SPIROCHÆTA MORSUS MURIS, N.SP., THE CAUSE OF RAT-BITE FEVER.

SECOND PAPER.

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PLATES 8 TO 10.

(Received for publication, August 2, 1916.)

We have already reported the finding of a new spirochete,[1] which we believe to be the cause of rat-bite fever and which was present in the blood, skin, and lymph glands of two out of four patients suffering from the disease. Five more cases, in each of which we have been able to find a spirochete, have come under our observation and in two of the patients we found the spirochete in the circulating blood derived from a vein or the punctured skin.

Description of the Spirochete.

Morphology.—The spirochete consists of a body and flagella. In stained film preparations of the blood of a patient or inoculated animal, the body is spiral and comparatively short and thick. The size varies from 1.5 to 2 μ to 5 to 6 μ; when the flagella at both ends are included, the length is 6 to 10 μ. Smaller bodies are found in the blood in greater numbers than large ones, while in lymph nodes slightly larger ones occur. In cultures still larger ones measuring up to 12 to 19 μ, have sometimes been observed; but minute ones of 1.5 to 2 μ also appear.

In our previous report we stated that the organism in the patient's lymph nodes was somewhat larger than *Spirochæta pallida*, but

[1] Futaki, K., Takaki, I., Taniguchi, T., and Osumi, S., *J. Exp. Med.*, 1916, xxiii, 249.

smaller than *Spirochætæ duttoni* and *obermeieri*. It is thicker also than *Spirochæta pallida* and presents usually one curve per micron, the curves being regular and sharp. The number of curves ranges from one and a half to six; occasionally ten to nineteen curves appear. Other exceptions are as follows: in cultures the angle of the curves is obtuse; some spirochetes show one curve in 2 μ. In rare instances, especially in cultures in which degeneration quickly occurs, the curves become irregular. The characteristics are the same whether living preparations or stained films are studied. An undulating membrane has not been found. The protoplasm stains equally well, regardless of the stain used. The ends of the body gradually become pointed and pass into the slender flagella. In most cases flagella project from both ends; sometimes from one only. Rarely two flagella occur at one end. The flagella have approximately the same thickness and measure 2 to 3 μ.

Staining.—The spirochetes stain readily. Giemsa's stain, Löffler's methylene blue, Ziehl's solution, and aqueous gentian violet all stain the organisms readily. They are, however, Gram-negative. Silver impregnation succeeds easily. The flagella may or may not become stained with Giemsa's stain or silver solution. India ink, used according to Burri, is very effective in revealing the flagella. With Giemsa's stain the bodies take a deep violet-red coloration. Levaditi's method of silver impregnation is most suitable for demonstrating the presence of the spirochete in the tissues. By this method the bodies appear thick and the curves sharper, giving the organism a spindle-like appearance. In this case, the spirochetes can be distinguished only by means of the flagella which assume a yellowish brown color. In some culture organisms one or more chromatin bodies have been detected by means of Giemsa's stain.

Locomotion.—Usually it is difficult to find the spirochetes in the blood or in the lymph of patients even by means of dark-field illumination, as the number is small. Recently, however, we observed in one case out of five, spirochetes in blood from the punctured skin. We have, on the other hand, experienced difficulty in finding the organisms in thick blood preparations made according to Koch's method. In the inoculated mouse it is not infrequent to find from one to five spirochetes in a single field of blood or lymph. Hence the number of

spirochetes is far less than in other spirochetal infections communicated to animals.

The light refraction is weak and the motion is like that of a vibrio. The organism moves rapidly across the field of vision; but the cultivated spirochetes with long bodies are, on the contrary, inactive, making only wriggling movements.

Movements may be clearly discerned in specimens studied according to Shimamine's[2] method. The agar medium, after having been dissolved and cooled to 45°C., is mixed with fresh blood containing spirochetes. This mixture is placed under a cover-glass and the spirochetes can be seen fixed in the agar; capillary preparations are then made. The movements are spasmodic, usually spiral, and freely pass backwards and forwards. Comparatively long organisms undergo flexion and rotation. Meanwhile, whipping, winding, stretching, and darting motions of the flagella can be seen. When the bodies are very small it is often impossible to distinguish the various movements.

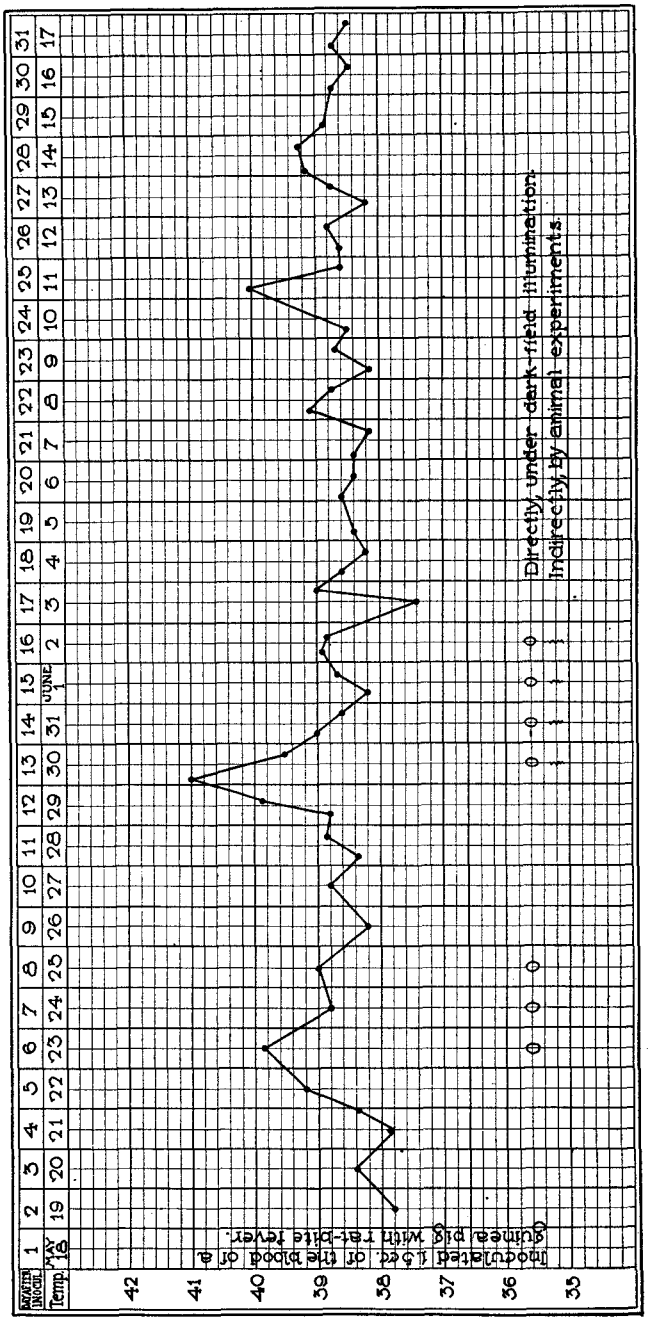
The spirochete examined microscopically under a strong light becomes quiescent comparatively soon. In animal blood at a temperature of 22–37°C., it ceases to move in about 24 to 48 hours. In this condition it is not recognizable under the dark-field microscope.

Mice, house rats, white rats, guinea pigs, and monkeys are suitable for the experiments, but mice are the best, especially when human material is to be inoculated directly into animals. White rats are the next best. Guinea pigs and monkeys frequently yield no result.

Spirochetes which have passed through the blood of mice or rats several times are highly virulent for other animals. This virulence depends, of course, largely upon the number of spirochetes inoculated.

After the inoculation of a mouse with human material, 7 days at the earliest, and several weeks or a month at the latest elapse before the organisms can be discovered in its blood. But if a mouse is in-

[2] Shimamine's medium is prepared as follows: 0.5 to 0.75 gm. of sodium nucleate and 100 cc. of horse serum are shaken until the former is completely dissolved, after which carbon dioxide is passed through the solution for 3 or 4 minutes, until the serum becomes transparent. The liquid is heated on 3 successive days, for about an hour at 60°C. On the 4th day it is heated at 65°C. for about 30 minutes, when it separates into a fluid and a coagulated portion.



TEXT-FIG. 1. *Pithecus fuscatus* (Blyth) inoculated with the blood of a guinea pig suffering from rat-bite fever.

oculated from an infected mouse, the spirochetes usually begin to appear in 4 days. Mice usually survive the experiment and retain the spirochetes for several months after the inoculation. Although the other animals generally survive the disease, death occurs now and then from various causes. Swelling of the spleen, hyperemia, congestion and swelling of the liver, or hemorrhage of the kidneys and lungs are often observed. Many of the organisms are also found in the liver or kidneys of animals as well as in their blood. The type of fever produced in the monkey, by the inoculation of spirochetes of rat-bite fever, is not typical of the fever in man. However, the type of fever in the monkey caused by inoculation with the spirochetes of relapsing fever is not typical of relapsing fever in man.

Propagation of the Spirochetes in the Human Body.

As cases of rat-bite fever are rare—only eight have come under our observation—and as we have not yet examined a fatal case,[3] our investigation is still far from complete. In the early stages the spirochetes are found in the local eruption of the skin and in the enlarged lymph nodes, whence they seem gradually to invade the blood.

In relapsing fever the organisms are numerous during the febrile, but decrease greatly in number or disappear altogether during the afebrile periods. When animals are inoculated with blood from a patient suffering from rat-bite fever during the afebrile period or when there is light fever, the spirochetes are present. However, it is always difficult to find them as they are few in number.

EXPERIMENTAL.

A. The striking clinical effect of salvarsan upon rat-bite fever has already been reported by Taniguchi and Hata,[4] whose experiments we have confirmed. We divided into three groups a number of mice in the blood of which the spirochetes had been demonstrated. The first group was kept as a control, and each animal in the other two

[3] A postmortem has recently been reported from the Kyushu Imperial University by Drs. Kaneko and Okuda. They claim to have found the same organism as ours in the intestines, and especially in the kidneys.

[4] Hata, S., *Münch. med. Woch.*, 1912, lix, 854.

groups was injected with $\frac{1}{600}$ to $\frac{1}{800}$ gm. of salvarsan for every 20 gm. of body weight (Table I).

TABLE I.

Experiments with Salvarsan.

Hypodermic Injection of 0.2 Cc. of Blood Containing Spirochæta morsus muris.

Mouse No. Body weight.	1	2 Control.	3	4 12 gm.	5 13 gm.	6 12 gm.	7 12 gm.	8 13 gm.	9 14 gm.
Dose per 20 gm. of body weight.				$\frac{1}{600}$ gm.			$\frac{1}{800}$ gm.		
No. of spirochetes.									
1st day (Mar. 2).	−	+	+	+	+	+	+	+	+
2nd "	+	+	+	−	−	−	−	−	−
3rd "	+	+	+	−	−	−	−	−	−
4th "	+	+	+	−	−	−	−	−	−
5th "	+	+	+	−	−	−	−	−	−
6th "	+	+	+	−	−	−	−	−	−
7th "	+	+	+	−	−	−	−	−	−
8th "	+	+	+	−	−	−	−	−	−
9th "	+	+	+	−	−	−	−	−	−
10th "	+	+	+	−	−	−	−	−	−
11th "	+	+	+	−	−	−	−	−	−
12th "	+	+	+	−	−	−	−	−	−
13th "	+	+	+	−	−	−	−	−	−
14th "	+	+	+	++	−	−	−	−	+
15th "	+	+	+	+	−	−	−	+	+
16th "	+	+	+	+	+	−	−	+	+

On the following day, though the spirochetes were found as before in the blood of the control animals, none were discovered in the other groups. This result continued until the 14th day, when about one-third of the animals showed a relapse; by the 17th day almost two-thirds had relapsed, and after 2 months 83 per cent showed spirochetes. A similar phenomenon has been witnessed in human beings.

Occurrence of the Spirochete in Nature.

We examined the blood of forty-three rats under dark-field illumination, and in one only, namely, a *Mus rattus alexandrinus* (roof rat) did we detect spirochetes. With the blood of this rat a white mouse was inoculated successfully. In respect to morphology, staining,

locomotion, and infectivity the spirochetes present in the roof rat are identical with those obtained from human cases of rat-bite fever. Yet we have not discovered the organism in the mouths of healthy house rats or in the saliva of house rats and other animals in whose blood the spirochete exists.[5] The spirochete found in a *Mus decumanus* by Carter[6] in 1887 and again by Mezinescu[7] in 1909 may be the same as ours. Moreover, Borrel,[8] Calkins,[9] and others have described spirochetes in cancerous and healthy mice. In one instance the description fitted the organism we are considering, but it has not been detected up to the present in healthy mice or guinea pigs in Japan.

B. We discovered our organism in November, 1915. Soon after, Ishiwara and his associates succeeded in cultivating outside the body a spirochete which was transmitted by the bite of a house rat to a guinea pig. In this case the spirochete-containing blood of the guinea pig was used. We have made similar observations on a somewhat larger scale. Some fifteen house rats having bitten guinea pigs, we examined the blood of the latter and found in one instance spirochetes which were identical with those present in rat-bite fever in man.

C. From the fact that patients suffering from rat-bite fever have invariably been bitten by rats and also that our spirochetes are found both in house rats and in human patients with rat-bite fever, it may be inferred that the spirochete is transmitted from these animals to the human body, and the experiments described above confirm this view. But as the organisms have not been detected in the saliva but only in the blood of rats, the source of the infectious organism probably is from the blood which, owing to hemorrhage from the gums, enters the saliva of the rat.

D. Various methods have been tested for the cultivation of the

[5] We owe to Drs. Ishiwara, Otawara, and Tamura valuable specimens of spirochetes from two rats—a *Mus decumanus* and a *Mus rattus alexandrinus*—in which they detected the organism.

[6] Carter, V., *Sc. Mem., Med. Officers Army of India*, 1887, iii, 45.

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

[8] Borrel, A., *Compt. rend. Soc. biol.*, 1905, lviii, 770.

[9] Calkins, G. N., and Clowes, G., *J. Infect. Dis.*, 1905, ii, 555.

spirochete, including especially Noguchi's and Shimamine's[2] employed for the cultivation of *Spirochæta pallida*. We have succeeded in cultivating our organism with the latter method, the first appearance of growth being noted after 14 days at 37°C. The inoculation is made by thrusting into the medium, thus prepared, a capillary tube impregnated with the blood of an animal containing the spirochetes. The tubes are not paraffined, and are left in the thermostat at 37°C. for 2 weeks. The growth is attended neither by liquefaction nor other obvious changes in the medium and no odor is developed. Multiplication is detected first by the dark-field microscope, and secondly by stained preparations. The pathogenicity of the cultures is under investigation.

The cultures show the following characteristics. The maximum number of spirochetes found in a microscopic field of the blood used for inoculation was three to four; of the culture, six to ten. The blood having been placed in the center of the medium, the spirochetes are diffused in the medium to within 1 cm. of the surface. Should the spirochetes not multiply in the culture those inoculated disappear in a week at the longest. Thus far a second culture generation has not been secured.

The size of the culture organisms varies greatly—the short ones measure from 1.5 to 2 μ, the large ones from 12 to 19 μ. The spirals are fewer in number (one curve in 2 μ) than in the blood spirochetes. The total number of curves varies from 1 to 1.5 to 19. The larger individuals in cultures have not been found either in the blood or lymph glands.

The culture spirochetes possess the same staining reaction as those of the blood. All the aniline dyes stain them, and Giemsa' solution gives them a deep violet-red tint. The flagella also stain distinctly. The larger organisms seem to divide transversely; vertical division has not been observed. Between the bodies slender, light colored mid-lines have been seen. In one instance one body was noted to give rise to four bodies. We have occasionally seen organisms with irregular spirals unevenly stained, which might be considered as degenerate forms. Once we observed from one to several deep red refractive chromatin corpuscles scattered among the culture spirals. In one instance only have we seen two flagella at one end of the body.

The culture organisms are motile—movements of the short individuals are brisk and similar to those of vibrios; the larger spirochetes exhibit slow, wriggling or whipping movements. 0.1 per cent sodium taurocholate and saponin dissolves the organism.

As regards classification, we believe that the spirochete belongs to the protozoa and not to the bacteria. We have not yet succeeded in obtaining a second generation of the culture.

Spirochetes have been described as occurring in rats or mice either in the blood or in tumors by many investigators (Carter,[6] Borrel,[8] Calkins[9] Wenyon,[10] Breinl and Kinghorn,[11] MacNeal,[12] Tyzzer,[13] Gaylord,[14] Deetjen,[15] Löwenthal,[16] Mezinescu,[7] Negre, (1910), and Hoffmann (1906). Although only a part of the descriptions suffices for certain identification, we have not been able to identify our organism with any of them. Moreover, in the case of none of the spirochetes described has the question of human infection entered. Hence we have concluded to offer as a designation of the spirochete of rat-bite fever the name *Spirochæta morsus muris.*

Ogata believes that rat-bite fever is caused by an aspergillus; Shikami[17] that it is caused by a telosporidia; Middleton,[18] by a diplococcus; Proescher,[19] by bacteria; and Schottmüller,[20] by a streptothrix. The streptothrix theory of Schottmüller was supported separately by Blake[21] and Tileston, and also by Tunnicliff.

By rat-bite fever we mean a disease having the symptoms described above, but the fever does not necessarily include all diseases related to rat bites. Hence we consider that the cause of rat-bite fever in this sense is the spirochete that we have described. In Schottmüller's two cases in which the victims fell ill without an incubation period, the disease may have been caused by a streptothrix, but it is not what we should call rat-bite fever. Tileston's cases were appar-

[10] Wenyon, C. M., *J. Hyg.*, 1906, vi, 580.
[11] Breinl, A., and Kinghorn, A., *Lancet*, 1906, ii, 651.
[12] MacNeal, W. J., *Proc. Soc. Exp. Biol. and Med.*, 1906–07, iv, 125.
[13] Tyzzer, E. E., *Proc. Soc. Exp. Biol. and Med.*, 1906–07, iv, 85.
[14] Gaylord, *Ann. Rep. Cancer. Lab., New York State Dept. Health*, 1907, viii, 34.
[15] Deetjen, H., *Münch. med. Woch.*, 1908, lv, 1167.
[16] Löwenthal, W., *Berl. klin. Woch.*, 1906, xliii, 283.
[17] Shikami, *Z. med. Ges. Tokyo*, 1909.
[18] Middleton, G. S., *Lancet*, 1910, i, 1618.
[19] Proescher, F., *Berl. klin. Woch.*, 1912, xlix, 841.
[20] Schottmüller, H., *Derm. Woch.*, 1914, lviii, Suppl. 77.
[21] Blake, F. G., *J. Exp. Med.*, 1916, xxiii, 39.

ently the same as ours, but he failed to detect the spirochete. Blake's case differs from our cases of rat-bite fever in that the lymph glands were not swollen, the rash was temporary, and the fever was not paroxysmal; it may properly be regarded as a case of streptothrix sepsis caused by the bite of a rat.

SUMMARY.

1. Since our first report on the discovery of the cause of rat-bite fever, we have been able to prove the existence of the same spirochete in five out of six more cases which have come under our observation.

2. The clinical symptoms of rat-bite fever are inflammation of the bitten parts, paroxysms of fever of the relapsing type, swelling of the lymph glands, and eruption of the skin, all occurring after an incubation period usually of from 10 to 22 days, or longer.

3. Our spirochete is present in the swollen local lesion of the skin and the enlarged lymph glands. But as the spirochetes are so few in number it is exceedingly difficult to discover them directly in material taken from patients. It is therefore better to inoculate the material into a mouse. In some cases the organism is found in the blood of the inoculated animal after a lapse of 5 to 14 days, or at the latest 4 weeks.

4. Generally speaking, the spirochetes present thick and short forms of about 2 to 5 μ and have flagella at both ends. Including the flagella, they measure 6 to 10 μ in length. Some forms in the cultures reach 12 to 19 μ excluding the flagella. The curves are regular, and the majority have one curve in 1 μ. Smaller ones are found in the blood and larger ones in the tissues.

5. The spirochetes stain easily. With Giemsa's stain they take a deep violet-red; they also stain with ordinary aniline dyes. The flagella, too, take Giemsa's stain.

6. The movements of our spirochetes are very rapid, resembling those of a vibrio, and distinguish them from all other kinds of spirochetes. When, however, the movements become a little sluggish, they begin to present movements characteristic of ordinary spirochetes.

7. For experimental purposes, mice, house rats, white rats, and monkeys are the most suitable animals. Monkeys have intermittent fever after infection, and spirochetes can be found in their blood, but

they are not so numerous as in the blood of mice. Mice are the most suitable animals for these experiments, and they appear, as a rule, to escape fatal consequences.

8. The spirochete is markedly affected by salvarsan.

9. The organism is not present in the blood of all rats, and there is no relation between the species of the rat and the ratio of infection. We have never found the spirochete in healthy guinea pigs or mice. By permitting a rat infected with the spirochete to bite a guinea pig, the latter develops the disease.

10. We have succeeded in cultivating the spirochete in Shimamine's medium.

11. Among the spirochetes described in the literature or discovered in the blood of rats and mice, there may be some resembling our spirochete, but none of the descriptions agree with it fully. Hence we have named our organism *Spirochæta morsus muris* and regard it as belonging to the Spironemacea (Gross) of the nature of treponema.

12. The spirochete can be detected in the bodies of patients. In seven cases out of eight, it disappears on recovery, only to reappear during the relapse.

13. The spirochete can be detected in about 3 per cent of house rats. These facts enable us to identify the cause of the disease.

14. There may be other causes than the spirochete for diseases following the bite of a rat. The cause, however, of rat-bite fever in the form most common in Japan is, we believe, the spirochete which we have described.

In conclusion we wish to express our indebtedness to Dr. Aoyama, former Director of the Imperial Institute for Infectious Diseases, and Dr. Hayashi, the present Director; to Dr. Miura who named the spirochete; to Professor Nagayo, Assistant Professor Ishiwara, and Drs. Manabe, Miyagawa, Mitamura, Matsuyama, and Katayama, for valuable suggestions; and to Dr. Oba, and Messrs. Itakura and Isosaki for the help they have given us.

EXPLANATION OF PLATES.

PLATE 8.

FIG. 1. Section of a lymph gland from a patient with rat-bite fever. Impregnated with silver nitrate according to Levaditi's method. × 1,250.

FIG. 2. Section of the lung of a mouse inoculated with venous blood from a patient with rat-bite fever. The length of the body of the spirochete is 2.2 μ; including the flagella it is 6 μ. Silver impregnation. $\times$ 1,500.

FIG. 3. A spirochete from a guinea pig with experimental rat-bite fever. The length of the body is 4μ.; with the flagella, 8.5. Giemsa's stain. $\times$ 1,500.

FIG. 4. Spirochetes from a guinea pig with experimental rat-bite fever. The length of the bodies varies from 2.2 to 4 μ. Giemsa's stain. $\times$ 1,250.

PLATE 9.

FIG. 5. *Spirochæta morsus muris* from a mouse inoculated with the blood of a patient with rat-bite fever. The length of the body is 4 μ; with the flagella it measures 9 μ. $\times$ 3,600.

PLATE 10.

FIG. 6. First column, *Spirochæta morsus muris* from the blood and tissues of human patients and inoculated animals. Second and third column, cultures of *Spirochæta morsus muris*.

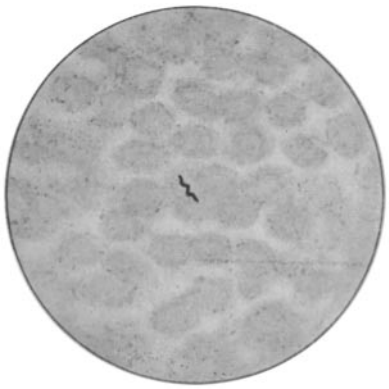


FIG. 1.

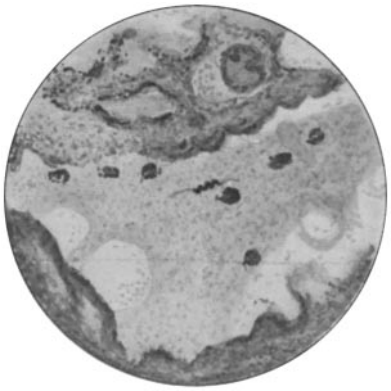


FIG. 2.

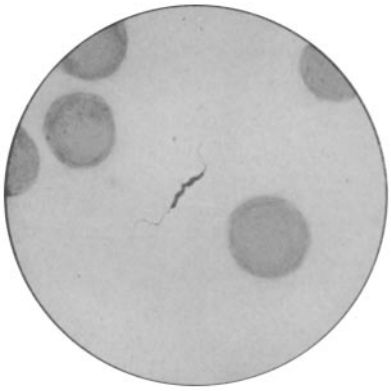


FIG. 3.

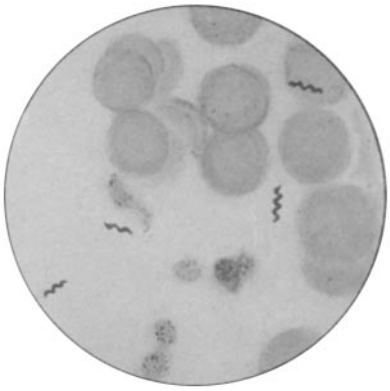


FIG. 4.

(Futaki, Takaki, Taniguchi, and Osumi: Cause of Rat-Bite Fever.)

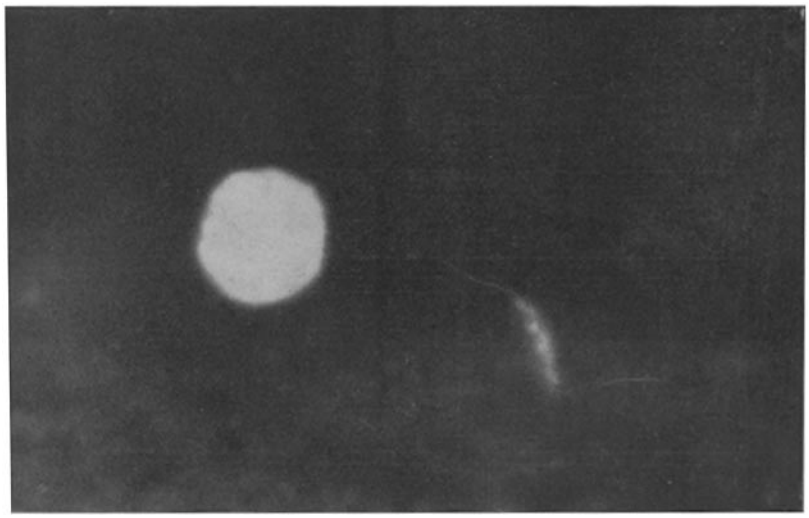


FIG. 5.

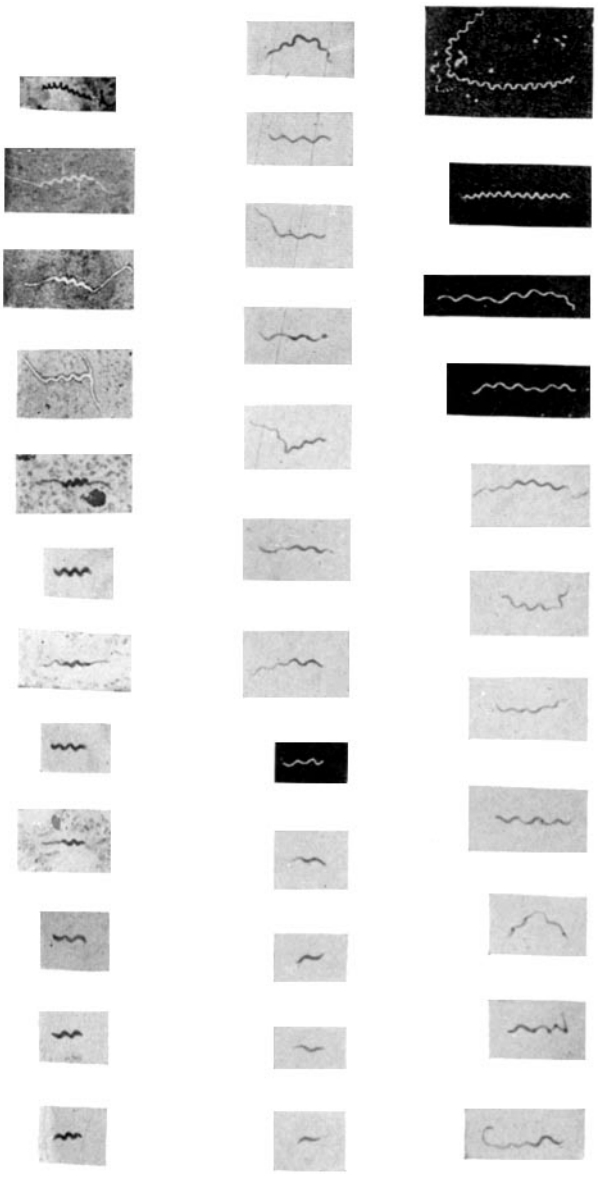


FIG. 6.

(Futaki, Takaki, Taniguchi, and Osumi: Cause of Rat-Bite Fever.)