

SPIROCHÆTA HEBDOMADIS, THE CAUSATIVE AGENT OF  
SEVEN DAY FEVER (NANUKAYAMI).

SECOND PAPER.

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INTRODUCTION.

Etiological studies on *nanukayami*, or seven day fever, have been conducted by us in the Prefecture of Fukuoka since the year 1915. In November, 1916, we observed a certain spirochete in a guinea pig which had received an inoculation of blood from a patient having this disease. The spirochete resembled *Spirochæta icterohæmorrhagica* in form and motion, but the results of animal experiments and immunological studies undertaken at that time showed it to be a new species and the causative agent of seven day fever. Hence we named it *Spirochæta hebdomadis*.

We have come to know since that this disease occurs not only in the Prefecture of Fukuoka, but also in those of Shizuoka, Okayama, Shiga, Nara, Hyogo, Hiroshima, Kochi, in Kyoto, and elsewhere. These facts were reported in April, 1917, at the meetings of the Nippon Medical Society, the Nippon Pathological Society, and the Nippon Hygienic Society.<sup>1</sup> But the cases of seven day fever which had come under our observation up to that time were few in number, and we were obliged to wait until the next epidemic season of the disease, which was the autumn of that year, in order to continue our investigation.

On September 7, 1917, we went to Sawara County in the Prefecture of Fukuoka. Here by animal experiments made in a number of cases

<sup>1</sup> *Nippon Naika Gakkai Zasshi*, 1917, v, No. 5.

of seven day fever we succeeded in establishing the presence of *Spirochæta hebdomadis* in the blood and in making the discoveries here reported.

A summary of the report made in April, 1917, regarding the causative agent of seven day fever follows:

1. Since 1915, animal experiments had been carried on with six cases of seven day fever. In two cases we used patients' blood taken on the 3rd and 4th days of the disease, in three cases blood from the 6th day, and in one case from the 8th day. The animals employed were rabbits, guinea pigs, white rats, and mice. Three guinea pigs, which had received injections of blood from a patient on the 6th day of the disease, developed fever and died. Spirochetes were found in their livers.
2. The spirochete found resembled *Spirochæta icterohæmorrhagiæ* in form and motion, but the results of animal experiments and immunological studies proved it to be a new species.
3. When a pure culture of this spirochete was inoculated into guinea pigs, they showed symptoms resembling those of seven day fever.
4. In the serum of a patient recovering from seven day fever immune bodies were found which killed and dissolved the spirochete.
5. We proceeded to search for the animal host harboring the organism and discovered the spirochete in the kidneys of wild mice (*Microtus montebelli*). In other words, it was found that wild mice are the carriers of the spirochete, and that they are constantly disseminating it through their urine.
6. There is a close relation between the districts infected with seven day fever and the regions infested with wild mice.

Of all these points, we place the greatest importance upon the immunological observations. The spirochete under discussion, as has been stated, closely resembles *Spirochæta icterohæmorrhagiæ* in form and motility. By means of Pfeiffer's test, however, we found that it was not affected by horse serum immunized with *Spirochæta icterohæmorrhagiæ* or by the serum of a patient convalescing from spirochætosis icterohæmorrhagica. On the other hand, the serum of goats immunized with this spirochete or the serum of a patient recovering from seven day fever failed to act upon *Spirochæta icterohæmorrhagiæ*.

We injected the two immunized sera into guinea pigs infected with either seven day fever or spirochætosis icterohæmorrhagica. The animals inoculated with the homologous immune serum recovered and no spirochetes could be found in their blood, while those

injected with the heterologous immune serum met with the characteristic death of the disease. The spirochetes in the blood were in no way affected by the serum. We inoculated the corresponding spirochetes into guinea pigs which had recovered from seven day fever or spirochætosis icterohæmorrhagica and found that they were not affected, while the animals inoculated with heterologous spirochetes contracted the corresponding disease.

From these observations and on the basis of the modern immunological point of view, we concluded that the spirochete under discussion represents a species different from *Spirochæta icterohæmorrhagiæ*.

We succeeded, therefore, in confirming the causative agent of seven day fever. But in the six cases of the disease which we observed, we were able to confirm the presence of the spirochete in only one. This was probably attributable to the fact that *Spirochæta hebdomadis*, in contrast to *Spirochæta icterohæmorrhagiæ*, does not affect all guinea pigs to the same degree. In heavy animals we found that the inoculation of liver emulsion or pure culture containing even a large number of the spirochetes would not uniformly lead to infection. Only in light weight guinea pigs were we able to produce experimental seven day fever by inoculation. The conclusion was based on the results of the animal experiments referred to, but to test its validity it was necessary that it should be supported by further experiments, with guinea pigs of light weight. As the disease occurs only in the autumn, we had no opportunity to conduct experiments until our visit to Sawara County in 1917.

#### EXPERIMENTAL.

The present researches in Sawara County covered the villages of Hara, Kanetake, Takuma, and Iki.<sup>2</sup> The cases of seven day fever which came under our observation from September 7, 1917, to the beginning of November, 1917, numbered twenty-six in all. We proceeded to the infected districts every other day and often made more than one animal experiment on the same patient. Serum was collected when necessary. Of the twenty-six patients under obser-

<sup>2</sup> The patients were studied through the kindness of Dr. Matsuguchi, Dr. Morita, and Dr. Tani of the district.

TABLE I.  
Guinea Pigs Inoculated with the Blood of Seven Day Fever Patients.

Case No.	Age.	Sex.	Day of first examination.	Length of time after onset.	No. of guinea pigs used.	Weight of animals.	Results.
	<i>yrs.</i>		<i>1917</i>	<i>days</i>		<i>gm.</i>	
1	26	Male.	Sept. 28	1	2	95	+
						65	+
2	32	"	Oct. 7	2	2	90	+
						130	+
3	25	"	Sept. 7	3	2	115	+
						135	+
4	19	"	" 13	3	2	116	+
						118	+
5	34	"	" 20	3	2	107	+
						110	+
6	17	"	" 26	3	3	100	+
						105	+
						105	+
7	20	"	Oct. 5	3	2	95	+
						110	+
8	31	"	" 5	3	1	120	+
9	26	"	" 5	3	2	100	+
						110	+
10	18	"	" 7	3	2	95	+
						110	+
11	10	"	Sept. 22	4	1	105	+
12	31	"	" 26	4	2	100	+
						110	+
13	42	"	" 28	4	2	85	+
						100	+
14	23	"	Oct. 5	4	1	100	+
15	18	Female.	" 9	4	1	140	-
16	16	Male.	Nov. 5	4	3	90	+
						115	+
						155	+
17	16	"	Sept. 7	5	2	98	+
						109	+
18	43	"	" 24	5	1	110	+
19	31	"	Oct. 5	5	2	95	+
						150	+
20	23	"	Sept. 11	6	4	85	+
						110	+
						115	+
						118	-
21	17	"	" 16	7	4	133	-
						142	-
						149	-
						114	-
22	16	"	Oct. 5	7	2	97	+
						105	-
23	20	"	" 7	7	2	110	-
						150	-

vation, we collected blood from twenty-three. The blood was injected into the peritoneal cavity of guinea pigs ranging in weight from 65 to 150 gm.; most of the animals averaged 100 gm. At least two animals were used for each test, for being small and light they frequently died in the course of the experiment. The quantity of blood injected was about 1 cc. for every 100 gm. of the animal's weight. Table I shows the results obtained from the twenty-three cases.

The twenty-three cases include one of blood taken on the 1st day of the disease, one on the 2nd day, eight on the 3rd day, six on the 4th day, three on the 5th day, one on the 6th day, and three on the 7th day. The guinea pigs inoculated with blood from Case 15, taken on the 4th day, and from Cases 21 and 23 taken on the 7th day, failed to develop fever and the experiments were negative. In the other twenty cases, however, the inoculated guinea pigs all developed fever in 5 to 7 days, and spirochetes were found in the blood. They were isolated in every case, almost in pure culture.

Daily records were kept of the temperatures of the guinea pigs. We also examined by dark-field illumination spirochetes in blood drawn from the ears, to ascertain whether the guinea pigs were affected. The animals were observed closely for symptoms. Only a few of the guinea pigs had icterus, and when it did occur, it was slight, far less severe than in spirochaetosis icterohæmorrhagica. The animals were much less apt to bleed. In some of the guinea pigs the spirochetes disappeared from the blood in the course of time; the congestion also disappeared from the orbital conjunctivæ, the temperature fell, and the animals were restored to perfect health. Results such as these have not been observed in animal experiments with *Spirochæta icterohæmorrhagiæ*.

It has been stated already that heavy guinea pigs, those weighing more than 200 gm., are rarely infected with the spirochetes of seven day fever, and even when infected, they seldom die of the disease. This was the finding when one animal was inoculated from another. It remained to be proved, however, whether it was also true of guinea pigs inoculated directly with the blood of patients. We determined to test this point, using guinea pigs of different weights, as shown in Table II, and injecting them with quantities of blood in accordance

with the weight of the animal. The results show that with direct inoculation also the smaller and lighter animals were more easily affected than the heavier ones. Even heavy animals were occasionally affected by the disease, but the percentage was far smaller than with lighter ones. This finding proves that our hypothesis as to the influ-

TABLE II.  
*Guinea Pigs of Different Weights Inoculated with the Blood of Seven Day Fever Patients.*

Experiment No.	Guinea pig No.	Weight.		Amount of blood injected intraperitoneally.	Spirochetes in blood.	Results.			
		gm.	cc.			Course.	Autopsy.	Spirochetes in liver.	Spirochetes in kidney.
1	1	115	2.0	+	+	Died on 10th day.	+	5-6 in field.	
2	2	118	2.0	-	-	Normal.			
3	3	110	2.0	-	-	"			
4	4	85	2.0	+	+	Died on 10th day.	+	10 in field.	
5	5	90	1.0	+	+	Died on 6th day.	+	10 in field.	
6	6	95	1.0	-	-	" " 2nd "	-	-	
7	7	115	1.2	+	+	" " 7th "	+	3-4 in field.	
8	8	155	1.5	+	+	" " 8th "	+	1-2 " "	
9	9	210	2.0	-	-	" " 16th "	-	-	
10	10	290	3.0	-	-	" " 15th "	+	-	1 in 1 specimen.
11	11	305	3.0	-	-	" " 4th "	-	-	
12	12	135	1.0	-	-	Died on 9th day.	+	1 in 20-25 fields.	
13	13	150	1.5	-	-	" " 9th "	±	-	
14	14	185	2.0	-	-	" " 6th "	-	-	
15	15	475	5.0	-	-	" " 10th "	+	1 in 20-25 fields.	
16	16	625	6.0	+	+	" " 29th "	+	-	1 in 1 specimen.

ence of weight was in the main correct. When inoculation is carried on in a series from animal to animal, the smaller animals are preferable.

The animal experiments demonstrate that the spirochete under discussion is not the same as *Spirochæta icterohæmorrhagiæ*, but that it agrees in every respect with *Spirochæta hebdomadis*, the discovery of

which we have reported.<sup>3</sup> There is no room left for doubt that the organism obtained from these twenty cases was *Spirochæta hebdomadis*, but for further proof immunological experiments were undertaken. Serum was collected from the twenty patients in the stage of convalescence. This serum, together with the spirochete obtained in 1916 from a patient having seven day fever, and also *Spirochæta icterohæmorrhagiæ*, was subjected to Pfeiffer's test.

Pfeiffer's tests were made in each of the twenty cases. As the results were identical in all, we have reported only five (Tables III and IV). The tests show that the serum of these patients had no effect on *Spirochæta icterohæmorrhagiæ*, while it killed and dissolved *Spirochæta hebdomadis*. It is evident, therefore, that the serum contained immune bodies.

We carried out Pfeiffer's test with the serum from convalescent patients having seven day fever, which contained strong immune bodies both against the spirochetes obtained in 1917 and those of the year previous, and immune serum of *Spirochæta icterohæmorrhagiæ*. As this experiment requires a comparatively large number of guinea pigs, it was confined to ten cases selected from the total number of twenty. We found that the spirochetes of the ten cases were not affected by the immune serum of *Spirochæta icterohæmorrhagiæ*, while the organisms were killed and dissolved by serum obtained from convalescent cases of seven day fever. This serum had already been shown to contain strong immune bodies against the spirochete of 1916.

Pfeiffer's tests were made in ten cases. The results being identical in all, we shall give the records for two only (Tables V and VI).

We believe that the animal experiments and the immunological studies made have proved conclusively that the organisms found in the twenty cases observed in 1917 differ from *Spirochæta icterohæmorrhagiæ*, and that they are of precisely the same species as *Spirochæta hebdomadis* discovered by us the previous year. Hence we are justified in concluding that the causative agent of seven day fever is undoubtedly *Spirochæta hebdomadis* and that the presence of this organism can always be proved by inoculating blood taken

<sup>3</sup> Ido, Y., Ito, H., and Wani, H., *J. Exp. Med.*, 1918, xxviii, 435.

TABLE III.  
*Pfeiffer's Tests. Spirocheta hebdomadis* Obtained in 1916 and Convalescent Serum from Seven Day Fever.

Guinea Pig No.	Weight. gms.	Intraperitoneal injection.		Spirochetes in peritoneal fluid.		Course.	Results.
		Serum used.	Spirochetes.	After 30 min.	After 2 hrs.		
Control.							
"	85	Isotonic salt solution, 1 cc.	<i>S. hebdomadis</i> , 1 cc.	1-2 in field.	1 in field.	No protection.	-
"	87	Immune horse, icterohemorrhagic, 1 cc.	" " 1 "	1-2 " "	1-2 " "	" "	-
"	143	Immune, patient's, icterohemorrhagic, 1 cc.	" " 1 "	1 " "	1 " "	" "	-
"	104	Immune, seven day fever (Case 24), 1 cc.	" " 1 "	0 " specimen.	0 " specimen.	Protection.	+
17	103	Immune, seven day fever (Case 17), 1 cc.	" " 1 "	0 " "	0 " "	" "	+
18	115	Immune, seven day fever (Case 3), 1 cc.	" " 1 "	0 " "	0 " "	" "	+
19	117	Immune, seven day fever (Case 20), 1 cc.	" " 1 "	0 " "	0 " "	" "	+
20	107	Immune, seven day fever (Case 12), 1 cc.	" " 1 "	0 " "	0 " "	" "	+
21	102	Immune, seven day fever (Case 4), 1 cc.	" " 1 "	0 " "	0 " "	" "	+



TABLE IV.  
Pfeiffer's Tests. *Spirocheta icterohemorrhagiae* and Convalescent Serum from Seven Day Fever.

Guinea pig No.	Weight. gm.	Intraperitoneal injection.		Spirochetes in peritoneal fluid.		Course.	Results.
		Serum used.	Spirochetes.	After 30 min.	After 2 hrs.		
Control.	150	Isotonic salt solution, 1 cc.	<i>S. icterohemorrhagiae</i> , 10 in field, 1 cc.	4-5 in field.	4-5 in field.	No protection.	-
"	135	Immune, patient's, icterohemorrhagic, 1 cc.	"	0 " specimen.	0 " specimen.	Protection.	+
22	120	Immune, seven day fever (Case 17), 1 cc.	"	4-5 " field.	2-3 " field.	No protection.	-
23	160	Immune, seven day fever (Case 3), 1 cc.	"	3-4 " "	2-3 " "	"	-
24	170	Immune, seven day fever (Case 20), 1 cc.	"	3-4 " "	1-2 " "	"	-
25	180	Immune, seven day fever (Case 12), 1 cc.	"	1-2 " "	2-3 " "	"	-
26	155	Immune, seven day fever (Case 4), 1 cc.	"	1-2 " "	1-2 " "	"	-

TABLE V.  
*Pfeiffer's Tests. Spirochetes from Case 4, and Immune Serum of Spirochæta hebdomadis and Spirochæta icterohæmorrhagica.*

Guinea pig No.	Weight. gm.	Intraperitoneal injection.		Spirochetes in peritoneal fluid.		Course.	Results.
		Serum used.	Spirochetes.	After 30 min.	After 2 hrs.		
Control.	95	Isotonic salt solution, 1 cc.	Spirochetes (Case 4), 10 in field, 1 cc.	1-2 in field.	1 in 1-2 fields.	No protection.	-
"	107	Case 4, 1 cc.	" "	0 in specimen.	0 " specimen.	Protection.	+
27	110	Immune, seven day fever (Case 25), 1 cc.	" "	0 " "	0 " "	"	+
28	110	Immune, seven day fever (Case 26), 1 cc.	" "	0 " "	0 " "	"	+
29	88	Immune, horse, icterohæmorrhagic, 1 cc.	" "	1 " 1-2 fields.	1 " 1-2 fields.	No protection.	-
30	100	Immune, patient's, icterohæmorrhagic, 1 cc.	" "	1 " 1-2 "	1 " 1-2 "	"	-

TABLE VI.  
*Pfeiffer's Tests. Spirochetes from Case 17, and Immune Serum of Spirocheta hebdomadis and Spirocheta icterohaemorrhagiae.*

Guinea pig No.	Weight. gm.	Intrapertitoneal injection.		Spirochetes in peritoneal fluid.		Course.	Results.
		Serum used.	Spirochetes.	After 30 min.	After 2 hrs.		
Control.	94	Isotonic salt solution, 1 cc.	Spirochetes (Case 17), 10 in field, 1 cc.	3-4 in 1 line.*	1 in a few fields.	No protection.	-
"	105	Case 17, 1 cc.	"	0 " specimen.	0 " specimen.	Protection.	+
31	103	Immune, seven day fever (Case 25), 1 cc.	"	0 "	0 "	"	+
32	120	Immune, horse, ictero- hemorrhagic, 1 cc.	"	1 " 7-8 fields.	2-3 " 1 line.	No protection.	-

\* Line means from one side to the other of the cover-glass (65 to 70 fields).

from a patient at a proper stage of the disease into guinea pigs of light weight.

In cooperation with Doctors Okuda and Kishimoto we conducted experiments to ascertain the particular day after the onset of the disease when the blood of patients is infective for guinea pigs. Blood was taken from all the patients, usually every other day, for a period of 10 days from the onset. The weight of the animals used for experiment and the method of inoculation were the same as already described. The results are given in Table VII.

TABLE VII.  
*Guinea Pigs Inoculated with Blood from 23 Cases of Seven Day Fever.*

Length of time after onset.	No. of guinea pigs.	Positive.	Negative.	Doubtful.	Per cent positive.	Guinea pigs developing spirochaetosis icterohaemorrhagica.	
						No. of guinea pigs.	Per cent positive.
<i>days</i>							
1	1	1	0	0	100		
2	1	1	0	0	100	40	100
3	8	8	0	0	100		
4	6	5	1	0	83.3		
5	4	4	0	0	100	7	88.9
6	7	2	4	1	33.3	19	73.7
7	12	1	8	3	11.1	15	46.7
8	5	0	4	1	0	7	13.3
9	1	0	1	0	0	6	16.7
10	1	0	1	0	0	1	0
Totals ...	46					95	

Up to the 3rd day from the onset of the disease ten cases were positive; *i.e.*, 100 per cent of the animals inoculated contracted the disease. On the 4th day some negative results appeared. On the 5th day all four cases were positive; the inoculation experiment showed 100 per cent positive. On the 6th day the percentage of guinea pigs infected with the disease fell considerably, being only 33.3. On the 7th day only 11.1 per cent were positive. On and after the 8th day all were negative, as may be seen in Table VII. The fact that after the 4th day of the disease the experiments on blood inoculation showed negative results may be explained as being attrib-

utable to the appearance in the blood of immune bodies against *Spirochæta hebdomadis*.

An experiment was made to ascertain the time of appearance in the blood of patients of immune bodies which kill *Spirochæta hebdomadis*. Table VIII shows the results.

It will be seen from the table that up to the 5th day Pfeiffer's test failed to prove the appearance of immune bodies. On the 6th and 7th days, however, the test showed the appearance of a large quantity of immune bodies, and on and after the 8th day immunity was complete. Pfeiffer's tests were made, as with *Spirochæta ictero-hæmorrhagiæ*, with serum obtained from patients and *Spirochæta hebdomadis*. The imperfect immunity referred to in Table VIII

TABLE VIII.  
*Time of Appearance of Immune Bodies in the Blood of Seven Day Fever Patients.*

Length of time after onset.	Positive reactions.	Incomplete reactions.	Negative reactions.	Totals.
<i>days</i>				
3			1	1
4			2	2
5			2	2
6	1	4	1	6
7	7	1	2	10
8	9			9
9	5			5
10 and over.	2			2

concerns two cases of seven day fever. For Pfeiffer's test in these cases the intraperitoneal fluid collected after 30 minutes and 2 hours respectively was examined by dark-field illumination. In one it was found to differ wholly from control experiments, for after 2 hours no spirochetes could be seen, although the guinea pig was later experimentally infected with the disease. In the other the intraperitoneal fluid also differed from the control. Spirochetes were not completely dissolved and even after 2 hours some of the organisms were still moving slightly.

It is evident that the blood in these cases had begun to lose its infecting power on the 6th day from the onset of the disease. Hence it was to be expected that on and after the 8th day it had become completely negative.

*Spirochæta hebdomadis*, like *Spirochæta icterohæmorrhagiæ*, is found occasionally, although in small numbers only, in film preparations of the blood of patients. We have observed it in four preparations stained by Giemsa's method, one of which was prepared on the 2nd day, two on the 3rd, and one on the 4th day of the disease.

With the cooperation of Doctors Okuda and Kishimoto we ascertained further that the spirochetes of seven day fever are excreted by patients in the urine. A quantity of 40 cc. of urine, taken aseptically from a patient, was centrifugalized and the deposit examined by dark-field illumination. When no spirochetes were observed the deposit was injected into the peritoneal cavity of guinea pigs. Usually after the 8th day of the disease microscopic examination showed the presence of spirochetes in the urine of patients having seven day fever. The only exception was a case where they were found on the 4th day. In the other twenty-one cases examined they were not observed until the 8th day. After the 9th day they were found in comparatively large numbers, and from about the 18th or 19th until the 25th day we found them uniformly. After that day the percentage decreased gradually, but as late as the 39th day we observed active spirochetes. We found a similar condition in spirochætosis icterohæmorrhagica, although in seven day fever the period in which the spirochetes are discharged is somewhat longer. In 40 cases microscopic examination failed to disclose spirochetes. In all these an injection of the deposit was made into the peritoneal cavity, but only two guinea pigs became infected.

Seven day fever is a disease found not only in the Prefecture of Fukuoka, but in other districts of Japan as well. We have established the fact that the "autumnal epidemic" in Shizuoka Prefecture, "*sakushu* fever" in Okayama Prefecture, "autumnal fever" in Kochi Prefecture, a fever found in Kyoto and in the Prefectures of Shiga, Hyogo, Nara, Hiroshima, Miyazaki, Nagasaki, and Formosa are in reality the same disease; *i.e.*, seven day fever. This statement is based on immunological investigations of the serum of patients.<sup>4</sup>

<sup>4</sup> Reported in April, 1917, at the meeting of the Nippon Naika Gakkai.

## CONCLUSIONS.

1. *Spirochæta hebdomadis* is always present in seven day fever and can be confirmed by animal experiments with guinea pigs of light weight.

2. The causative agent of this disease can also be found in film preparations of the blood of patients, though it is not present in large numbers.

3. *Spirochæta hebdomadis* is discharged in the urine of patients having seven day fever. The number of spirochetes in the urine is great during convalescence.

4. Seven day fever is a disease found not only in the Prefecture of Fukuoka, but in many other districts of Japan as well.

We take pleasure in expressing our indebtedness to Professor Inada, under whose direction the studies were undertaken. We also desire to thank our colleagues, Doctors Okuda and Kishimoto, for their assistance.

The studies here reported were carried on in the Laboratory for the Serum Therapy of Spirochætosis Icterohæmorrhagica in connection with Dr. Inada's clinic, established by the Association Commemorating the Tercentenary Anniversary of Toshogu. The experimental animals used were supplied by the Association. We extend our thanks to Prince Iesato Tokugawa, the President of the Association.