FLAGELLUM OF THE MICROORGANISM OF RAT-BITE FEVER.

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PLATES 81 AND 82.

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Since 1915 when Futaki and Ishiwara found, independently, a microorganism as the cause of rat-bite fever, various investigators have published their results on this subject. The present paper is a report of observations recently made by the writer on the microorganism.

Material.

Two strains were kept in the laboratory, one from an infected child, and the other from a rat. Besides these, four were obtained from fourteen rats. Each of these strains was injected cutaneously into two guinea pigs. In a few days the site of injection became hard, the lymphatic glands were swollen, and the animals developed fever and died. Death was caused by the injected organism.

Method.

The blood of mice and guinea pigs injected with the strains described above was observed under dark-field illumination. The flagellum of the microorganism was fixed best with vapor of osmic acid (Figs. 1 to 4). Fixation with bichloride of mercury was unsuitable, since it caused contraction. Fixation with methyl alcohol was simple and brought out clearly a large flagellum. Before the cover-glass preparation was dried it was exposed to the vapor of osmic acid (1 gm. of osmic acid + 100 cc. of distilled water + 10 drops of 5 per cent bichloride of mercury) for from 30 seconds to 1 minute. Giemsa's solution or a modified reagent was used for staining. Gen-

erally the microorganism took a long time to stain, though the time required depended upon the power of the solution, which varied with the proportion of its components, "methylene azur," methylene blue, and eosin. The proportion of the components of the solution was not quite the same as that of Giemsa's solution, which did not give the best results, the commercial product often failing to stain the flagellum. A better solution for staining was obtained by adding a little alkali to Akashi's solution which is a modification of Giemsa's stain. It is worth mentioning that not only did the staining power of the preparation become very poor a few weeks after it was made, but also it was not always possible to obtain the same staining power, though the method of preparation was always the same. This point will be studied further.

OBSERVATIONS.

The microorganism consisted of the body and the flagella, as first recognized by Ishiwara and his coworkers and confirmed by many investigators. The body was short and thick; there were two or three windings which were thick, regular, and spiral, but not wave-like. Its length was about 3 microns. The microorganism moved energetically among the blood corpuscles when it was observed under the dark-field microscope, but its form was not changed during movement.

As to the number of the flagella, most writers have observed one flagellum at one end or at both ends. Matsusaki and his coworkers and Otawara observed one at one end and two at the other. In stained preparations the flagellum was observed at both ends of the microorganism or sometimes at one end only. In fresh preparations under dark-field illumination, however, they could always be seen at both ends. It is probable, therefore, that the flagella are generally present at both ends. In preparations fixed with methyl alcohol I found one flagellum at one or both ends. But in one preparation fixed with vapor of osmic acid, one flagellum was shown at one or both ends of some microorganisms, while many flagella were detected at one or both ends of others. These two types of microorganisms were always found by this method of staining, and the better the staining power of the solution the greater was the number with many

flagella. This was true even in the same material and preparation and seems to indicate that the number of flagella demonstrable depends on the staining power of the solution. These facts were confirmed by a study of the six strains already mentioned. The largest number of flagella counted was seven at one end. There were a few instances in which more than seven flagella were present, but they were so dense and tangled that they could not be counted. On the other hand, in one type of microorganism which showed one stained flagellum at one or both ends of the body, only one flagellum was found even when the staining power of the solution was best.

It therefore seemed advisable to investigate further the relation of these two types. 100 microorganisms with one flagellum at one or both ends and 100 with two or more flagella at one or both ends were studied. It was found that there was no relation between the length of the body or number of windings and number of flagella. It seemed possible, however, that the number of flagella might be related to stages of development. With this idea in mind streak preparations of blood taken from the ear vein of infected guinea pigs were made. Even in these cases two types were always found; that is, some microorganisms had one large flagellum at one or both ends and others had two or more flagella at one or both ends. But the ratio of the two types of microorganisms during one period showed no special relation to that at any other period.

The diameter of the end of the body of the microorganisms with one large flagellum gradually decreased to the flagellum, while in specimens with two or more slender flagella the ends of the body were blunt. Sometimes it was observed, however, that those with one slender flagellum were also blunt at the end of the body. In this case the number of flagella which were attached at the blunt end increased with better staining.

There were some peculiar specimens in which two flagella were fused into one (Fig. 8, *a* and *b*). In some instances the flagellum was divided into two or three parts at certain points (Fig. 8, *d* to *f*). Or again one flagellum was divided into two or three branches and one of the branches was again divided into two (Fig. 8, *f*). The part of the flagellum which was divided into two or three was generally

thicker than any other part. Furthermore, the diameters of the flagella of a microorganism might vary. In this case one flagellum which was larger than the others was sometimes divided into two or three (Figs. 5, *e*, 8, *d* and *f*, and 10, *a*).

Generally the flagellum was not straight; in the living specimen it was spiral. If the object-glass was skillfully moved in following a swift movement of the microorganism so as to keep it in the center of the dark-field, the movement became slow and the winding of the flagellum was readily observed, as the microorganism was weak to resist direct light; motion ceased even though the light was removed. In this case the flagellum became comparatively straight, L-shaped, or bow-like instead of spiral. Such was always true of stained preparations fixed with methyl alcohol. After fixation with osmic acid the flagellum was generally spiral just as in living microorganisms. Often the direction of the spiral was the same as that of the winding of the body. The spiral consisted of one and a half or two windings. When many flagella occurred at one end, the spiral of each was independent of the others. The direction of the spirals differed, but in many instances they faced one another. Two flagella, for example, which were extended at one end, were situated at an angle of 180° to each other (Fig. 5, *b* and *c*), or three flagella were kept at 120° (Figs. 5, *d* and 8, *e*).

The direction of the flagellum from the body varied with the movement. Futaki observed in streak preparations that the direction of the flagellum varied and thought that it was due to technique. This is not, however, always the case, because variation is observed even in a fresh preparation seen under the dark-field microscope. Often, however, the flagellum was extended in the same direction as the body as observed in a forward or backward movement (Figs. 1 and 5, *a*), though this did not always occur (Fig. 6, *a* to *c*). In some instances the flagellum was tangled around the body (Fig. 7, *b* and *c*), so that the microorganism appeared to have an undulating membrane. Furthermore, sometimes it was noticed that long microorganisms had one or two flagella in the center or near the center of the body. The center from which the flagellum originated was generally, though not always, small in comparison with other parts of the body. Moreover, this part was commonly slightly bent. In addition, two micro-

organisms, each of which had two or three windings, were connected with a thread and a flagellum at the connected ends (Fig. 10, *c* and *d*). Short microorganisms with one or more flagella at one or both ends were often observed (Fig. 9, *c* and *d*). These facts suggest that the microorganism divides transversely, though it is generally assumed that it divides longitudinally.

CONCLUSIONS.

The work of previous investigators was confirmed in that the microorganism of rat-bite fever was found to have flagella which are clearly visible by dark-field illumination and which can be stained. The best staining is obtained with alkalinized Akashi solution, which is a modification of Giemsa's solution; the vapor of osmic acid gives the best fixation.

The number of demonstrable flagella seems to vary with the technique. Sometimes many slender flagella unite into one large one or one large flagellum divides into several smaller ones. It may be concluded that commonly many slender flagella occur at the ends and that these may unite into one or several large flagella. In the living microorganism the flagella appear to be spiral. Their form in fixed preparations depends upon the method employed. Some long forms have flagella arising at the middle of the body; this seems to indicate that division is transverse and not longitudinal as generally believed. The rigid body, the signs of transverse division, and multiple flagella seem to distinguish the forms reported here from spirochetes and indicate that they are spirilla.

The work was done under the direction of Professors Miyairi and Ogawa.

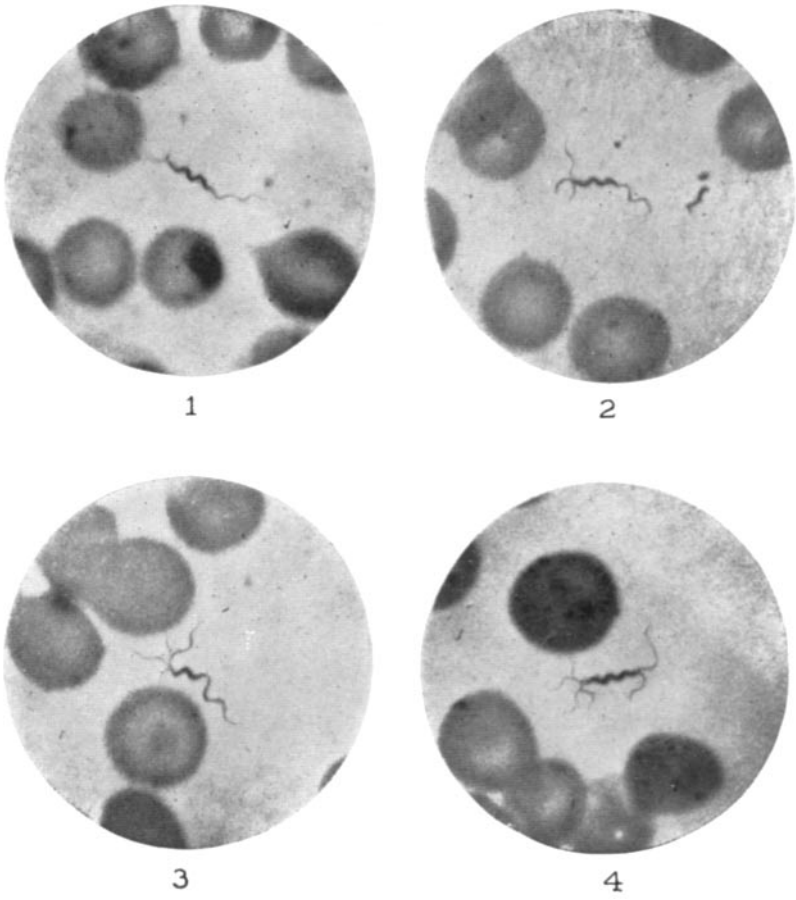
EXPLANATION OF PLATES.

PLATE 81.

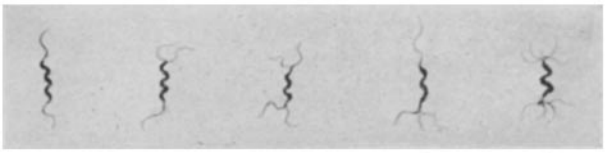
FIGS. 1 to 4. Photographs of microorganisms which were found in the blood of mice previously injected. Vapor of osmic acid fixation. × 2,000.

PLATE 82.

FIGS. 5 to 10. Free-hand drawings of microorganisms found in the blood of mice and guinea pigs previously injected. All flagella are drawn on the same plane, though they were not so in reality.



(Adachi: Microorganism of rat-bite fever.)



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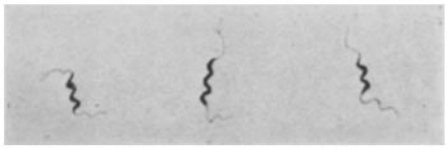

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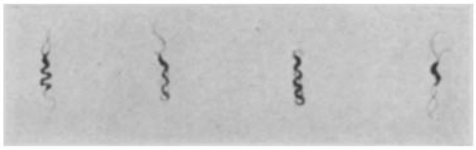

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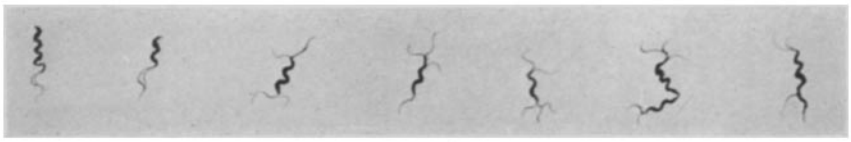

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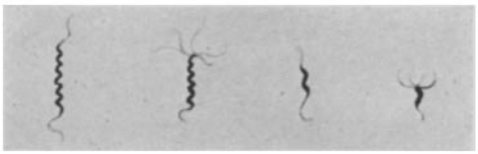

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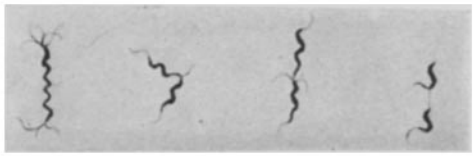

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(Adachi: Microorganism of rat-bite fever.)