EXPERIMENTAL RAT-BITE FEVER.

First Report.*

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Plate 11.

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INTRODUCTION.

At least eighty-one cases of human rat-bite fever have, according to Blake,[1] been reported by European, British, American, and Japanese investigators. Only two of the reports, however, those of Miura and Toriyama[2] and of Blake, include the pathological anatomy of the disease. Ogata[3] attributes rat-bite fever to an aspergillus, Shikami[4] attributes it to a telosporidia, Middleton[5] to a diplococcus, Proescher[6] to a bacillus, and Schottmüller[7] and Blake to a streptothrix.

Ogata inoculated two guinea pigs with a freshly excised piece of a swollen lymph gland taken from a patient with rat-bite fever; both animals died about 3 weeks later. The swollen glands of the animals were inoculated into other guinea pigs which also died about 3 weeks after the inoculation. In 1909 Ogata caused a *Mus decumanus* to bite two guinea pigs in the leg; the guinea pigs developed fever and swelling and congestion of the bitten parts and finally succumbed. Autopsy of all these animals showed swelling and congestion of the lymph glands and swelling of the kidneys to be the chief lesions, although pneumonia was present in some instances. From these experiments Ogata concluded that rat-bite fever could be produced experimentally in animals by the bite of rats.

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* This paper was presented at the meeting of the Japanese Hygienic and Bacteriological Society on March 3, 1916.

[1] Blake, F. G., *J. Exp. Med.*, 1916, xxiii, 39.

[2] Miura, M., and Toriyama, N., *Z. med. Ges. Tokyo*, 1897, xi, 1059.

[3] Ogata, *Mitt. med. Fakult. Univ. Tokyo*, 1911, ix, 1913; 1913–14, xi, 179.

[4] Shikami, *Z. med. Ges. Tokyo*, 1909.

[5] Middleton, G. S., *Lancet*, 1910, i, 1618.

[6] Proescher, F., *Berl. klin. Woch.*, 1912, xlix, 841.

[7] Schottmüller, H., *Derm. Woch.*, 1914, lviii, Suppl. 77.

One of us had the opportunity of assisting at these experiments, and noted pathological changes in the adrenals of the animals, but Ogata made no mention of them in his report.

In March, 1915, one of us undertook the serological study of guinea pigs bitten by rats. In November of the same year Futaki and his collaborators examined under dark-field illumination and by Burri's method the tissue fluid of excised skin and that of punctured lymph glands of two patients suffering from rat-bite fever. They also examined the excised lymph gland of one of the patients, stained by Levaditi's method, and found that it contained a number of spirochetes. About the same time we examined the excised viscera of guinea pigs which we had kept for study from the spring of that year and found a spirochete in sections of the adrenals of several animals, stained according to Levaditi's method. Futaki[8] and his coworkers announced the results of their investigation at a meeting of the Tokyo Medical Society on November 20, 1915, and we reported our findings at the same meeting. We promptly found the same spirochetes in living animals which were under observation and described them in a second report made before the Tokyo Medical Society on February 5, 1916.

Ogata was the first to transmit rat-bite fever to guinea pigs by causing rats to bite them, and we have confirmed his experiments.

EXPERIMENTAL.

We caught many rats by means of wire nets and kept them under observation in the laboratory. The rats were divisible into two classes, those that would and those that would not bite when irritated from time to time by means of a small metal rod. Rats which did not bite in one test would, as a rule, not bite in another. Moreover, only a part of the rats were capable of conferring the rat-bite infection, because a rat which did not confer the disease to one animal by biting did not confer it to another; conversely a rat which conveyed the disease by biting one animal would also convey it by biting another. This led us to conclude that only certain rats tend to bite and of those only a part convey the infection of rat-bite fever.

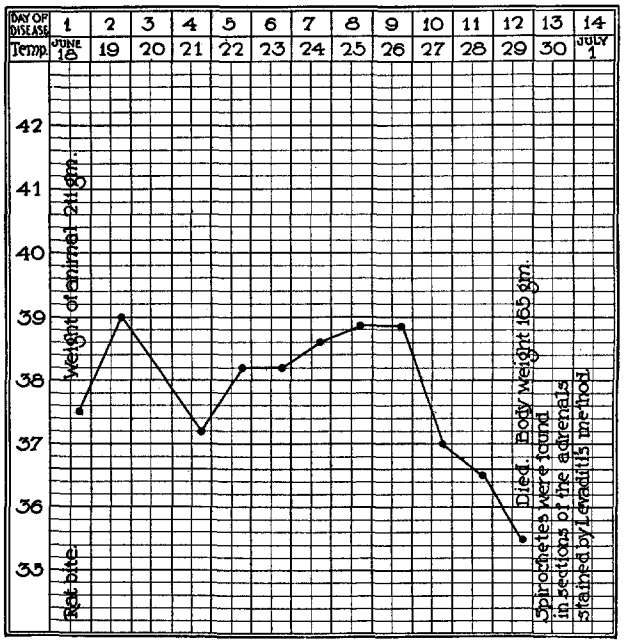
The test animals were guinea pigs the legs of which were exposed. About 80 rats were employed, one-half of which were made to bite, and of this number only about ten individuals, all *Mus decumanus*, caused experimental rat-bite fever. Thus far we have not discovered the exact conditions under which the disease is transmitted, and we have not detected the specific spirochete in the mouths

[8] Futaki, Takaki, Taniguchi, and Osumi, *Z. med. Ges. Tokyo*, 1915.

of rats. In one rat, however, we detected a few spirochetes of the same form in the blood.

Clinical Course.

The symptoms and course of the disease in the guinea pig were as follows: The bitten parts became swollen within 2 or 3 days, and in severe cases the affected legs reached three times the natural size. The bite healed promptly, but the swelling continued, sometimes de-

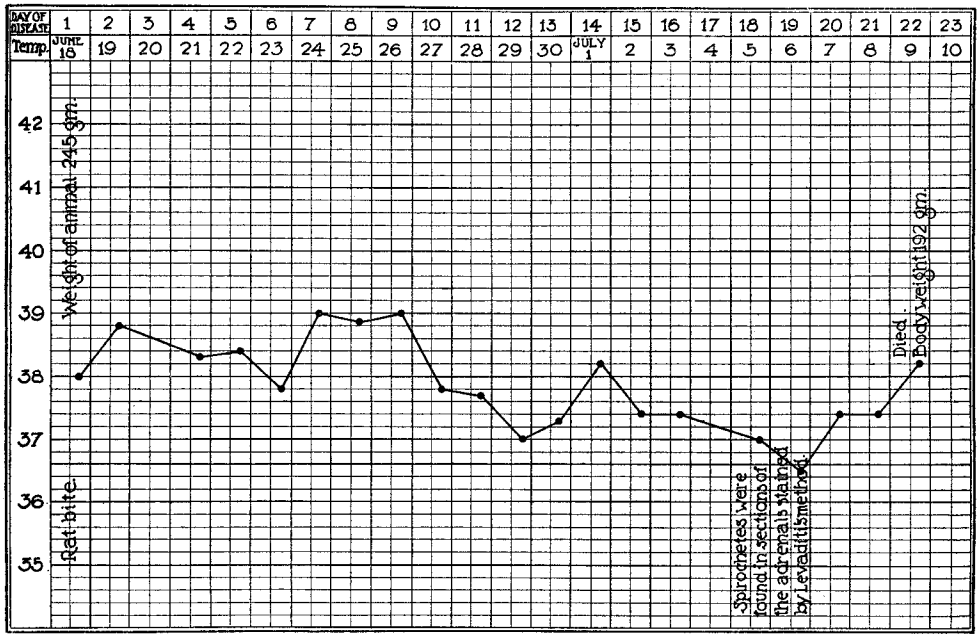


TEXT-FIG. 1. Temperature chart of Guinea Pig 1 (original generation).

creasing, until the death of the animal. The skin of the leg became cyanosed and the subcutaneous lymph glands became palpable. Within 24 hours after the bite a rise in temperature occurred, but this was soon followed by a fall to normal. A second rise of temperature took place 6 to 7 to 10 days later, only to fall again soon. This rise and fall might be repeated, but in this respect the experimental condition does not run so regular a course as human rat-bite fever.

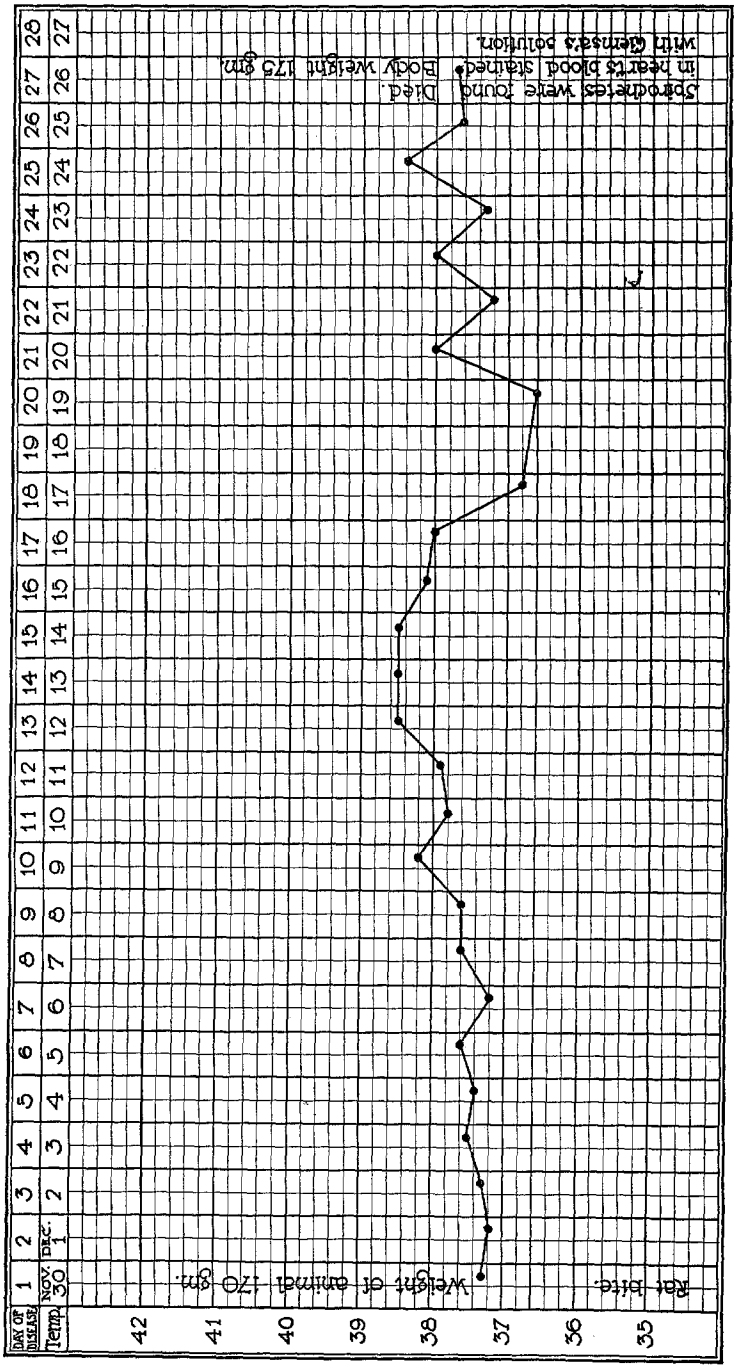
Weight was gradually lost, but an erythema did not appear. The guinea pigs which showed this condition invariably died. The course of the fever was not uniform, but tended to extend over 3 weeks. The longest course was something over 5 weeks, the shortest less than 2 weeks. Text-figs. 1, 2, and 3 show the progress of events in three instances.

Guinea Pig 1 (Text-fig. 1) was bitten by *Mus decumanus* 4 on June 18, 1915. On the 2nd day the bitten parts became swollen and on the 4th day the enlarged inguinal glands on the affected side were palpable.



TEXT-FIG. 2. Temperature chart of Guinea Pig. 2 (original generation).

The anatomical appearances are described below. Two fresh guinea pigs were inoculated with emulsions made from the lymph glands and two with emulsions made from brain tissues of this animal. Spirochetes were found in sections of the adrenals stained according to Levaditi's method. Aerobic cultures made after the animal's death from the intraperitoneal exudate, heart's blood, fluids of brain tissues and excised tissues taken from the section through the bitten parts were negative, as were also the stained cover-glass preparations.



TEXT-FIG. 3. Temperature chart of Guinea Pig 3 (original generation).

Before inoculation we always examine the animals' blood microscopically, after treating it with acetic acid.

Guinea Pig 2 (Text-fig. 2) was bitten by the same rat on the same day as No. 1. On the 2nd day the bitten parts became swollen; the swelling decreased soon after, but it increased again on the 9th day. Enlargement of the inguinal glands could be detected on palpation. On the 14th day the swelling of the bitten parts again decreased. The anatomical appearances were identical to those described below. Aerobic cultures made from the excised tissues of the bitten parts, the intraperitoneal fluid, heart's blood, and brain tissue fluid were negative. Stained cover-glass preparations were also negative. Two fresh guinea pigs were infected with emulsions made from lymph glands and brain tissues. Excised tissues of the adrenals stained according to Levaditi's method were positive for spirochetes.

Guinea Pig 3 (Text-fig. 3) was bitten on November 30, 1915, by another *Mus decumanus*. The next day the leg was swollen to three times the normal size and developed a dark cyanotic color. From the 10th day on the fever progressed as shown in Text-fig. 3. The animal died on the 28th day after being bitten.

Autopsy.—Weight of animal not decreased. The leg remained swollen. Swelling and congestion of the subcutaneous and internal lymph nodes were also present. Both adrenals were markedly swollen and congested. The kidneys of both sides showed congestion of the cortex. Aerobic cultures from the peritoneal fluid and heart's blood were sterile. Further inoculation into guinea pigs with the heart's blood and emulsion of the mesenteric lymph nodes gave positive results. Spirochetes were found in the heart's blood stained by Giemsa's solution.

Anatomical Changes.

The subcutaneous lymph glands and the inguinal and axillary glands were swollen and congested. The periglandular tissues and the mesenteric and retroperitoneal glands were also congested. The liver and lungs were congested, the latter often showing pneumonic foci. The spleen showed no apparent macroscopic change. The kidneys were more or less swollen, hemorrhage being often noted under the capsule. The sectioned surface showed edema, and small hemorrhagic spots were observed in the cortical parts. The adrenals were congested and showed small hemorrhagic spots under the capsules. In the peritoneal cavity a hemorrhagic exudate was sometimes found. The bladder and gall-bladder were usually full. The pia mater was congested. The important points to be noted among the postmortem appearances are: swelling of the bitten parts, swelling and congestion of the lymph gland system, and congestion and hemorrhage of the

adrenals and kidneys. The changes in the adrenals were not remarkable in cases where the infected guinea pigs died after a long illness.

Microscopic sections of the kidneys stained with hematoxylin and eosin showed the capillary vessels, especially those of the cortex, to be enlarged and congested, with occasional hemorrhagic spots. The convoluted uriniferous tubules and glomeruli showed a degree of hyaline degeneration of the cells, as in acute nephritis. In the adrenals the capillary vessels were enlarged and congested, and small hemorrhagic spots were observed. The capillary vessels in the lungs and liver were also distended and congested.

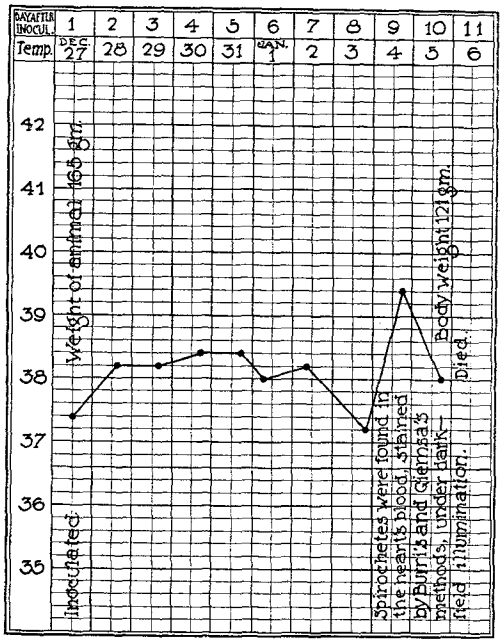
Further Inoculation Experiments.

When a guinea pig died of the fever, emulsions were made from the lymph and adrenal glands, brain substance, heart's blood, etc. The inoculation of these emulsions into the subcutaneous tissues or peritoneal cavities of fresh guinea pigs, always caused the death of the animals after giving rise to such symptoms as high temperature, swelling of the lymph glands, and a decrease in body weight. In these animals, which we called the first generation, the disease ran a shorter course than in the original generation. Some of the experiments are given below.

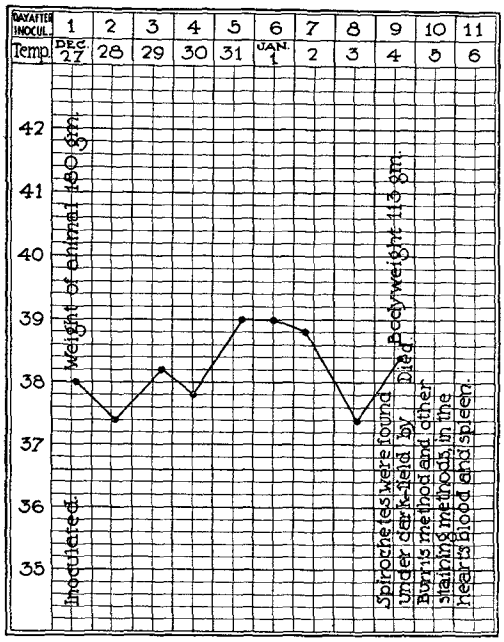
Guinea Pig 4, first generation, (Text-fig. 4) was inoculated on December 27, 1915, by injecting the heart's blood taken from Guinea Pig 3, original generation, intraperitoneally. At autopsy swelling and congestion of the inguinal, axillary, mesenteric, and retroperitoneal glands were found. No changes were observed in the peritoneal cavity where the inoculation had been made, but the changes in the kidneys and adrenals were typical. Spirochetes were found in the preparations made from the heart's blood stained with Burri's and Giemsa's solutions and also examined under dark-field illumination. Four fresh guinea pigs were inoculated with the heart's blood of this animal.

Guinea Pig 5, first generation, (Text-fig. 5) was inoculated subcutaneously with an emulsion made from the mesenteric lymph node of Guinea Pig 3, original generation, at the same time as Guinea Pig 4.

Autopsy.—The inguinal, axillary, and mesenteric lymph nodes were swollen and congested. The pathological changes of the kidneys and adrenals were typical. Spirochetes were found under dark-field illumination, by Burri's method and other staining methods, in the heart's blood and spleen.



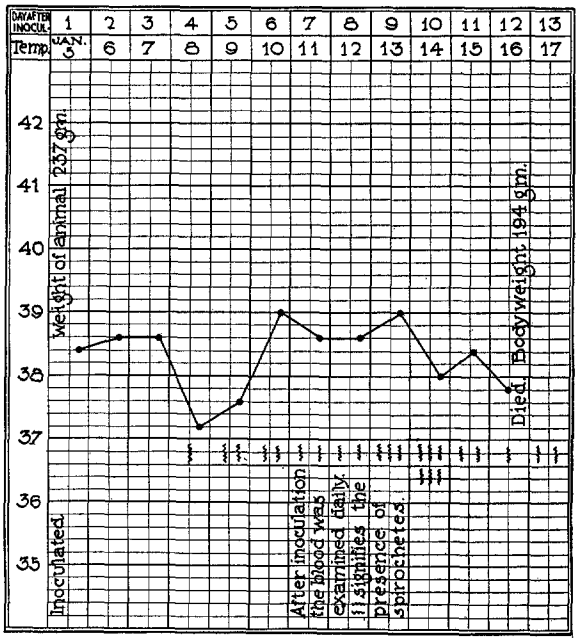
TEXT-FIG. 4. Temperature chart of Guinea Pig 4 (first generation).



TEXT-FIG. 5. Temperature chart of Guinea Pig 5 (first generation).

Further inoculation into mice, white rats, and guinea pigs gave positive results. Spirochetes were found in the heart's blood and films of the spleen under dark-field illumination, with Burri's and Giemsa's stains.

Guinea Pig 6, second generation, (Text-fig. 6) was one of the guinea pigs inoculated into the peritoneal cavity with the heart's blood of Guinea Pig 4 of the first generation. After inoculation we drew blood daily from the ear-lobes, examined it under dark-field illumination, and made stained preparations with Burri's and Giemsa's methods, and other stains. Spirochetes were present in all. The autopsy showed typical pathological changes.



TEXT-FIG. 6. Temperature chart of Guinea Pig 6 (second generation).

At autopsy the guinea pigs of the first generation showed swelling and congestion of the lymph glands and marked changes in the kidneys and the adrenals, as in the original generation.

The inoculation of fresh guinea pigs with the peripheral blood, heart's blood, and emulsion made from lymph glands taken from the guinea pigs of the first generation gave rise to the same symptoms

as in the first generation, and caused the death of the animals. The course of the disease and the result of the postmortem examination were similar to those of the first generation. We obtained the same result from the experiments with material from the animals of the 2nd, 3rd, 4th, and further generations, which indicates that there was no change in the pathogenicity of the spirochetes throughout the generations, which were many in number. It is thus seen that the guinea pig is highly susceptible to the disease.

Experiments on White Rats.

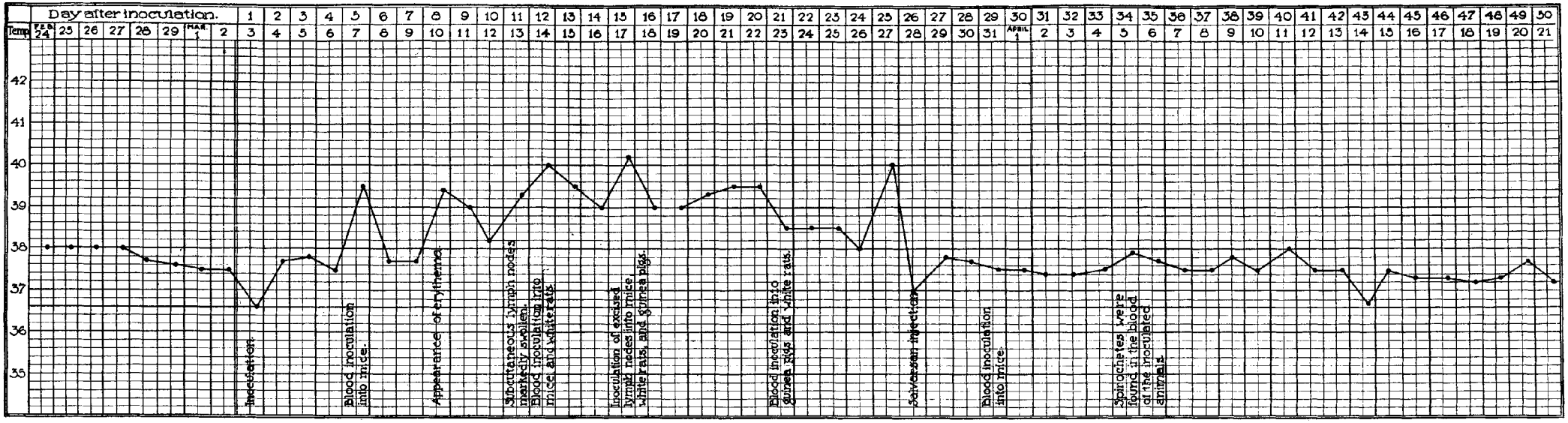
We inoculated a number of white rats subcutaneously or intraperitoneally with blood taken from an infected guinea pig. The inoculated animals showed no symptoms, but the spirochetes were found to multiply in their blood, as described below. The white rats did not die as a result of the inoculation, but spirochetes always developed in the blood of rats of the new generation. The inoculation into fresh guinea pigs of blood taken from the white rats of the new generation always gave rise to the usual symptoms, causing the death of the animals. In every instance spirochetes were found in the blood. Spirochetes were also found in the blood of mice inoculated with blood from the white rats.

We inoculated white rats with blood containing spirochetes from an infected guinea pig. After spirochetes were observed in the blood of the rats, the legs of two fresh guinea pigs were scratched with the rats' teeth until they bled slightly. One of the guinea pigs died several days later, but no spirochetes were found in the blood. The other guinea pig had fever on the 6th, 11th, 20th, and 25th days, and died on the 27th day.

At autopsy the usual findings were present, though in less degree, and many spirochetes were observed in the heart's blood. We therefore concluded that the cause of infection existed in the mouths of experimentally infected white rats.

Experiments on White Mice.

Spirochetes were also found in the blood of white mice inoculated with blood from an infected guinea pig. Only a few of the mice died



TEXT-FIG. 7. Temperature chart of Monkey 2 (*Pithecus rhesus* (Audebert)) inoculated intraperitoneally with blood from a guinea pig with experimental rat-bite fever.

and no noteworthy macroscopic changes were found at autopsy. Guinea pigs inoculated with the blood of an infected mouse developed the usual symptoms and died. Spirochetes were always found in the blood. White rats inoculated with the blood of the mice also became infected. Many of the spirochetes in the blood of the mice had a large number of spirals.

Experiments on Rabbits.

We inoculated two rabbits, and no spirochetes were detected in their blood. The number of animals used, however, was too small to allow us to arrive at a definite conclusion.

Experiments on Monkeys.

We inoculated one *Pithecus irus* (F. Cuvier) and one *Pithecus rhesus* (Audebert) with blood from a guinea pig with experimental rat-bite fever (No. 7). The first monkey developed no symptoms of the disease, but spirochetes were found in the blood of mice and rats inoculated with the monkey's blood. In the *rhesus* monkey intraperitoneal inoculation caused a high temperature on the 5th day (the incubation period), and the lymph glands became swollen and were palpable. The swelling of one or two of the inguinal lymph nodes was especially marked. Erythema was also present. On the 8th day after the inoculation round red patches from 1 to 2 mm. in diameter appeared on the loins and the lower parts of the buttocks. These patches gradually increased in number, 40 to 50 being present on the 12th day. Most of them appeared close together on the skin, and finally coalesced into one another. The surface of the patches was slightly swollen, the red color fading on pressure. There were also some patches on the abdomen. The temperature rose every 2 or 3 days, and recurred six times in 25 days. The fever curve in the *rhesus* resembled that of human rat-bite fever (Text-fig. 7). No spirochetes were found in the films made almost every day from the peripheral blood or in the films from swollen lymph glands and excised lymph node tissue. Spirochetes were present, however, in rats and mice inoculated with the blood and emulsions made from excised glands.

Cultivation Experiments.

When an infected guinea pig of the original generation died, we made ordinary aerobic cultures of tissues taken from the site of the bite, the heart's blood, lymph glands, and fluids from the viscera and peritoneal cavity. In these cultures we sometimes found bacilli resembling *Bacillus coli* in form, movement, manner of staining, and pathogenicity. In our opinion, these contaminating bacilli in the cultures came from the animals. No such growth was noticed in other cases. We have not yet succeeded in making cultures from blood containing spirochetes.

Description of the Organism.

Localization of the Spirochetes.—The first spirochetes that we found were in Guinea Pig 1, bitten by a rat on June 18, 1915. The symptoms, course of the disease, and the postmortem examination indicated that the infection produced in the animal was a typical example of experimental rat-bite fever. Spirochetes were detected in the cortical capillaries and the parenchyma near the capsule in preparations made from excised tissue of the adrenals and stained by Levaditi's method. Spirochetes were observed later in the preserved adrenals of other guinea pigs. In the living animals they were demonstrated in the peripheral blood under dark-field illumination with Burri's and Giemsa's stains and with aniline dyes. At autopsy they were also found in cover-glass preparations of the heart's blood and spleen tissues. The spirochetes were present in all generations, but they were not so numerous as the recurrent spirochetes in the blood of infected mice. The largest number of spirochetes observed in blood films was five or six in one field (Zeiss, oc. 4, oil immersion $\frac{1}{12}$).

Time of the Appearance of the Spirochetes.—The symptoms were irregular in the original generation of guinea pigs, as stated above. Spirochetes were found in the peripheral blood only at the last stage of the disease, and before that none could be observed with any degree of accuracy. The animals of the first and later generations, however, displayed the usual symptoms, and spirochetes were observed in the peripheral blood 4 days, rarely 2 days, after inoculation. They gradually multiplied in the blood of guinea pigs, mice, and

white rats, reaching the highest numbers in 8, 10, or 12 days. In white rats and white mice, the spirochetes could be detected more than 2 months after the date of inoculation. Guinea pigs always died within 2 weeks after inoculation.

Movement.—In preparations made from the peripheral blood of experimental animals and examined under dark-field illumination, the spirochetes were seen to be transparent, and to possess an active progressive movement. As they passed swiftly out of the field, accurate observation of their morphological characteristics was impossible. They became sluggish after some time, and made spiral, rotating, or other movements. At this stage, the spirochete resembled a woodlouse in shape and appeared to consist of several joints of transverse lines. It was almost impossible to recognize the original shape of a spirochete, and its spiral form may be observed only at a much later period. The spirochetes were still moving actively in the blood 24 hours after the death of the animal.

Form.—Preparations made from the heart's blood of guinea pigs and stained according to Giemsa's method showed that the size of the spirochetes varied from 1.6 to 3 μ in length, 2 μ being the average size. The width was approximately from 0.4 to 0.5 μ. They tapered slightly at both ends. There were from two to six spirals or more, the average being two or three. Under dark-field illumination they were found to possess a flagellum at each end, which was filamentary and two or three times as long as the body. Sometimes the flagellum was present at one end only. It was clearly seen in preparations made by Burri's method (Fig. 1), or impregnated with silver according to Levaditi (Fig. 2). The undulating membrane has not yet been observed.

Staining of Spirochetes.—The spirochetes stain uniformly, easily absorbing the color, when dyed according to Giemsa's method (Fig. 3) or with aniline dyes, or with Löffler's methylene blue, aniline gentian violet, or carbol-fuchsin, and they are Gram-negative. When the number of spirochetes contained in the blood is very small, the blood should first be thickly smeared over the cover-glass, then treated with 1 to 2 per cent acetic acid by Koch's method, and finally dyed with gentian violet. In one instance two spirochetes apparently in the process of division seemed to be joined together (Fig. 3). We shall report more fully on the morphology later.

Experiments with Salvarsan.

Fourteen inoculated white rats, in the blood of which spirochetes were observed, were divided into three groups and given subcutaneous injections of salvarsan. The groups received 0.2, 0.1, and 0.06 gm., respectively, per kilo of body weight. Although many spirochetes were present in their blood before inoculation, none were detected on the 1st, 2nd, 3rd, 6th, and 10th days after inoculation. On the 17th day only a small number of spirochetes was found in one of the white rats into which 0.06 gm. per kilo had been injected, while in the control rats they were constantly present. We also obtained the same result by injecting into thirty-six mice a quantity of salvarsan varying from $\frac{1}{400}$ to $\frac{1}{1,000}$ gm. per 20 gm. of body weight. The details of this experiment are shown in Tables I and II.

TABLE I.

Injection of Salvarsan into White Rats.

No. of animal.	Weight.	Dose per kg.	Spirochetes before injection.	Spirochetes after injection. Day.							
				1	2	3	6	10	17	26	33
	gm.	*gm.*									
1	87	0.2	+++	−	−	−	−	−	−	−	−
2	78	0.2	+++	−	−	−	−	−	−	−	−
3	46	0.2	+++	−	−	−	−	−	−	−	−
4	53	0.2	+++	−	−	−	−	−	−	−	−
5	41	0.2	+++	−	−	−	−	−	−	−	−
6*	43	0.2	+++	++	+	+	+	+	+	+	+
7	43	0.2	+++	−	−	−	−	−	−	−	−
8	58	0.2	+++	−	−	−	−	−	−	−	−
9	172	0.1	+++	−	−	−	−	−	−	−	−
10	137	0.1	+++	−	−	−	−	−	−	−	−
11	59	0.1	+++	−	−	−	−	−	−	−	−
12	40	0.1	+++	−	−	−	−	−	−	−	−
13	115	0.06	+++	−	−	−	−	−	−	−	−
14	104	0.06	+++	−	−	−	−	−	+	−	−
15	77	−	+++	+++	+++	+++	+++	++	++	+	+†
16	51	−	+++	+++	+++	+++	+++	++	++	+	+†
17	40	−	+++	+++	+++	+++	+++	++	++	+	+†

* The dose of salvarsan was insufficient, owing to a technical mistake.

† Spontaneous decrease of spirochetes.

TABLE II.

Injection of Salvarsan into Mice.

No. of animal.	Weight.	Dose per 20 gm.	Spirochetes before injection.	Spirochetes after injection. Day. 1	2	3	4	7	10	14	17	19
	gm.	*gm.*										
1	15	1/400	+++	Died.								
2	15	1/400	+++	−	−	−	−	−	−	−	−	+
3	18	1/400	+++	−	−	−	−	−	−	−	−	+
4	15	1/400	+++	−	−	−	−	−	−	−	−	+
5	15	1/400	+++	−	−	−	−	−	−	−	−	+
6	15	1/400	+++	−	−	−	−	−	−	−	−	+
7	15	1/400	+++	−	−	−	−	−	−	+	−	+
8	15	1/400	+++	−	−	−	−	−	−	−	−	+
9	15	1/400	+++	−	−	−	−	Died.				
10	15	1/600	+++	−	−	−	−	−	−	−	−	+
11	15	1/600	+++	−	−	−	−	−	−	−	−	+
12	17	1/600	+++	−	−	−	−	−	−	−	+	+
13	15	1/600	+++	−	−	−	−	−	−	−	−	+
14	17	1/600	+++	−	−	−	−	−	−	−	−	+
15	19	1/600	+++	−	−	−	−	−	−	−	−	+
16	15	1/600	+++	−	−	−	−	−	−	−	−	+
17	19	1/600	+++	−	−	−	−	−	−	+	−	+
18	15	1/600	+++	−	−	−	−	−	−	−	−	+
19	15	1/800	+++	−	−	−	−	−	−	+	+	+
20	17	1/800	+++	−	−	−	−	−	−	+	+	+
21	15	1/800	+++	−	−	−	−	−	−	Died.		
22	15	1/800	+++	−	−	−	−	−	−	+	+	+
23	15	1/800	+++	−	−	−	−	−	−	−	+	+
24	15	1/800	+++	−	−	−	−	−	−	−	+	+
25	15	1/800	+++	−	−	−	−	−	−	+	+	+
26	15	1/800	+++	−	−	−	−	−	−	−	−	+
27	15	1/800	+++	−	−	−	−	−	+	+	+	+
28	15	1/1000	+++	−	+	−	+	+	+	+	+	+
29	17	1/1000	+++	−	+	+	+	+	+	+	+	+
30	15	1/1000	+++	−	−	−	−	−	−	+	+	+
31	17	1/1000	+++	−	−	−	−	−	−	−	+	+
32	15	1/1000	+++	+	−	−	−	+	+	−	+	+
33	15	1/1000	+++	+	−	−	−	−	−	+	+	+
34	17	1/1000	+++	+	−	−	−	+	+	+	+	+
35	18	1/1000	+++	+	−	−	−	+	+	+	+	+
36	17	1/1000	+++	−	−	−	−	−	−	+	+	+
Controls. 37	15	−	+++	+++	−	++	++	+	+	+	−	+
38	18	−	+++	+	Died.							
39	13	−	+++	+++	+++	+	+	+	+	+	+	+
40	17	−	+++	++	++	+	+	+	++	+	+	+
41	15	−	+++	+++	+++	++	+	+	+	+	+	+
42	17	−	+++	+++	+++	+	++	+	+	Died.		
43	15	−	+++	++	++	+	++	++	++	+	+	+
44	17	−	+++	+++	+++	+	+	++	++	+	+	+
45	15	−	+++	++	++	+	+	+	+	+	+	+
46	15	−	+++	+++	+++	+	+	+	+	+	+	+

Identification with Other Species.

Pathogenic and non-pathogenic spirochetes have been observed in many animals. We shall compare the various spirochetes with ours—except those which clearly differ in form—the animals in which they exist, and the results of experiments on animals.

(1) Wenyon's *Spirochæta muris*[9] is extremely motile. He attributes this to the flagella, though he does not claim to have proved it. The undulating membrane was not observed. The number of spirals varies between six and two according to the size of the spirochetes, which measure 6 to 7, or 3 to 4 μ in length and 0.2 μ in breadth. When inoculated into mice, they begin to appear in the blood in 5 or 6 days, and though few at first, they attain the greatest number about the 10th day and then decrease again in number. In 2 or 3 months they disappear entirely from the blood and at no period do they produce pathogenic symptoms in the mice.

Wenyon's spirochete and that found by us are similar in form, motion, the absence of pathogenicity for mice, and the manner of multiplication. In his experiment, however, guinea pigs did not become infected even with the whole blood of a mouse—a point on which Wenyon's spirochete differs from ours, since our spirochete invariably infects a guinea pig and can be seen in its blood. Wenyon further states that his spirochete can infect young rats but not full grown ones. Our spirochete, on the other hand, infects both young and adult rats.

(2) Breinl and Kinghorn's[10] *Spirochæta laverani* was found in a white mouse infected with *Trypanosoma dimorphon* sent by Laveran from the Pasteur Institute in Paris, and in the blood of two of the six wild mice caught in the neighborhood of the Liverpool School of Tropical Medicine. It is very motile, readily stains with the aniline dyes, and all the modifications of Romanowsky's method. It is generally short and round, but some are long, measuring between 1.8 and 3.75 μ in length and 0.1 and 0.2 μ in breadth. The number of spirals varies between two and four. Only a few of these parasites are found in the blood. It readily infects mice and rats, but gives rise to no pathogenic symptoms. Breinl and Kinghorn did not find many of the spirochetes in the blood, but could prove their existence in the peripheral blood 2 months after inoculation. They regard their spirochete as a distinct variety and have named it *Spirochæta laverani*. It seems to resemble our spirochete in form, movement, staining reaction, and its condition in mice and rats, but the result of the attempts to transmit it to guinea pigs is unknown.

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[9] Wenyon, C. M., *J. Hyg.*, 1906, vi, 580.

[10] Breinl, A., and Kinghorn, A., *Lancet*, 1906, ii, 651.

(3) Several investigators have found spirochetes in the cancer of mice. Borrel,[11] who was probably the first to do so, observed them in four mice suffering from primary cancers. After him Gaylord[12] examined forty-eight mice with cancer and more without cancer, chiefly by Levaditi's method, and observed numerous spirochetes. According to him, they stain readily by Levaditi's method, but not by Giemsa's; they are motile, but no flagella are seen; they vary between 2.5 and 7.8 μ in length, and have from four to thirteen spirals. Calkins[13] and Tyzzer[14] have reported the same spirochete.

Deetjen[15] likewise made a study of mouse cancer and observed a species of highly motile spirochetes. It stained readily by Giemsa's method, measured from 1.5 to 5 μ, and had from one to five spirals and a flagellum about 3.0 μ long at each end. He states that the flagella occasionally failed to stain except by Levaditi's method.

Löwenthal[16] observed small spirochetes from 2.6 to 6 μ in length and 0.25 or 0.2 μ in breadth with four to twelve spirals in ulcerated cancer from human beings and dogs. They stained readily; no flagella and no undulating membranes were seen, but Löwenthal believes that they are provided with the latter. He called this species *Spirochæta microgyrata*.

It is difficult to identify the species of spirochetes, since the size of a spirochete or the number of its spirals varies according to the method of treatment. In experiments on animals, too, different results do not prove conclusively the difference of species. Deetjen, however, thinks that his spirochete is identical with Wenyon's *Spirochæta muris* and Breinl and Kinghorn's *Spirochæta laverani*, and that although it is difficult to identify the spirochetes found by Borrel, and Gaylord, and Tyzzer with his, they are undoubtedly similar species. Wenyon, on the other hand, considers that his species is distinct from Carter's.[17]

We have examined the blood of a large number of normal mice and white rats, treated with acetic acid, but have not found a single spirochete. We have not, however, examined the cancer of mice; we cannot, therefore, attempt to identify our spirochete with those observed by the investigators mentioned above.

[11] Borrel, A., *Compt. rend. Soc. biol.*, 1905, lviii, 770.
[12] Gaylord, *Ann. Rep. Cancer Lab. New York State Dept. Health*, 1907, viii, 34.
[13] Calkins, G. N., and Clowes, G., *J. Infect. Dis.*, 1905, ii, 555.
[14] Tyzzer, E. E., *Proc. Soc. Exp. Biol. and Med.*, 1906–07, iv, 85.
[15] Deetjen, H., *Münch. med. Woch.*, 1908, lv, 1167.
[16] Löwenthal, W., *Berl. klin. Woch.*, 1906, xliii, 283.
[17] Carter, V., *Sc. Mem., Med. Officers Army of India*, 1887, iii, 45.

We should mention also the spirochetes in rats found by Carter[17] (1887), MacNeal[18] (1907), and Mezinescu[19] (1909). As we have stated, Wenyon finds it impossible to identify his spirochete with Carter's, and Breinl and Kinghorn also believe that Carter's spirochete, which is larger and has more spirals, must be different from Wenyon's. Mühlens,[20] however, is inclined to believe that they all belong to the same species. In only one instance, among all the normal rats whose blood we have examined, did we find spirochetes similar in form. Since we made no further test, we cannot, however, go into the details of the subject. The spirochetes found by MacNeal, Mezinescu, and others do not give rise to pathogenic symptoms in the animals into which they are inoculated. However, the spirochetes of these investigators and the spirochete found by us appear from inoculation tests to be similar in form. As their tests were not the same as ours, it is difficult to compare the spirochetes. Unlike the investigators referred to above, we first undertook the bite experiments and observed the course of the infection after the bite and the pathological appearance at autopsy, and finally the pathogenicity of the virus for the guinea pig and monkey. The favorable reaction with salvarsan seems also to be important.

As we have stated, we always observed traces of acute changes in the kidneys and adrenals of the guinea pigs bitten by rats and of guinea pigs inoculated from the original generation. We are, however, at present unable to compare experimental and human rat-bite fever since only two reports on the anatomical view of the latter have appeared. Blake believes that Schottmüller's *Streptothrix muris ratti* is the causative agent of the disease. His diagnosis was "acute ulcerative endocarditis; subacute myocarditis; subacute interstitial hepatitis; subacute glomerular and interstitial nephritis, subacute perivascular exudate of adrenals; infarcts of spleen and kidney; congestion, hemorrhage, and edema of lungs; atrophic leiomyoma of the uterus."[21] Blake's report, however, affords us little help in our anatomical study of guinea pigs, inasmuch as we cannot be certain whether the abnormalities in the kidneys and the adrenals were due to rat-bite fever or to a streptothrix. The other report, which is by Miura and Toriyama,

[18] MacNeal, W. J., *Proc. Soc. Exp. Biol. and Med.*, 1907, iv, 125.

[19] Mezinescu, D., *Compt. rend. Soc. biol.*, 1909, lxvi, 58.

[20] Mühlens, P., in Kolle, W., and von Wassermann, A., Handbuch der pathogenen Mikroorganismen, Jena, 2nd edition, 1913, vii, 921.

[21] Blake, *J. Exp. Med.*, 1916, xxiii, 47.

does not mention the condition of the kidneys and adrenals. Crohn[22] described fifty-two cases of human rat-bite fever, and although he did not have the result of the examination of urine in every case, he enumerates nine cases of nephritis. From this it seems to be evident that human rat-bite fever also frequently affects the kidneys.

SUMMARY.

1. We have confirmed Ogata's results in experimental rat-bite fever caused by the bite of rats.

2. In our experiments with guinea pigs, swelling and congestion of the bitten parts, swelling of the subcutaneous lymph nodes, fever, and loss of weight were the typical symptoms. The progress of the fever was not so regular as in human cases, but we find records in the literature of patients who showed irregular fever types or were afebrile. The chief points that we noted in the anatomical view of the guinea pigs were swelling and congestion of the lymph gland system and acute changes in the adrenals and kidneys.

3. If an emulsion made from the lymph glands, cerebral substance, or the adrenals, or the heart's blood of a guinea pig of the original generation is inoculated subcutaneously or intraperitoneally into a fresh guinea pig, the animal invariably dies with the usual symptoms of fever and swelling of the lymph glands. The anatomical changes in this case were the same as those of the original guinea pig, except that the course was shorter and more regular. The same result was observed in further generations. No change was observed in pathogenicity, and in the guinea pigs of the original and further generations a species of spirochete as the causative agent was always observed. The incubation period in the original generation was from 1 to 2 weeks, and in further generations about 1 week.

4. When a mouse or white rat was inoculated, spirochetes always appeared in the peripheral blood, but no other symptoms developed. When peripheral blood drawn from a mouse thus treated was inoculated into a fresh mouse or a fresh white rat and peripheral blood drawn from the mouse and rat was inoculated into a fresh guinea pig, they all became infected and the guinea pigs always died. Thus we found that rats and mice are media but not victims of the disease, while guinea pigs are both media and victims of it.

[22] Crohn, B. B., *Arch. Int. Med.*, 1915, xv, 1014.

5. In the *rhesus* monkey on which we made our experiment we witnessed a process similar to that of human rat-bite fever, and our spirochetes were observed in other animals into which blood drawn from the monkey was inoculated.

6. In the original animals spirochetes were seen chiefly toward the end of the process and the conditions as to the period previous to it are not yet clearly known. In further generations of all the animals we used, spirochetes were found in the peripheral blood 4 or 5 days after inoculation, and gradually multiplied until the greatest number was reached about the 10th day after inoculation. They then began to decrease; yet spirochetes could be observed over 2 months later.

7. We have found spirochetes chiefly in the adrenals of the animals by Levaditi's method, but have not yet ascertained their distribution in other organs.

8. Our spirochete is short, round, and highly motile; it stains readily, and has few spirals. We have not yet observed an undulating membrane, but have seen what we believe to be a flagellum at each end.

9. The identification of our spirochete with other species must be left for further study. The spirochete which Futaki, Takaki, Taniguchi, and Osumi[8] found in two patients with rat-bite fever, seems to differ from ours in form.

10. Spirochetes disappear from the blood of the animals as a result of the injection of salvarsan, thus indicating that the spirochete is arsenotropic.

In conclusion we wish to express our indebtedness to Professor M. Ogata, to Professor Aoyama, Director of the Imperial Institute for Infectious Diseases, and to Professor Hayashi and Drs. Miyagawa and Mitamura, for their valuable assistance.

EXPLANATION OF PLATES.

PLATE 11.

FIG. 1. Spirochetes in the blood of a guinea pig infected with experimental rat-bite fever. Stained by Burri's method.

FIG. 2. A spirochete in a section of the adrenal of the same guinea pig as in Fig. 1. Stained by Levaditi's method.

FIG. 3. Spirochetes in the blood of a guinea pig infected with experimental rat-bite fever. Giemsa's stain. Zeiss, oc. 4, obj. $\frac{1}{12}$ oil immersion.

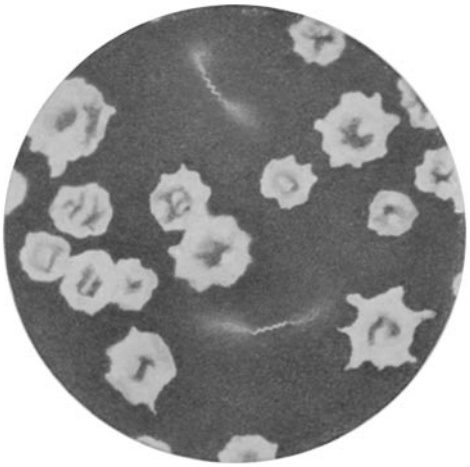

FIG. 1.

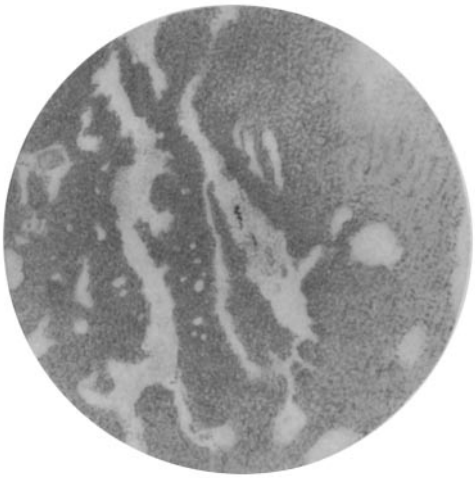

FIG. 2.

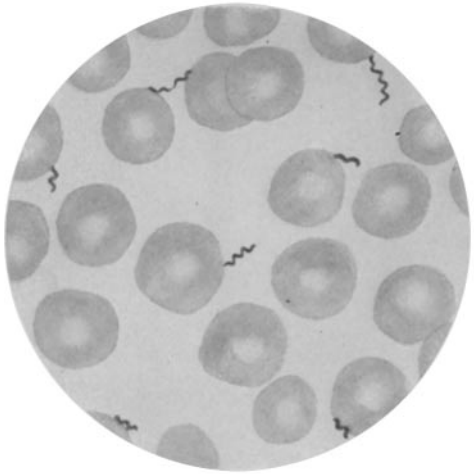

FIG. 3.

(Ishiwara, Ohtawara, and Tamura: Experimental Rat-Bite Fever.)