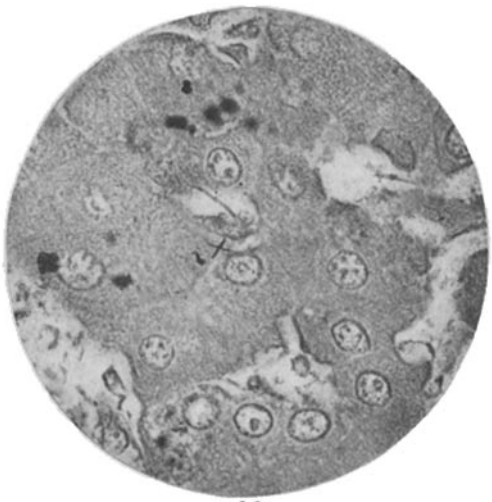


17

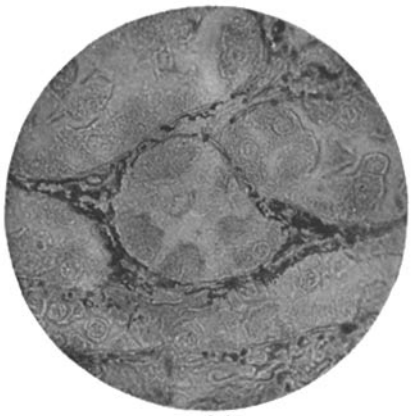


18

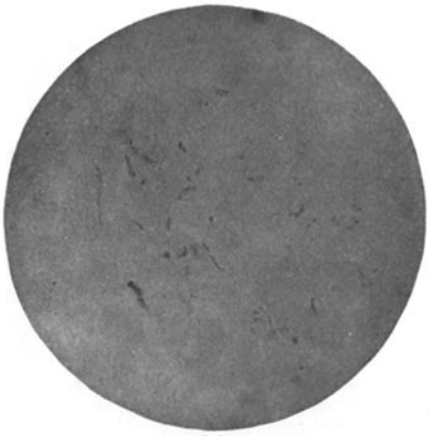
(Inada, Ido, Hoki, Kaneko and Ito: Weil's Disease.)



19



20



21

22

(Inada, Ido, Hoki, Kaneko and Ito: Weil's Disease.)

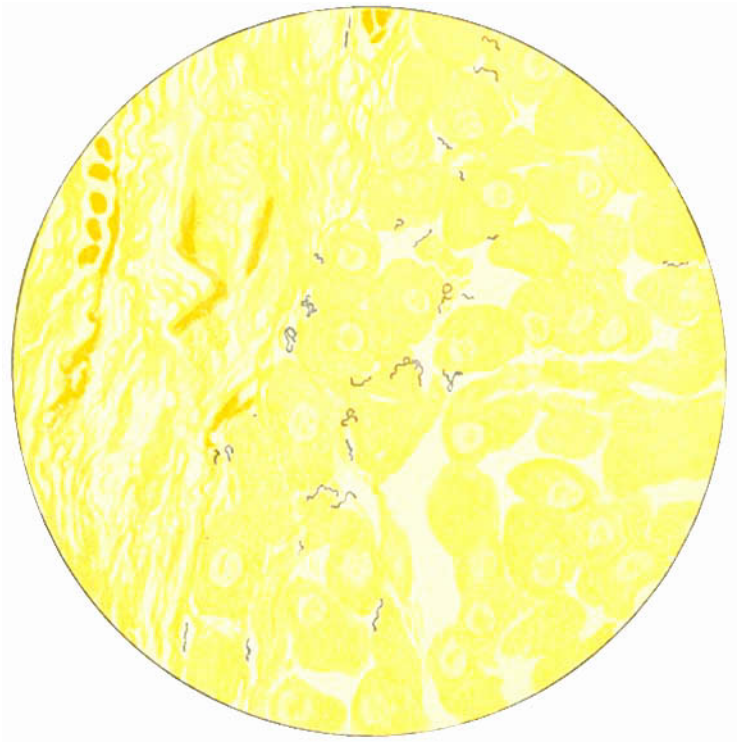
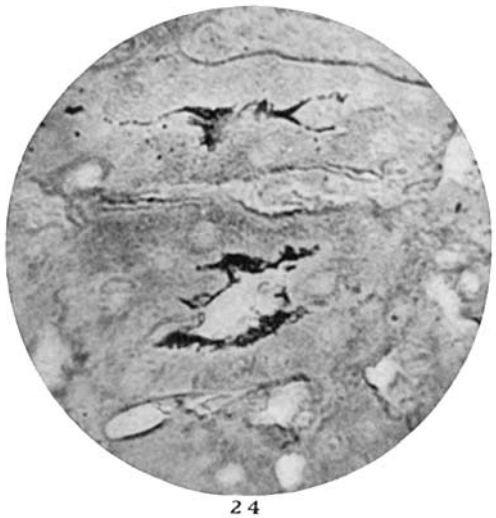


FIG. 23.



24

(Inada, Ido, Hoki, Kaneko and Ito: Weil's Disease.)

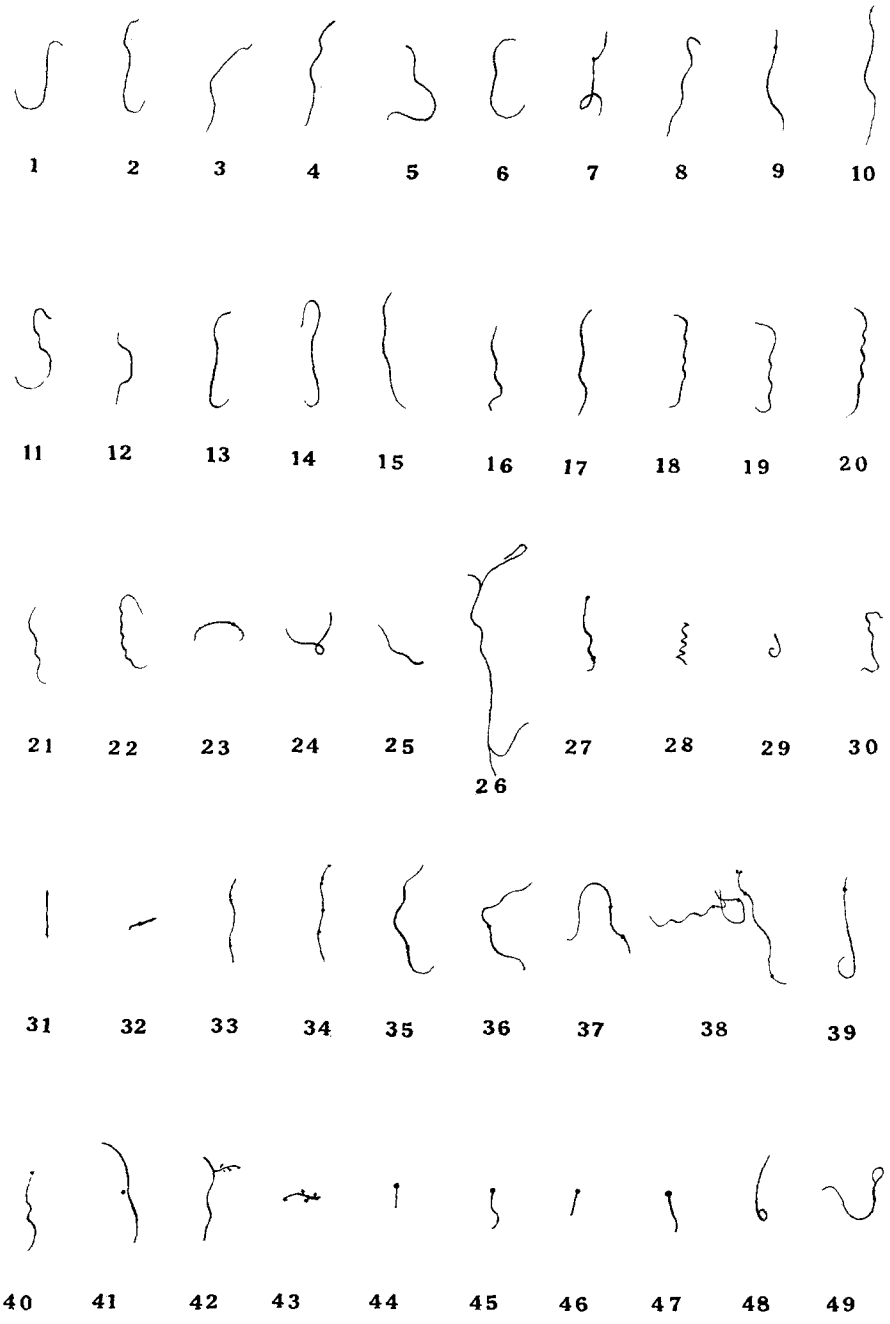
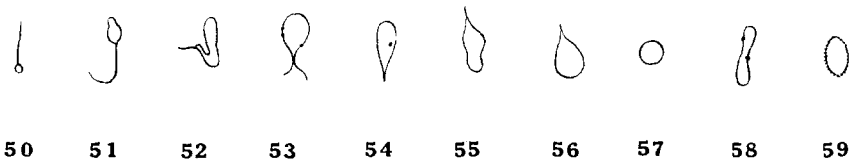
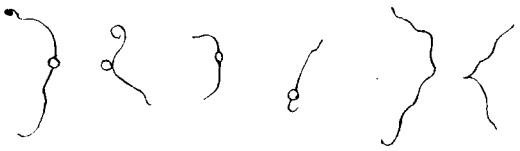

1 2 3 4 5 6 7 8 9 10
11 12 13 14 15 16 17 18 19 20
21 22 23 24 25 26 27 28 29 30
31 32 33 34 35 36 37 38 39
40 41 42 43 44 45 46 47 48 49

FIG. 25a.



50 51 52 53 54 55 56 57 58 59



60 61 62 63 64 65

66 67 68 69 70 71 72 73

74 75 76 77 78 79 80 81

82 83 84 85 86 87

FIG. 25 b.
(Inada, Ido, Hoki, Kaneko and Ito: Weil's Disease.)

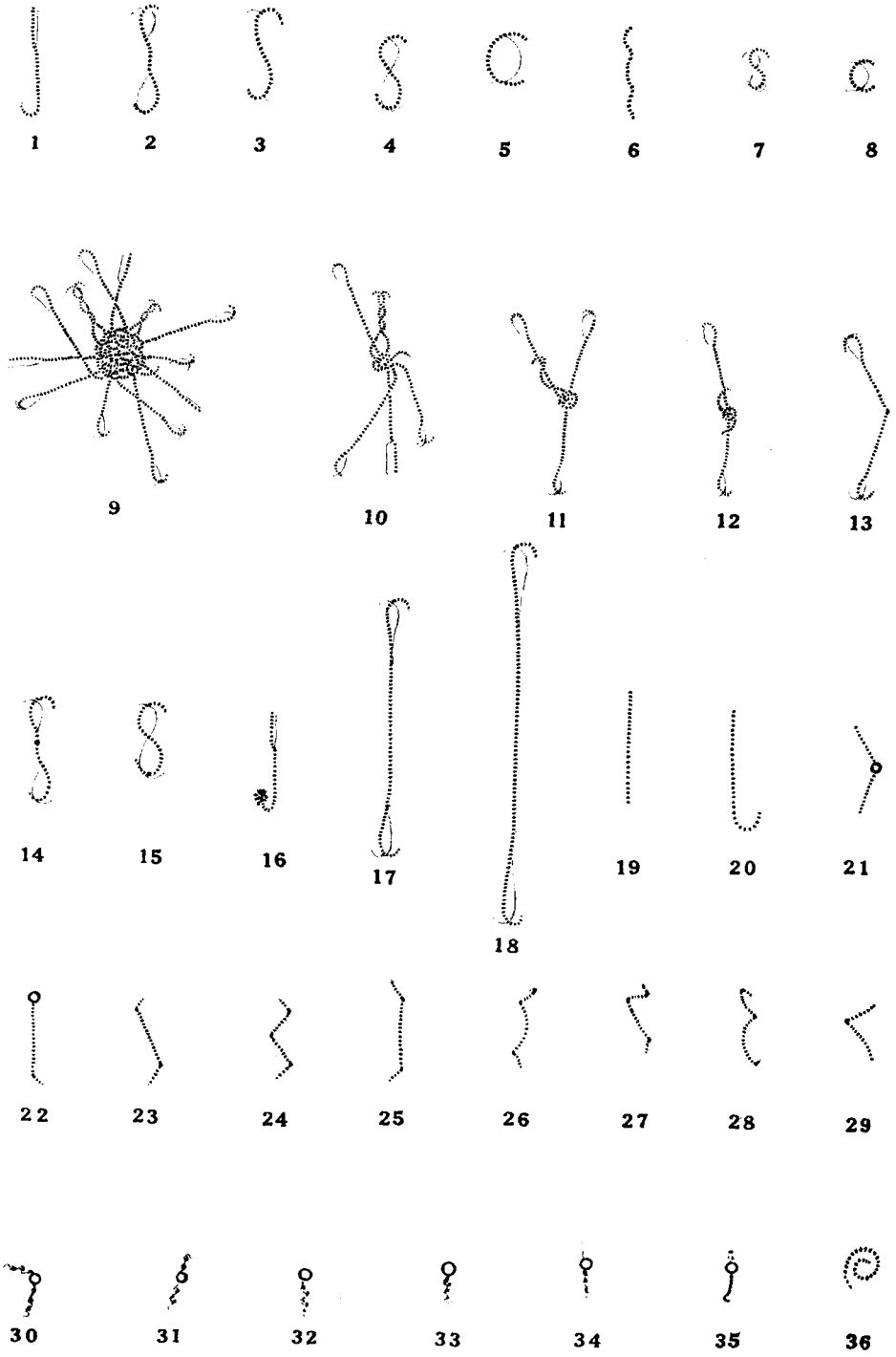


FIG. 26.

(Inada, Ido, Hoki, Kaneko and Ito: Weil's Disease.)