

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

FIRST PAPER.

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PLATE 46.

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INTRODUCTION.

There prevails in the province of Fukuoka a disease known as *nanukayami*, or seven day fever, the symptoms of which are like those of atypical Weil's disease. The latter does not usually show any icterus. Seven day fever has a sudden onset with fever, languor, congestion of the conjunctivæ, muscle pain, disorders of digestion, and swelling of the lymphatic glands. Moreover, albuminuria and leukocytosis appear in the earlier stages. Subsequently clouding of the vitreous humor may arise. The disease does not prevail in the cities, e.g. Fukuoka, but is restricted to the country. It runs a short course and apparently causes no fatalities. It has been disputed for a long time whether seven day fever and Weil's disease are identical or distinct entities.

Inada examined many cases of seven day fever in 1909 and 1910, and in the latter year he published his views to the effect that the two diseases were independent. As at that time the causative agent of Weil's disease, *Spirochæta icterohæmorrhagiæ*, had not yet been discovered, a final decision was not possible. Ido and Wani observed many cases of seven day fever in the autumn of 1916, and determined through epidemiological and immunological studies that this affection was distinct from Weil's disease.

We have been engaged since 1916 in the study of the etiology of seven day fever, and in October of that year ascertained that the causative agent of seven day fever is also a spirochete which resembles

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Spirochæta icterohæmorrhagiæ, but is separable from it. Further study showed that the field mouse (*Microtus montebelli*) is a carrier of this spirochete.

The first of our studies were carried out, as stated, in 1915, and were confined to six human cases of seven day fever. In the autumn of 1917, we were enabled to study twenty-five more cases, and material from twenty-three of the cases was used to inoculate guinea pigs. In twenty instances the results were found to be positive either by detection of the spirochetes or determination of the occurrence of immune reactions. The twenty successful cases had been examined within the first 5 days of the appearance of symptoms of the disease. Moreover, we discovered that the spirochetes in the blood of the patients (Figs. 1 to 3) and also in the urine of a convalescent patient stained by Giemsa's solution. More complete data concerning these cases will be published later.

EXPERIMENTAL.

Isolation and Identification of the Spirochete.

The method of investigation followed was similar to that employed by Inada and Ido in the study of Weil's disease. Six cases have been studied since 1915. The blood and urine of patients were injected intraperitoneally into various animals—guinea pigs, rabbits, mice, and rats.

A guinea pig, inoculated October 1, 1916, with the blood of Patient 1, died on October 27 without having shown conspicuous symptoms. A few spirochetes were found in the kidneys, stained according to Levaditi's method.

In the meantime we observed still another patient (Case 2). The blood from Patient 2 was injected intraperitoneally into three guinea pigs, two rabbits, and three mice. Of these, the three guinea pigs showed fever on the 5th to the 6th day and died on the 7th to 9th day. One of the guinea pigs exhibited a slight icterus and all showed hemorrhage in the lungs. The liver showed many spirochetes on dark-field illumination.

As the form and movement of the spirochetes were similar to those of *Spirochæta icterohæmorrhagiæ*, we at first mistook them for that

organism, and accordingly regarded the condition as Weil's disease. But a more exact observation of the postmortem examinations of the guinea pigs brought out a striking difference in comparison with those of the guinea pig which had died of infection with known *Spirochæta icterohæmorrhagiæ*. Thus, although one of the guinea pigs manifested icterus, it was of very slight degree; and the hemorrhages were much less than in experimental spirochætosus icterohæmorrhagica. Moreover, the lymphatic gland swelling was very prominent. On microscopic examination the lymphatic enlargement was traceable to hyperplasia, while in experimental Weil's disease the swelling is due to hyperemia and hemorrhage. Hence it may be said that the post-mortem appearances are more suggestive of seven day fever than of Weil's disease.

We next undertook to determine whether the spirochetes present in the liver of the experimentally infected guinea pig (Fig. 4) were actually *Spirochæta icterohæmorrhagiæ* or another variety. For this purpose we employed convalescent serum of cases of Weil's disease and immune serum of the horse inoculated with *Spirochæta icterohæmorrhagiæ*. These sera contain spirochetolytic and spirocheticidal substances active against this spirochete. A goat was immunized with the new spirochete and its serum tested against *Spirochæta icterohæmorrhagiæ*. We therefore carried out Pfeiffer's tests in two ways. The results in both instances were negative: anti-*icterohæmorrhagiæ* serum was without effect on the new spirochete, and the antiserum from the goat was equally ineffective upon *Spirochæta icterohæmorrhagiæ*.

The next step was to determine whether cross-immunity existed *in vivo*. Guinea pigs which have recovered from experimental spirochete infection are refractory to reinoculation. Hence we injected intraperitoneally the spirochetes of seven day fever into three healthy guinea pigs and let them recover from the infection induced. After the disappearance of the spirochetes from the blood, they were injected with liver emulsion or pure cultures containing many *Spirochæta icterohæmorrhagiæ*; they developed typical experimental Weil's disease. Again we induced in four guinea pigs experimental Weil's disease, and treated them with the specific antiserum. The animals recovered, after which they were injected intraperitoneally with liver

emulsion which contained many of the new spirochetes. All the guinea pigs developed typical experimental disease corresponding to seven day fever.

These experiments indicate that cross-immunity in the guinea pig to the two spirochetes is absent. Hence it would follow that *Spirochæta icterohæmorrhagiæ* and the spirochete obtained from patients with seven day fever are independent organisms, although in both their form and movements they are strikingly alike.

The next point was to establish the fact that the spirochetes present in the guinea pigs actually came from the patients. In this connection we had already learned that this species of spirochete is never present in healthy guinea pigs, while it is not possible to produce the effects described in guinea pigs by the injection of the blood of healthy persons or of patients suffering from other diseases. Hence we conclude that the spirochetes actually existed in the blood of the seven day fever patients and were transferred by injection to the guinea pigs in which the experimental form of the disease was induced.

We carried the proof still further. We studied the effect of serum of convalescents from seven day fever upon this spirochete, for we assumed that it contained a specific immune body. Should this be the case, then the simultaneous injection of spirochetes and the convalescent serum must fail to induce infection in the guinea pig. This proved to be true, as the guinea pigs so treated remained well; while other guinea pigs inoculated with a mixture of the same spirochetes and the *icterohæmorrhagiæ* serum developed the typical disease and died. From these facts we believe that the spirochetes derived from patients with seven day fever are the causative agent of that disease.

But if this spirochete is the causative agent of seven day fever as it occurs in Fukuoka, it must be present in all cases. We tested this point by injecting the blood of patients into guinea pigs; in two cases on the 3rd and 4th days, in three cases on the 6th, and in one case on the 8th day of the disease. We obtained a positive result only in the case tested on the 6th day. At first this was an inexplicable result but it was cleared up later when we came to study the susceptibility of the guinea pig to this spirochete. The young is much more sensitive than the old guinea pig. The latter does not respond either to inoculation

with the hepatic emulsion or with pure culture containing abundant spirochetes; while, on the contrary, young animals easily react typically. Only older animals were used in the first experiments, hence the failures. Unfortunately this point cannot be completely investigated until next autumn as seven day fever prevails only at that time of the year. In the meantime we arrived at the conclusion regarding the spirochete as the cause of seven day fever by indirection by studying the spirochetolytic and spirocheticidal action of the blood serum of the eighteen persons who had once suffered from seven day fever. We made the Pfeiffer test with the serum with our spirochete and for control with *Spirochæta icterohæmorrhagiæ*. Of the eighteen cases, fourteen yielded an immune body which acted upon these spirochetes, while in no instance was a specific immune antibody for *Spirochæta icterohæmorrhagiæ* found. Hence we conclude that the spirochete is the causative agent of seven day fever.

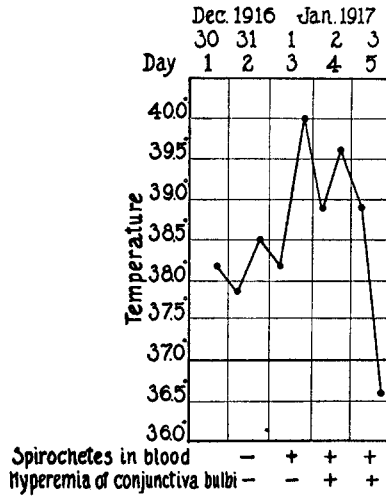
We could now carry our experiments a step further to the detection of the natural carrier of the organism, which proved to be the field mouse (*Microtus montebelli*), just as the rat is the carrier of *Spirochæta icterohæmorrhagiæ*.

According to the tests performed up to the present, 3.3 per cent of the field mice examined show the spirochete in the kidneys (Fig. 5) and in the infected animals they are present in the urine. In movement they resemble *Spirochæta icterohæmorrhagiæ*. Finally, it may be stated that regions in which field mice abound are the regions in which seven day fever occurs. We have called the causative organism *Spirochæta hebdomadis*.¹

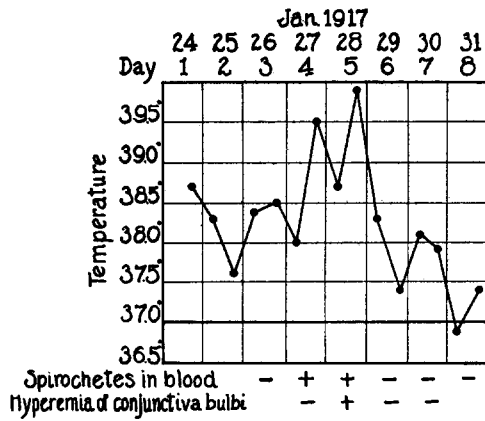
As has been stated, three guinea pigs which developed symptoms following blood inoculation showed spirochetes in the liver by dark-field illumination. The inoculation of urine into guinea pigs was without effect. The results in guinea pigs both with blood and urine were different from those following inoculation from cases of Weil's disease, as fewer infections were obtained with the blood of cases of seven day fever, probably because of the age and size of the guinea pigs employed, and no infections were obtained from the urine.

Transfer from an infected guinea pig to a healthy one sometimes succeeds with the spirochete of seven day fever, but not always.

¹ The author has adopted the corresponding designation of *Spirochæta nanukayami* in the Japanese form of his paper.

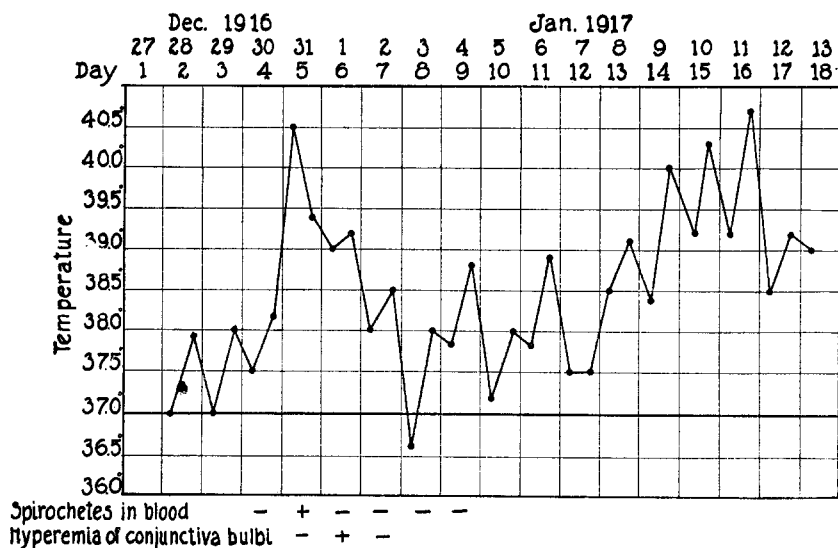


TEXT-FIG. 1. Temperature curve of a guinea pig experimentally infected with seven day fever. Died on the 5th day. No icterus. Postmortem examination positive. Spirochetes abundant in the liver.



TEXT-FIG. 2. Temperature curve of a guinea pig experimentally infected with seven day fever. Died of icterus on the 8th day. Postmortem examination positive. No spirochetes in the liver.

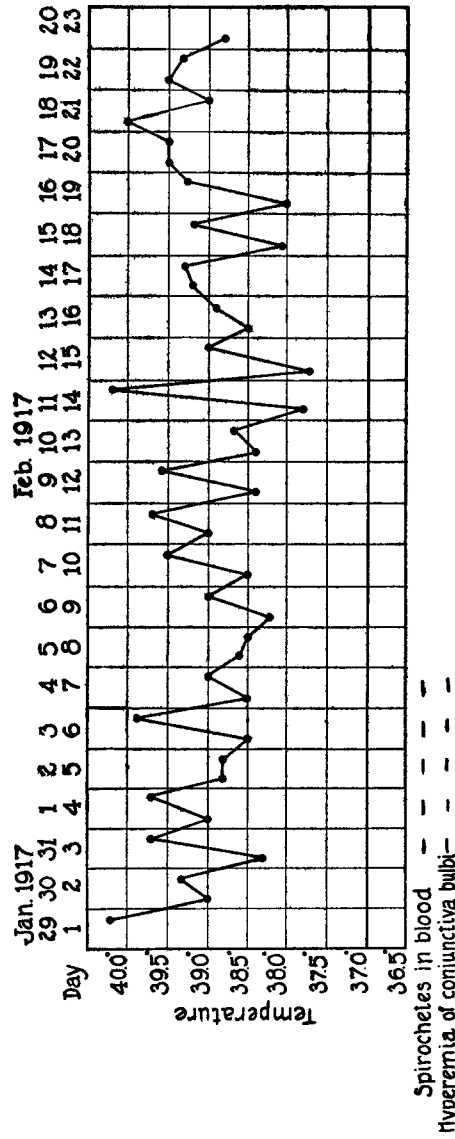
This is another point of difference from *Spirocheta icterohæmorrhagica*. Moreover, in certain guinea pigs in which the spirochete of seven day fever appeared in the blood and fever was present, the spirochete afterwards disappeared spontaneously. These animals either recovered entirely or died subsequently although no spirochetes were present (Text-figs. 1 to 4). As has been stated, we came to the conclusion that the larger guinea pigs were insusceptible and hence the failure to infect them. We arranged a set of experiments so as to



TEXT-FIG. 3. Temperature curve of a guinea pig experimentally infected with seven day fever. Died on the 18th day. No icterus. Postmortem examination positive. Spirochetes found in the kidney but not in the liver.

inject a quantity of hepatic emulsion, 1 cc. for every 100 gm. of animal weight. Most of the guinea pigs weighing more than 200 gm. either did not become sick or did not die, while guinea pigs of about 150 gm. weight became infected and died (Table I).

Because of the inconstancy of the infection in guinea pigs, it is difficult or impossible to keep a strain of the spirochete alive by successive transfer to these animals. But this deficiency can be overcome by artificial culture. In order to accomplish transfer from guinea pig to guinea pig the surest way is to inject into the peritoneal cavity



TEXT-FIG. 4. Temperature curve of a guinea pig experimentally infected with seven day fever. Killed on the 23rd day. Postmortem examination: Hemorrhage in the lungs. Spirochetes found in the kidney and urine and immune body in the blood serum.

$\frac{1}{2}$ cc. of blood containing the spirochete taken by cardiac puncture. Successful infections can also be accomplished in some instances by skin inoculation with and without previous abrasion, or even *per os*.

The incubation period in guinea pigs fluctuates between 2 and 8 days, being shortest after intraperitoneal injection of cardiac blood and longest after dermal inoculation.

The symptoms in the guinea pig recall those of seven day fever in man. There are anorexia, fever, congestion of the conjunctivæ, emaciation, anemia, hemorrhages, and leukocytosis. But all the

TABLE I.

Intraperitoneal Injection of Spirochetes of Seven Day Fever into Guinea Pigs.

Experiment No.	Body weight.	Amount of hepatic emulsion injected into the peritoneal cavity.	Spirochetes in the blood.	Icterus.	Course.	Autopsy.	Spirochetes in the liver.
	<i>gm.</i>	<i>cc.</i>					
1	490	4.9	—	—	Normal.		
2	470	4.7	—	—	Died on 9th day.	+	—
3	440	4.4	—	—	Normal.		
4	259	2.6	+	—	"		
5	250	2.5	+	—	Died on 7th day.	+	—
6	232	2.3	+	+	" " 7th "	+	—
7	167	1.6	+	—	" " 6th "	+	+
8	144	1.4	+	—	" " 4th "	+	+

symptoms are slighter than those that appear after inoculation of *Spirochæta icterohæmorrhagiæ*. The temperature is 38–40°C., and continues from 1 to 3 days, when it falls by crisis. The jaundice, when it appears, comes on with the fall in fever. Hemorrhages are slight, as a rule, except in the lungs. Besides being present in the lungs the hemorrhages appear in the abdominal walls, retroperitoneal connective tissues, and the serous membranes; but they are relatively small and few. The external hemorrhages are inconspicuous (Table II).

When spontaneous recovery ensues, the spirochetes first disappear from the blood, then the congestion of the conjunctivæ abates, and

lastly the fever abates. Immune bodies appear in the blood in about 1 week and about that time the spirochetes may be found in the urine.

Besides guinea pigs we inoculated rabbits, mice, and rats with the blood of patients with seven day fever (Table III). With the exception of the rabbits, none of the latter animals showed any symptoms.

Rabbits.—Sixteen rabbits were injected intraperitoneally with 1 cc. of hepatic emulsion per 100 gm. body weight. On the 2nd to the 6th day spirochetes were present in the blood, fever appeared on the 2nd or 3rd day, rarely on the 4th day, and then fell. Young animals of 200 to 500 gm. in weight were more responsive than old animals, and they sometimes succumbed on the 4th or 5th day when

TABLE II.

Percentage of the Mortality, Icterus, and Hemorrhage of Guinea Pigs Experimentally Infected with Seven Day Fever and Spirochætosis Ictero-hæmorrhagica.

	Spirochætosis ictero-hæmorrhagica.	Seven day fever.
	<i>per cent</i>	<i>per cent</i>
Mortality.....	100.0	60.8
Icterus.....	99.0	17.4
Nosebleed.....	78.0	4.4

spirochetes can be found in the liver. From the 6th day no more could be detected. In older animals the spirochetes have entirely disappeared from the blood on the 4th or 5th day. Young rabbits may show icterus but usually no hemorrhages; old rabbits develop no typical symptoms.

Mice.—Three mice were given $\frac{1}{2}$ cc. of blood or hepatic emulsion intraperitoneally. On the 4th day spirochetes were detected in the blood, but they soon disappeared; no symptoms developed.

Field Mice.—Ten field mice were injected intraperitoneally with $\frac{1}{2}$ to 1 cc. of blood or hepatic emulsion. None showed symptoms, but five showed spirochetes in the urine.

House Rats.—Two house rats only were studied but no results were obtained.

Dermal Infection.—Thirteen guinea pigs were employed for this purpose. The animals are fixed so that they cannot lick off the inoculated material. The hair is clipped from the abdomen and the skin moistened with sterile water. In six animals the skin was scarified so that the abrasions barely reached the corium. In seven guinea

TABLE III.

Animal Experiments with the Blood and Urine of Patients with Seven Day Fever.

Case No.	Age.	Date.	Day of dis- ease.	Experimental animal.	Material for inoculation.	Dose.	Result.	Icterus.	Hemorrhage.	Spirochetes in the liver.	Spirochetes in the kidney.
3	14	1915 Aug. 22	6	Guinea pig (2).	Blood.	2.0	—	—	—	—	—
4	41	Sept. 11	3	Guinea pig (2).	Blood.	2.0	—	—	—	—	—
				Rabbit (1).	"	5.0	—	—	—	—	—
				White rat (1).	"	1.0	—	—	—	—	—
				Mouse (2).	"	0.5	—	—	—	—	—
5	34	1916 Sept. 11	6	Guinea pig (2).	Blood.	2.0	—	—	—	—	+ (1)
				" "	Urine.	3.0	—	—	—	—	— (1)
				Mouse (2).	Blood.	0.5	—	—	—	—	—
				"	Urine.	0.5	—	—	—	—	—
1	20	Sept. 11	4	Guinea pig (3).	Blood.	2.0	—	—	—	—	
				Mouse (2).	"	0.2	—	—	—	—	—
2	53	Oct. 16	6	Guinea pig (3).	Blood.	2.0	+	+ (1) — (2)	+	+	+
				Rabbit (2).	"	5.0	—	—	—	—	—
				Mouse (3).	"	0.5	—	—	—	—	—
6	19	Nov. 10	8	Guinea pig (3).	Blood.	2.0	—	—	—	—	

pigs no scarification was made. On each was dropped $\frac{1}{2}$ cc. of hepatic emulsion containing about 10 spirochetes per microscopic field. It was allowed to dry for about half an hour, when the animals were released and returned to their cages. Among the six scarified animals four became infected; among the seven not scarified five developed symptoms. Of the four former three died on the 5th to the 6th day,

and of the five latter all died on the 8th to the 9th day. Pure cultures have also been used for dermal infection.

Oral Infection.—Six guinea pigs were each given by mouth 2 cc. of an hepatic emulsion similar to the one used for dermal inoculation. Of these, five developed symptoms and died on the 6th to the 13th day of the typical disease. We conclude, therefore, that the spirochete can penetrate the intact gastrointestinal mucosa.

Portal of Entry.—The portal of entry of the spirochete into the human body has not yet been minutely studied. The literature of the disease gives certain suggestions; namely, entrance by way of the skin surfaces. In such instances swelling of the regional lymphatic glands was present, and the patients had been workers in the fields or forests, and often suffered skin abrasions. In conformity with this is the observed fact of a case of laboratory infection from the pricking of the finger with the needle of a syringe contaminated with the blood of an infected guinea pig. The attack was typical of seven day fever. We made certain studies on the incubation period of the disease arising in man through dermal infection and concluded that it was about 6 days.

Channels of Excretion.—We also undertook to study whether the spirochetes leave the human body, and if so how. Urine, bile, and feces from inoculated animals were studied by dark-field illumination. In nine out of thirteen guinea pigs the spirochete was detected in the urine: thus in three of six animals dying on the 6th, in two of three killed on the 16th, and in one each of animals killed on the 11th, 18th, 19th, and 23rd day. The urine of nine infected guinea pigs was injected intraperitoneally into nine other guinea pigs of which five acquired the typical disease. Bile, feces, and intestinal contents never conveyed the infection. The conclusion reached is that as with *Spirochata icterohæmorrhagiæ*, the chief excretory path is by way of the urine. If the spirochetes are present in the bile, feces, or intestinal contents, they are too few to cause infection on inoculation.

CONCLUSIONS.

A new species of spirochete which we have called *Spirochata hebdomadis* has been described as the specific etiological agent of

seven day fever, a disease prevailing in the autumn in Fukuoka and other parts of Japan.

This spirochete is distinguishable from *Spirochæta icterohæmorrhagiæ* to which it presents certain similarities.

Young guinea pigs are susceptible to inoculation with the blood of patients and to pure cultures of the spirochete, and those developing infection exhibit definite symptoms suggestive of those of seven day fever in man.

The blood serum of convalescents from seven day fever contains specific immune bodies acting spirochetolytically and spirochetidally against the specific spirochetes, but not against *Spirochæta icterohæmorrhagiæ*.

The field mouse (*Microtus montebelli*) is the normal host of the spirochetes, which have been detected in the kidneys and urine of 3.3 per cent of the animals examined.

The endemic area of prevalence of seven day fever corresponds with the region in which field mice abound.

We wish to express our appreciation to Professor R. Inada for his assistance in this work.

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EXPLANATION OF PLATE 46.

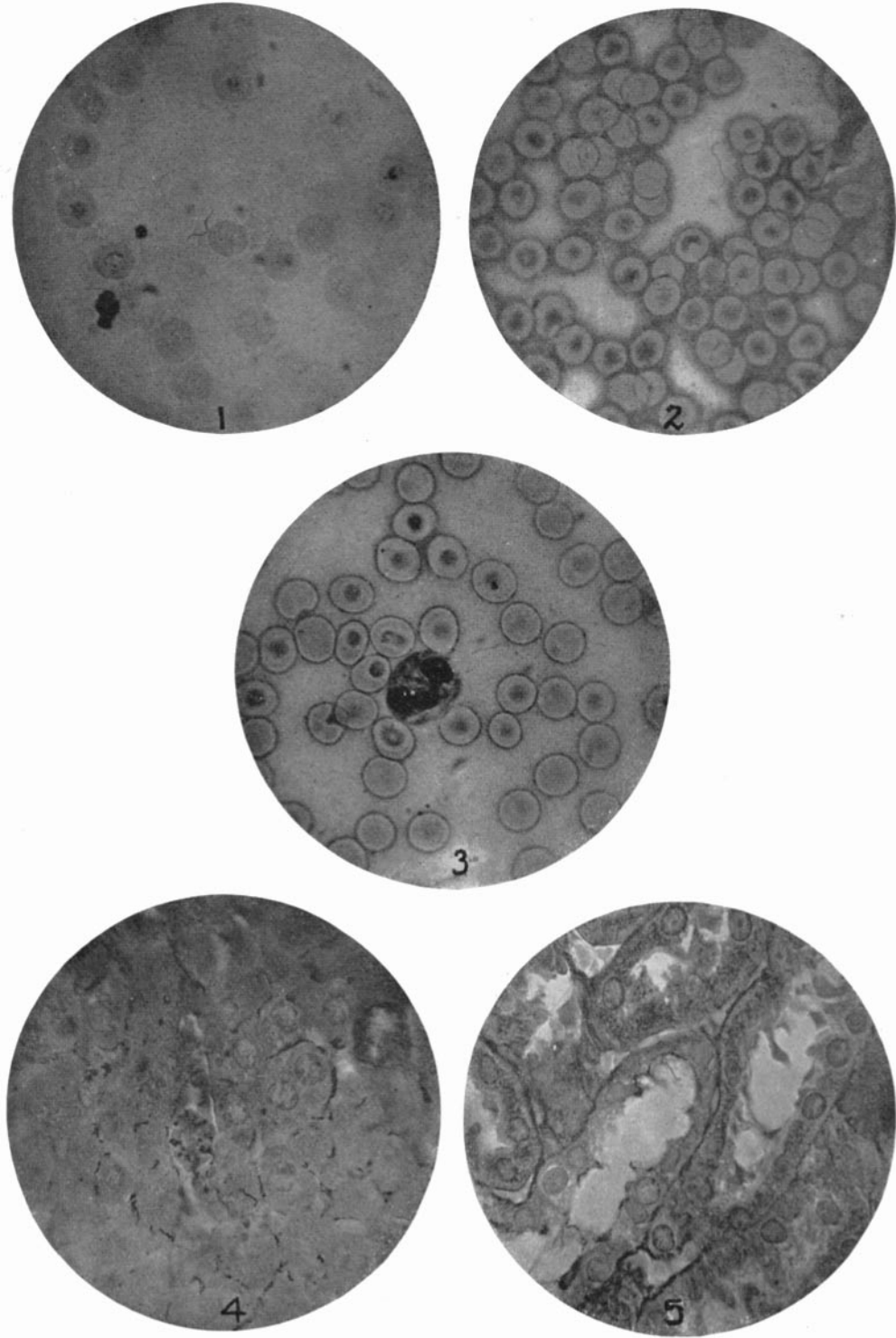
FIG. 1. Microphotograph of *Spirochæta hebdomadis* in human blood. Giemsa stain. 4th day of illness. $\times 700$.

FIG. 2. Microphotograph of *Spirochæta hebdomadis* in human blood. Giemsa stain. 4th day of illness. $\times 700$.

FIG. 3. Microphotograph of *Spirochæta hebdomadis* in human blood. Giemsa stain. 3rd day of illness. $\times 700$.

FIG. 4. Spirochetes in the liver of a guinea pig. Silver impregnation.

FIG. 5. Spirochetes in the kidney of a field mouse. Silver impregnation.



(Ido, Ito, and Wani: Seven day fever.)