THE RAT AS A CARRIER OF SPIROCHÆTA ICTERO-HÆMORRHAGIÆ, THE CAUSATIVE AGENT OF WEIL'S DISEASE (SPIROCHÆTOSIS ICTEROHÆMORRHAGICA).*

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In a previous communication[1] on the prophylaxis of spirochætosis icterohæmorrhagica, we discussed briefly the excretion and means of invasion of the causative agent. It was clear that soil and water, particularly stagnant water, are related to the infection, but where the spirochetes have their habitation outside of the human body and proliferate until they again attack man remained a difficult problem of prophylaxis.

When Inada at the annual meeting of the Kitasato Institute for Infectious Diseases, in the spring of 1915, gave a comprehensive report of studies made on spirochætosis icterohæmorrhagica in his clinic, Miyajima called attention to the fact that in his investigations on tsutsugamushi, he had found on several occasions spirochetes resembling *Spirochæta icterohæmorrhagiæ* in the kidneys of the field mouse, *Microtus montebelloi*. On the basis of these findings, we conducted during the following year an investigation on twenty-two house and roof rats, *Epymis alexandrinus* and *Epymis norvegicus*. We discovered on one occasion in the kidney of one of the animals a specimen of *Spirochæta icterohæmorrhagiæ*. There was at that time doubt in our minds whether the organism in question had actually resided in the kidney or was a contamination introduced from the outside,

* Published in *Tokyo Iji-Shuho*, July, 1916, Nos. 1978, 1979.

[1] Ido, Y., Hoki, R., Ito, H., and Wani, H., The prophylaxis of Weil's disease (spirochætosis icterohæmorrhagica), *J. Exp. Med.*, 1916, xxiv, 471.

inasmuch as we were working with *Spirochæta icterohæmorrhagiæ*. The problem was then left for future investigation.

In the spring of 1916, Miyajima again reported that he had found in *Microtus montebelloi* spirochetes resembling *Spirochæta icterohæmorrhagiæ*, which injected into guinea pigs produced fever and hemorrhage, and after a number of generations, icterus.[2] As the immune serum of *Spirochæta icterohæmorrhagiæ* was capable of destroying the organisms in question, he concluded that they were identical with *Spirochæta icterohæmorrhagiæ*.

From our clinical experience we had already surmised the relation of the rat to the infection in man. Cooks working in kitchens frequented by rats often became ill with spirochætosis icterohæmorrhagica. At the beginning of the year we observed two typical cases following the bite of rats. We were led to the conclusion that the rat plays an important part in the transmission of the infection, and that the spirochete previously found by us in the kidney of a rat was not a contamination, but came from that organ. On the basis of this assumption we undertook an investigation of the house and roof rats in the city of Fukuoka and its vicinity.

EXPERIMENTAL.

The rats examined were *Mus alexandrinus* and *Mus decumanus*. We were able to find virulent *Spirochæta icterohæmorrhagiæ* in the kidneys, in 40.2 per cent out of 149 *Mus decumanus*, and in 0.8 per cent of 24 *Mus alexandrinus*.[3] The morphological examinations and

[2] The findings of the Japanese workers have been confirmed in Europe by Dr. Stokes, who conducted an investigation of cases of Weil's disease occurring in the British army. He was able to demonstrate *Spirochæta icterohæmorrhægiæ* in the kidneys of field rats, and infected guinea pigs with the organisms (Stokes, A., Ryle, J. A., and Tytler, W. H., Weil's disease (spirochætosis icterohæmorrhagica) in the British Army in Flanders, *Lancet*, 1917, i, 142). In the United States, Dr. Noguchi has confirmed the work by his finding of *Spirochæta icterohæmorrhagiæ* in domestic wild rats (Noguchi, H., *Spirochæta icterohæmorrhagiæ* in American wild rats, and its relation to the Japanese and European strains, *J. Exp. Med.*, 1917, xxv, 755).

[3] The determination of the animal species was made by Mr. Namiye, assistant in the Zoological Institute of the Imperial University at Tokyo, to whom we desire herewith to express our thanks.

specific differences in the immune serum proved that the spirochete found by us in the kidneys of house and wild rats is identical with *Spirochæta icterohæmorrhagiæ*.

We also examined six specimens of field mice.[4] In one instance we produced by intraperitoneal injection of mouse kidney emulsion, icterus and hemorrhages in the guinea pig, and identified numerous *Spirochæta icterohæmorrhagiæ* in the liver, though we found none by dark-field illumination in fresh kidney preparations of the field mouse. In five other cases, we could find no spirochetes, either by dark-field illumination or through intraperitoneal injection of kidney emulsion.

In our experiments with rats we employed the following method. A rat was permitted to bite a guinea pig in the hind leg. The rat was then killed, and a search was made by dark-field illumination for spirochetes in the blood, liver, and kidneys. When no organisms were found in this way, we injected intraperitoneally into the guinea pigs the blood, urine, liver, and kidney emulsion of the rat, awaiting the development of icterus and hemorrhages. As a rule, we examined three preparations by dark-field illumination, and if no organisms were found, we considered the result negative.

Experiment 1. Kidneys.—The number of rats employed was 92. In 26 of the animals, or 28.3 per cent, spirochetes could be demonstrated in the kidneys. We injected kidney emulsion from 8 of the 26 animals intraperitoneally into guinea pigs. 7 of these died on the 8th to the 13th day with marked icterus and hemorrhages; numerous organisms were found in their blood and liver. From 59 animals showing microscopically no organisms, we prepared kidney emulsion, which we injected intraperitoneally into guinea pigs; 5 (8.5 per cent) animals died with typical symptoms on the 8th to the 11th day. The number of spirochetes in the kidneys varied between one to a preparation and fifteen to sixteen in an optical field. Occasionally we found a tuft of spirochetes. All specimens showed brisk movements.

Experiment 2. Urine.—We examined the urine of 71 rats. In 22, or 31 per cent, spirochetes were present. In 19 rats, in which we found the organisms in the kidneys, they were contained also in the urine. In 3 out of 52 in which we were unable to demonstrate spirochetes in the kidneys, we discovered them in the urine. The urine sediment of 2 rats, in which no spirochetes had been found by either method, was injected intraperitoneally into guinea pigs. One

[4] These mice were furnished us through the kindness of Dr. Miyakawas.

of the experimental animals died with typical symptoms on the 17th day. To test the virulence of the spirochetes, we then injected 0.1 to 0.2 cc. of urine containing spirochetes, from 5 rats, intraperitoneally into 5 guinea pigs. The animals died with marked icterus and hemorrhages 8 to 10 days later, thus proving a high degree of virulence for the organisms.

The spirochetes excreted in the urine appear for the most part in the nubecula, though some are found free. Their number fluctuates from fifteen to sixteen specimens in one nubecula to one or two in a preparation. The spirochetes in the nubecula are usually motionless, while the freely floating spirochetes make brisk movements. Occasionally degenerative forms are seen.

Experiment 3. Blood and Liver.—We examined the blood of 64 rats by dark-field illumination. In 20 of these we had found spirochetes in the kidneys and the urine, but we were unable to find them in the blood in a single instance. The intraperitoneal injections of guinea pigs with the blood of 6 rats, in which organisms were found in the kidneys, proved negative, as well as five blood injections from animals having no spirochetes in their kidneys. Similar experiments were conducted with liver emulsion and intestinal contents. We examined the livers of 62 rats, and the large intestines of 10. All these experiments were negative.

Experiment 4. Rat Bite.—As we had observed two cases of typical spirochætosis icterohæmorrhagica following the bite of a rat, we proceeded to reproduce the conditions experimentally. Rats were permitted to bite guinea pigs in the leg. Of 50 experiments thus made, only one guinea pig died of icterus and hemorrhages on the 11th day following the bite. In this case, numerous *Spirochæta icterohæmorrhagiæ* were found in the blood and the liver. In two guinea pigs we observed in the blood another form of spirochete which Futaki and Ishiwara, with their associates, have assigned as the cause of rat-bite fever. It may be mentioned in passing that Drs. Kaneko and Okuda[5] of our clinic were able to confirm the view of the other Japanese investigators in this respect by finding the spirochetes in the kidneys of a patient dying from rat-bite fever. By our finding of spirochetolytic and spirocheticidal immune bodies in the serum of individuals who had recovered from rat-bite fever, we were able to affirm that the above mentioned spirochete is the causative agent of rat-bite fever.

Mizukuchi reported before the Pathological Anatomical Congress held in Tokyo in April, 1916, an interesting fact which grew out of an experimental investigation of rat-bite fever. He permitted guinea

[5] Kaneko, R., and Okuda, K., The distribution in the human body of *Spirochæta icterohæmorrhagiæ*, *J. Exp. Med.* 1917, xxvi, 325.

pigs to bite rats, and certain of the animals died later, as the result of a peculiar icteric and hemorrhagic condition. In the liver of these animals he found a spirochete which, according to our view, is identical with *Spirochæta icterohæmorrhagiæ*. Mizukuchi believed the spirochete to be the cause of rat-bite fever, a conclusion with which we cannot agree. It is highly probable that the microorganism was *Spirochæta icterohæmorrhagiæ*, and that it was communicated to the guinea pigs in the same manner as in our experiments.

The manner in which *Spirochæta icterohæmorrhagiæ* is conveyed by the bite of a rat to man or the guinea pig is not yet clear. As no spirochetes were found either in the mouth or the blood of the rat, it is not likely that they are carried directly through biting. It may be assumed, however, that they are conveyed indirectly from rat urine, with which the mouth of the rat may be contaminated, into the wound created by the bite of the rat.

As stated above, the spirochetes found in the kidneys and the urine of rats resemble in form and movement *Spirochæta icterohæmorrhagiæ*. When injected intraperitoneally into the guinea pig, the animal succumbs after a time, with icterus and hemorrhages, symptoms which are identical with those of spirochætosis icterohæmorrhagica. These facts seem to prove without a doubt that the spirochete in question is identical with *Spirochæta icterohæmorrhagiæ*, the causative agent of Weil's disease, but further experiments to confirm this point are cited below.

Action of Spirochæta icterohæmorrhagiæ Immune Serum on Spirochetes Found in Rats.

For Pfeiffer's tests we used guinea pig liver emulsion rich in spirochetes, and immune horse serum of *Spirochæta icterohæmorrhagiæ*. Control experiments were also carried out. These were made (1) with rat spirochetes, *Spirochæta icterohæmorrhagiæ*, and isotonic salt solution; and (2) with *Spirochæta icterohæmorrhagiæ* and its immune horse serum. The results are shown in Table I.

As shown in the table, no spirochetes were contained in the peritoneal fluid in from 30 minutes to 2 hours after injection in the main experiments conducted with rat spirochetes and immune horse serum,

TABLE I.

Results by Pfeiffer's Method with Rat Spirochetes and Immune Horse Serum of Spirochæta icterohæmorrhagiæ.

Animal No.	Case No.	Intraperitoneal injections into guinea pigs. Spirochetes. Generation.	No.	Amount injected.	Serum. Type.	Amount injected.	No. of spirochetes in the peritoneal fluid. After 30 min.	After 2 hrs.	Course of experimental animal.	Autopsy.	Spirochetes in liver.	Results.
				cc.		*cc.*						
1	219	R 16/1 III Rat spirochetes.	10 in one field.	1	Horse serum.	1	2 in one preparation.	0 in one preparation.	Well for 1 mo.			+
2	220	" " "	10 " "	1	" "	1	0 " " "	" " " "	" " 1 "			+
					Control experiments.							
3	217	R 16/1 III Rat spirochetes.	10 in one field.	1	Sodium chloride.	1	3–6 in one field.	1–2 in field, lively.	Died 5th day, icterus and hemorrhage.	+	Numerous.	
4	218	Pure culture.	10 " "	1	" "	1	3–6 " " "	1–2 " "	" 5th " " " "	+	"	
5	221	" "	10 " "	1	Horse serum.	1	1 in preparation, lively.	None in preparation.	Well for over 1 mo.			+

and in an experiment made with *Spirochæta icterohæmorrhagiæ* and immune horse serum, while numerous spirochetes were found in the control experiments conducted with isotonic salt solution. These tests show that the serum of horses immunized with *Spirochæta icterohæmorrhagiæ* has a spirocheticidal and spirochetolytic action upon the spirochetes of the rat.

Experiments to Determine Whether Goat Serum Obtained by Immunizing with Rat Spirochetes Will Destroy Spirochæta icterohæmorrhagiæ.

We immunized a number of goats with a pure culture of the rat spirochete or with liver emulsion containing the organism, and were able to obtain an effective serum. We then made Pfeiffer's tests with the goat serum and *Spirochæta icterohæmorrhagiæ* and the rat spirochete in the same order as the previous experiments. Table II shows the results.

The result of these experiments shows that the goat serum obtained by immunizing with rat spirochetes is capable of destroying *Spirochæta icterohæmorrhagiæ.*

On the basis of these experiments, we may conclude that the spirochetes found in the kidneys of house and wild rats are identical with Inada and Ido's *Spirochæta icterohæmorrhagiæ.*

We examined the kidneys of 6 field mice and injected their kidney emulsion into the peritoneal cavity of guinea pigs. One of the animals died on the 25th day of icterus and hemorrhages, but here the period of incubation was too long, and the hemorrhages were insignificant. As up to the present we have not observed stall infection among the guinea pigs, we must conclude that though the field mouse harbors our spirochetes in the kidneys, they are not of great virulence in that animal. Miyajima's findings also are in accord with our observations, for he observed in the kidneys of field mice spirochetes which appeared to be far less virulent than those observed by us in the kidneys of house and wild rats.

TABLE II.

Pfeiffer's Tests Made with Spirochæta icterohæmorrhagiæ and Immune Goat Serum from Rat Spirochetes.

Animal No.	Case No.	Intraperitoneal injections into guinea pigs. Spirochetes. Generation.	No.	Amount injected.	Serum. Type.	Amount injected.	No. of spirochetes in the peritoneal fluid. After 30 min.	After 2 hrs.	Course of experimental animal.	Autopsy.	Spirochetes in liver	Results.
				cc.		*cc.*						
1	223	R 30/1 IX Rat spirochetes.	10 in one field.	1	Goat.	1	0 in two preparations.	0 in two preparations.	Well for over 1 mo.			+
2	224	B.-C. IX 682 *S. icterohæmorrhagiæ.*	10 " " "	1	"	1	0 " " "	0 " " "	" " " 1 "			+
3	225	" " "	10 " " "	1	"	1	1–2 in specimen,* moving slowly.	0 " " "	" " " 1 "			+
		Control experiments.										
4	226	B.-C. IX 682 *S. icterohæmorrhagiæ.*	10 in one field.	1	Sodium chloride.	1	2–3 in one field, lively.	1 in one field, lively.	Died 5th day, icterus and hemorrhages.	+	Numerous.	
5	229	R 30/1 IX Rat spirochetes.	10 " " "	1	" "	1	2–3 " " "	1 " " " "	" 5th " " " "	+	"	

* Specimen covered about 70 optical fields, Leitz oc. 3, $\frac{1}{12}$ oil immersion.

DISCUSSION.

1. In 34 out of 92 cases, or 37 per cent, spirochetes were present in the kidneys or in the urine, as demonstrated directly by dark-field illumination and indirectly by inoculation.

2. The form, movement, virulence, and immune serum prove that our spirochete is identical with *Spirochæta icterohæmorrhagiæ*. The organisms cannot be demonstrated in the blood and the liver, but in the urine of rats harboring *Spirochæta icterohæmorrhagiæ* in the kidneys, they are present without exception.

3. Urine containing spirochetes, even in small amounts (0.1 to 0.2 cc.) infects guinea pigs when injected intraperitoneally.

4. *Spirochæta icterohæmorrhagiæ* are rarely conveyed directly to the guinea pig by the bite of the rat.

5. In Japan, the rat is undoubtedly a carrier of the causative agent of spirochætosis icterohæmorrhagica. *Mus decumanus* was found to be a carrier in 40.2 per cent of 149 cases, *Mus alexandrinus* in 0.8 per cent of 24 cases.

Whether in addition to *Epymis norvegicus* and *alexandrinus* and *Microtus montebelloi* there are still other carriers of Weil's disease is not known.

The Transmission of Spirochæta icterohæmorrhagiæ Infection by the Rat and the Soil.

It is noteworthy that our spirochete appeared only in the kidneys of rats—not in their blood and liver. This peculiarity of distribution is observed also in man during the convalescent stage of Weil's disease and in the guinea pigs treated with immune serum. As already stated, when the antibodies have been fully developed, the spirochete remains only in the kidneys. As, however, the rats examined by us were apparently in a good state of health, notwithstanding the numerous spirochetes found in their kidneys, we may assume that the organisms do not cause any, or only a slight degree of illness. The probability is that, entering the animal by the mouth or skin, after a time they find their way into the kidneys.

We conducted a number of experiments on mice and white rats, injecting 14 mice with 0.5 cc. of blood or liver emulsion from an

infected guinea pig. Four of the animals died with icterus, the others remaining well. Dr. Kaneko found spirochetes only in the kidneys. Of four white rats, one died of icterus; here also spirochetes were found only in the kidneys. It is possible that ordinary rats possess a greater degree of resistance to the infection than mice and white rats.

The behavior of the spirochete within the rat is open for further study, but we know that the rats harboring spirochetes always excrete them in the urine. The organisms thus find their way to the ground, where they may infect other rats as opportunity offers. In all probability they are disseminated by means of rats, the soil and the animals forming a circle of habitation for the spirochetes. It happens rarely that human beings are infected directly through the bite of rats, the infection being usually transmitted from the soil, where evidently the excreted spirochetes lodge and thrive. On these grounds we can explain the epidemics of spirochætosis icterohæmorrhagica which occur in coal mines and among the farmers in the vicinity. Rats are constant tenants of the mines, and it is known that the miners go barefoot. A similar statement may be made concerning the transmission of Weil's disease on the battle-fields of Europe. There the rats living in the trenches infect the soldiers.

It has been stated that the spirochetes of the field mice are less virulent than those of house and wild rats. This fact does not seem to harmonize with the infection of farmers in the fields; but on the other hand, it must be mentioned that wild rats are also found in the fields to some extent. It was certainly very striking that cooks and maids working in kitchens showed so high a percentage of spirochætosis icterohæmorrhagica lesions. Of 84 patients admitted to our clinic, 23 were in occupations which subjected them to contact with rats; *i.e.*, 8 were cooks, 6 maids, 3 pastry cooks, 3 "kamaboko" (bone meal) manufacturers, 2 vegetable dealers, and 1 fish dealer. The clinical observations alone made it probable that there was some connection between the rat and spirochætosis icterohæmorrhagica, though cases in which the infection was traced directly to the bite of a rat are rare. Brief protocols of the histories of two such patients are given below.

Case 1.—Male, age 26; riksha man. Admitted Nov. 24, 1915. On Nov. 11 was bitten by a rat in the little toe of the left foot. The wound bled and was painful, but improved. After a few days, the patient was quite well. On Nov. 17 (7 days after the rat bite) he became ill, with marked general weakness, chills, headache, and pain in thighs. Typical spirochætosis icterohæmorrhagica developed. The incubation period was 6 days.

Case 2.—Male, age 25; waiter in a restaurant. Admitted July 9, 1915. On June 24 had been bitten by a rat in the left first and middle fingers, which bled profusely. On July 2, 1 p.m., patient suddenly had chills, followed by high fever, marked headache, pain in thighs, and general weakness. Typical spirochætosis icterohæmorrhagica. The incubation period was 8 days.

On classifying the rats in respect to their spirochetal content, and the territory from which they came, we find the results shown in Table III.

TABLE III.

Geographical Distribution of Rats Harboring Spirochetes.

Region where rats were caught.	No.	Cases in which spirochetes were found.	Per cent of positive cases.
Fukuoka and vicinity	52	22	42.3
Coal mines in Province of Fukuoka. Tagawa	9	5	55.5*
Coal mines in Province of Fukuoka. Hondo	8	2	25.0
Coal mines in Province of Fukuoka. Akaike	5	4	80.0
Naokata, Province of Fukuoka	3	0	0
Otsu, Province of Shiga	6	0	0
Tsuchiura, Province of Ibaraki	1	1	100.0
Mimasaku, Province of Okayama	6	0	0
Tottori, Province of Tottori	1	0	0
Totals	91	34	37.4

* Of the twenty-two cases in this group eleven were positive (50 per cent).

It may be mentioned that Dr. Saito, of the First Medical Clinic, traced *Spirochæta icterohæmorrhagiæ* in the kidneys or urine of 50 per cent of the rats in Kyoto.

As shown in the table, the percentage of rats harboring spirochetes is high in those regions where Weil's disease is prevalent, while it is low in regions free from that disease. But it must be remembered that the number of rats examined from the latter regions was small, and that the difference may be attributable to this cause. Miyajima

reported that he found *Spirochæta icterohæmorrhagiæ* in the field mice of a region free from Weil's disease, and it is possible that the rats of a healthy region may also harbor the spirochetes in their kidneys and excrete them with the urine.

The life conditions of the spirochetes seem to be manifold. It was brought out by epidemiological studies that a certain degree of moisture in the soil and a certain temperature are necessary for proliferation. Damp coal mines particularly are favorable places of infection, while the disease is relatively rare in coal mines that are dry. As far as temperature is concerned the optimum in the cultivation of the spirochetes is 22–25°C. Weil's disease in Japan occurs but rarely in the height of summer and the coldest part of winter, but mostly at the end of spring, in early summer, and particularly in the autumn. In coal mines, which have an even temperature, the disease is equally prevalent at all seasons of the year.

That the soil plays an important part in the life of cholera bacilli was emphasized by Emmerlich; and it is known that not only cholera bacilli, but other bacteria and protozoa as well fail to thrive in an acid soil. Our spirochetes also die in a weakly acid medium. This fact led us to trace a connection between the endemic appearance of spirochætosis icterohæmorrhagica and the composition of the soil. Through the kindness of Dr. Takaishi, a member of the Agricultural Institute, we were able to obtain information concerning the distribution of acid, alkali, and neutral soils in the Province of Fukuoka. Comparing the spread of spirochætosis icterohæmorrhagica with the chemical condition of the soil, we discovered the interesting fact that on the whole, the disease occurs rarely in regions having an acid soil, while it is endemic in alkali and neutral soils. The composition of the water and soil of coal mines was also found to vary, an alkali reaction being obtained from the soil of the Ita, Hondo, and Nishizinmachi coal mines, while the earth of the Miike, Yamano, and Akaike mines showed an acid reaction. In Miike no cases of spirochætosis icterohæmorrhagica are known to occur, while in the course of 18 months over 300 cases occurred in Ita, and in Hondo annually from 80 to 100 are recorded. Although there was a high percentage of rats harboring spirochetes in Akaike (80 per cent), only 7 or 8 cases of the disease occur there per year. In the Yamano mine the yearly number is from 5 to 20 cases.

CONCLUSIONS.

1. On the basis of these findings, we conclude that the extermination of rats and field mice is a highly important prophylactic measure against Weil's disease.

2. The chemical composition of soil and water plays an important part in the development of *Spirochæta icterohæmorrhagiæ*, and consequently in the spread of the disease of which it is the causative agent.

We desire to express to Professor Ryokichi Inada our appreciation of the guidance which he has given to our work.