FLAGELLUM AND MOTILITY OF SPIRILLUM SERPENS

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Bacterial flagella are still treated and discussed as if they were all homologous. This is due to superficial resemblances. The flagellum of *Vibrio metschnikovii* was shown to be intrinsically different from similar structures on *Salmonella typhosa* (Pijper and Nunn, 1949), and both kinds of flagella have little in common with the flagellum of *Spirillum volutans* (Pijper, 1949*a*,*b*).

Conclusions derived from observations on one kind of flagella are not relevant to flagella of other bacteria. Ignoring this has caused misunderstandings and confusion.

The reality of some flagella, apart from their nature, is still in doubt. Morton *et al.* (1951) saw flagella on spirochetes; Bradfield and Cater (1952) did not.

TERMINOLOGY

Flagellum is used here without prejudice as to function. Knaysi (1951) wanted "spiral" as used in bacteriology replaced by "helix" and "helicoidal". Many a "helix", however, eventually may prove a "spiral". Neumann (1929) showed *Treponema pallidum* to be too flat to be called a helix. For the sake of convenience and tradition the word spiral might stand for a while.

MATERIALS AND METHODS

Our strain of *Spirillum serpens* was isolated from the local Apies River by adding 6.0 g calcium lactate, 0.3 g ammonium chloride, 0.15 g magnesium sulfate, and 0.15 g dipotassium phosphate to 300 ml of water samples for enrichment, and obtaining pure colonies on 2 per cent agar (tap water; 1 per cent peptone (Difco); and 1 per cent calcium lactate). Motility was maintained very well on this mixture without the agar, and it was used nearly exclusively. The pH was unimportant and varied from 6.0 to 8.8.

Our spirillum corresponded culturally to *S. serpens* in *Bergey's Manual* (Breed *et al.*, 1948) and in the monograph by Giesberger (1936).

Our chief microscopic method was sunlight dark ground as described previously (Pijper, 1938, 1940). The sun's brilliancy made flagella easily visible in their natural state and allowed the making of a 16 mm film of motile phenomena.

MORPHOLOGY

Bodies. Bergey's Manual (Breed *et al.*, 1948) apparently following Giesberger (1936) described *S. serpens* as curved rods, 0.8 to 1.0 μ in diameter; wavelength, 8 to 9 μ; width of spiral 1.5 to 1.8 μ. Our measurements on spirilla lying still in the liquid medium (figure 1) gave a wavelength varying between 7.1 and 9.7 μ, the mean being 8.2 μ, and a width of spiral varying from 1.5 to 3 μ, the mean being 2.1 μ.

The variations need comment. Spirilla are traditionally called "rigid", "nonflexible", "nonflexuous", and "inflexible", and this feature is used in classification (Wilson and Miles, 1946; Dubos, 1945). Exceptions are Knaysi (1951) ascribing occasional flexibility to spirilla and Bisset (1952) who said their cell wall was either rigid or soft. A bunch of polar flagella usually is supposed to rotate the rigid spiral body which then would move through water like a screw through wood.

We observed that motility is accompanied by, and we think it is caused by, changes taking place in the coils of the spirillum. Moving spirilla alternatingly stretched and tightened their coils constantly, and during life there were no static dimensions. Measurements on spirilla lying still or dead reflected the phase of activity that they were in when movement stopped, and not so much shapes and sizes of individual spirilla as possible changes in shape and size. This lent these features a new significance without diminishing their diagnostic importance.

Flagellum in dark ground. The bunch of polar flagella familiar from stained preparations and

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electron micrographs made spirilla lophotrichate. Dark ground microscopy of motile *S. serpens* showed a polar flagellum, usually one at each pole (figures 2 and 3). Splitting of this polar flagellum was a post-mortem or a pathological phenomenon, observed in aged or dying forms. It usually produced two, sometimes three, occasionally four or five wavy threads, preserving the curve of the original flagellum (figure 4). In electron microscopy the wavy threads resulting from splitting are more numerous and thinner (van Iterson, 1947). The polar flagellum obviously consists of a very large number of wavy threads all twisted together during life. Splitting was an irreversible abnormal happening, and so *S. serpens* must be regarded as mono- or amphitrichate but not lophotrichate.

Splitting as we saw it was often accompanied, followed, or just preceded by, whiplike movements, either of the resulting wavy threads or for a short while of the still intact whole flagellum. Separate threads often did not move in unison, or one might lie still while others performed whiplike movements, often each in its own tempo. This did not suggest a common blepharoblast. These whiplike movements, so different from normal activities, never set the body in motion, but rather suggested inner tensions in the flagellum set up by the untwisting of numerous threads. This kind of movement might explain the "independent" movement of flagella belonging to bacteria that lie still, as reported by Johnson and Baker (1947) and Mallett *et al.* (1951), and the rotating movements featured by Kingma Boltjes (1948*a*,*b*). We saw such whiplike movements start in flagella of *S. serpens* that had been under the microscope for more than 48 hours, with spirilla motionless for at least a day, and presumably dead.

The single polar flagellum of *S. serpens* always showed a very definite shape and a very sharp outline (figures 2, 3, 4, 5, and 9). This contrasted markedly with the fuzzy appearance and unsharp contours of the tail of *S. typhosa* and similar bacteria, photographed for previous publications (Pijper, 1940, 1946, 1949*b*).

With *S. typhosa* the appearance of tails depended on unknown factors (Pijper, 1949*b*). The flagellum of *S. serpens* was visible in any medium and did not disappear in distilled water or saline as the tail of *S. typhosa* often did. It looked tough and horny, not soft and soggy like the tail of *S. typhosa*. Its constant length, tapering into a sharp point, its characteristic curvature, and definite attachment by a thin stem were all features absent in *S. typhosa*. In *S. typhosa* tail attachment was indistinct. During reversals the tail material floated to the other pole to form a new tail, and the bacterial body could perform somersaults without affecting the tail. The thin stem of *S. serpens* (figures 2, 3, 5, 6, 7, 9) though noticeable in all good photomicrographs of previous authors has been ignored by them. *S. volutans* also has it (Pijper, 1949*a*).

In *S. volutans* the flagellum was visible constantly because it nearly always stood off from the body. In *S. serpens* the flagellum tended to become wound round the body, as suggested by figures 5 and 8. During rapid movement the flagellum in front became so closely wound round the anterior part of the body that it became invisible. The flagellum at the back then became visible as a tail. With reversal of movement, front becoming rear and *vice versa*, the tail flagellum quickly wound round the new anterior end and disappeared from view while the other flagellum presented itself as a visible tail. This phenomenon often went on at high speed very many times. Although noticed by Reichert (1909) its bearing on motility has been overlooked. Many spirilla have one flagellum only. Knaysi (1951) thought that an amphitrichate state indicated cellular division. Monotrichate spirilla reversed as rapidly and frequently as amphitrichates. (There was no indication of polarity as suggested by Miss van Iterson (1947) on the strength of one electron micrograph.) In monotrichate spirilla when the one and only flagellum was in front, it became coiled round the body and there was no tail; when during reversal it found itself at the back, it showed up as a tail. The speed of movement was the same at all times. A flagellum wound tightly around the body could hardly exert motive power.

S. serpens often moved too fast for proper analysis of its movements. It slowed down with age. Various colloid solutions have different effects on different bacteria. Methylcellulose slowed down young *S. serpens* just as it did *S. typhosa* (Pijper, 1947) but had no effect on *S. volutans* (Pijper, 1949*a*). On *S. serpens* it had two separate effects: It slowed down movement through increased viscosity and it precipitated

onto the bodies and flagella, causing first a granular precipitate (figure 6) and later a continuous sheath (figure 7) making the flagellum more conspicuous, but preserving the curious shape and making it more difficult to get wound round the body (figure 8). Replacing the most suitable 0.5 per cent solution in saline of the 15 cp type of "methocel" (Dow Chemical Company) by a 20 per cent sugar solution of similar viscosity (about 2 centipoises) also slowed down movement but did not thicken flagella. Their greater conspicuousness in methylcellulose therefore was due to thickening by a precipitate and not to increased viscosity.

On nutrient agar bacteria live in a syneresis fluid which contains agar, and this also precipitates on *S. serpens*, producing pictures very much like figures 6, 7, and 8. Spirilla grown on agar are live artifacts but with the advantage that the thickened flagella are visible readily with a weak source of light.

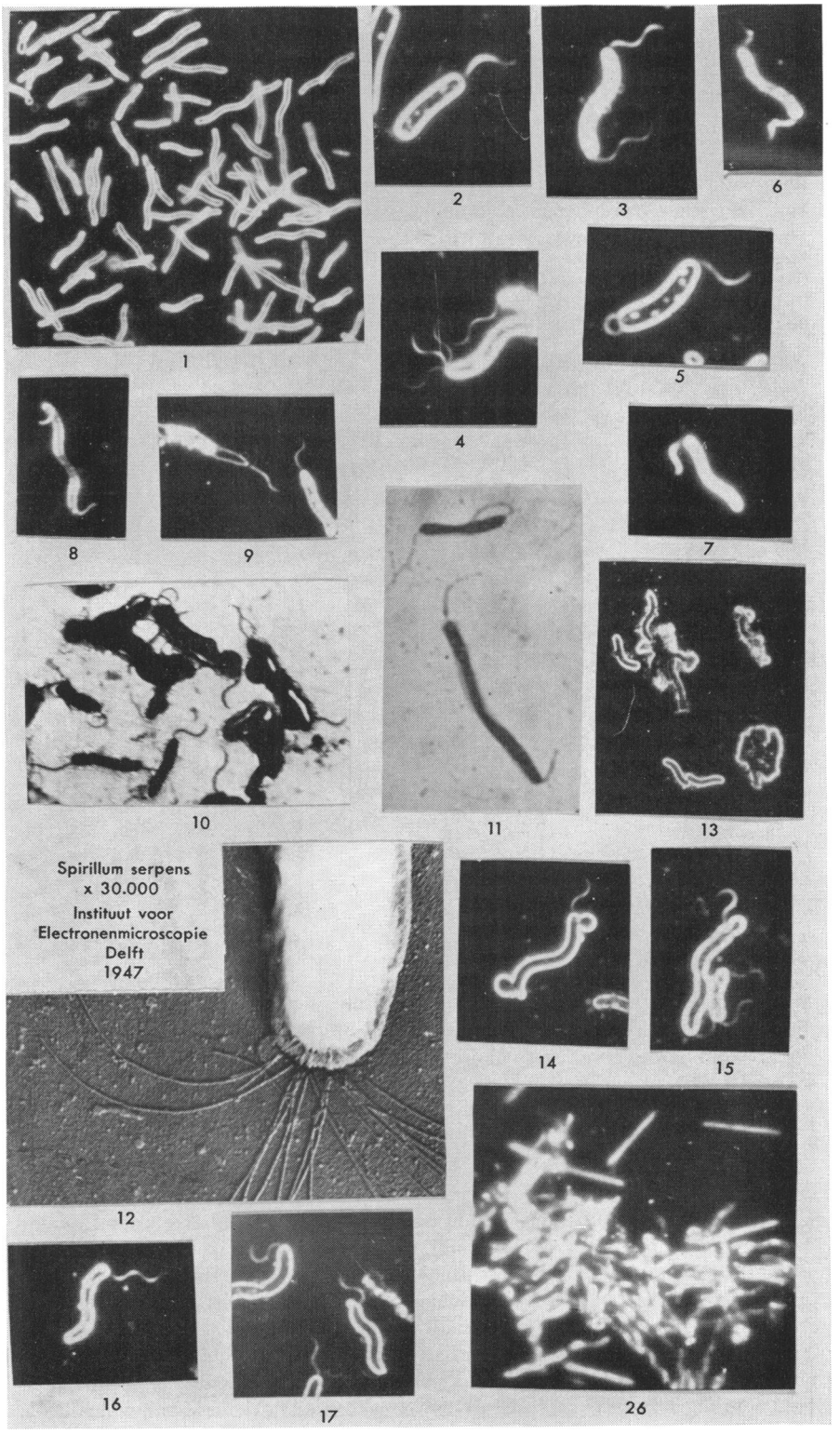
For *S. volutans* the flagellum was claimed to be cell wall drawn out into a fine point (Pijper, 1949*a*). The continuity of the flagellum of *S. serpens* with the cell wall showed up when it was kept under the microscope for a day or two. Autolysis then left an empty shell, presumably the tough cell wall, with flagellum attached and continuous (figure 9).

Staining of flagellum. The flagellum of *S. serpens* stained differently from the tail or flagella of *S. typhosa*. Flagella staining in *S. typhosa* is notoriously difficult, involved, and usually disappointing. In *S. serpens* the flagellum stained regularly with the simple cell wall staining method of Knaysi (1941) (figure 10), and also with that of Gutstein (1926) (figure 11). Knaysi's method gave a blue cell wall, red contents, and a blue flagellum. These staining results confirmed that the flagellum was a continuation of the cell wall.

Flagellum and cell wall. Fuhrmann (1910) claimed piercing of cell wall by flagellum in *S. volutans*, which was refuted by Meyer (1912). Miss van Iterson's electron photograph of *S. serpens* which she thought proved that flagella went through the cell wall (1947) is reproduced here with her permission (figure 12). This popular picture was used for the same purpose again by Houwink and van Iterson (1950), later by Bisset (1950, 1951), and twice borrowed by Kingma Boltjes (1948*a*,*b*). Miss van Iterson saw "rhizoid protoplasmic extensions" from which the flagella had their origin, in this picture, and also "conical basal parts of the flagella" (1947). Doubts about these interpretations were expressed early (Pijper 1949*a*). One must keep in mind (1) that the flagellum of *S. serpens* is attached polarly and at one point only by the thin stem mentioned above, and (2) that dying spirilla are likely to blow out their cell wall as a whole or as localized bubbles of various size and orientation.

In old spirilla an open space between cytoplasm and cell wall usually is explained as retraction of the cytoplasm. It may just as well be a bulge of the cell wall. The slim shape of *S. serpens* in figure 1 a few hours later had become like figure 13 with general swelling and local bulging to some

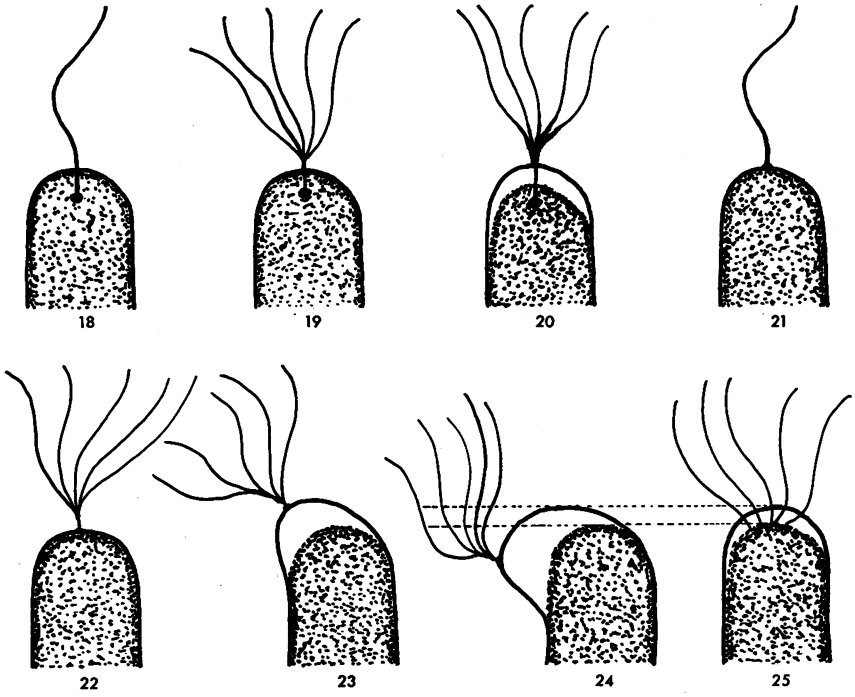
Figure 1. Spirillum serpens, in lactate medium, showing variety in shape. 600 ×.
Figure 2. Spirillum serpens, with typical polar flagellum. 1,200 ×.
Figure 3. Spirillum serpens, with two polar flagella. 1,200 ×.
Figure 4. Spirillum serpens, flagellum split into three. 1,200 ×.
Figure 5. Spirillum serpens, one free flagellum and one getting wound round body. 1,200 ×.
Figure 6. Spirillum serpens, in methylcellulose solution, granular precipitate on body and flagella. 800 ×.
Figure 7. Spirillum serpens, flagellum in sheath of methylcellulose. 600 ×.
Figure 8. Spirillum serpens, in methylcellulose, flagellum showing twist round body. 600 ×.
Figure 9. Spirillum serpens, autolysed, cell wall and flagellum left. 600 ×.
Figure 10. Spirillum serpens, stained with Knaysi's cell wall stain. 900 ×.
Figure 11. Spirillum serpens, stained with Gutstein's cell wall stain. 900 ×.
Figure 12. Copy of Miss van Iterson's electron micrograph of *Spirillum serpens*. 13,000 ×.
Figure 13. Spirillum serpens, having blown out its cell wall. 600 ×.
Figure 14. Spirillum serpens, blown out at pole, flagellum not displaced. 900 ×.
Figure 15. Spirillum serpens, blown out at pole, flagellum much displaced. 800 ×.
Figures 16 and 17. Spirillum serpens, blown out at pole, flagellum displaced. 600 ×.
Figure 26. Spirillum serpens, showing curved forms in clump and straight fast moving forms outside. 600 ×.



Figures 1–17, 26

unrecognizable shapes. In figure 14 the bulge had just left the flagellar attachment in place, but in figures 15, 16, and 17 it was displaced.

Figure 18 is a diagram of a polar end of *S. serpens* with its cytoplasm in place, and, for the sake of argument, a flagellum piercing the cell wall complete with blepharoblast was drawn in. In figure 19 the flagellum has split into a number of wavy threads. If now the cytoplasm withdrew from the cell wall, it might pull the stem of the flagellum through the cell wall, giving rise to figure 20. It could hardly give rise to figure 25 which is a diagram of Miss van Iterson's electron micrograph. If it did, it would mean that the retracting cytoplasm in pulling all the wavy threads separately through the cell wall had torn the cell wall wide open at the pole. It should not be ignored that in *S. serpens* the flagellum is attached by one stem, whether it is split or not. A new and better explanation of Miss van Iterson's picture starts with figure 21 with the flagellum attached to the cell wall of which we think it is a continuation. In figure 22 this flagellum has split. If now the cell wall started bulging as in figure 23 and continued to do so as in figure 24, the point of attachment of the flagellum or of the wavy threads would be much displaced. Looking at figure 24 in the plane of the paper, one would get figure 25 which is the diagram of the original picture to be explained. In Miss van Iterson's case the spirillum, having undergone the bulging disfigurement of figure 24, evidently came to lie with its bulge downwards or upwards on the screen of the electron microscope. The artifact of figure 12 illustrates again the necessity of interpreting electron micrographs from live observations (Pijper, 1949*b*).

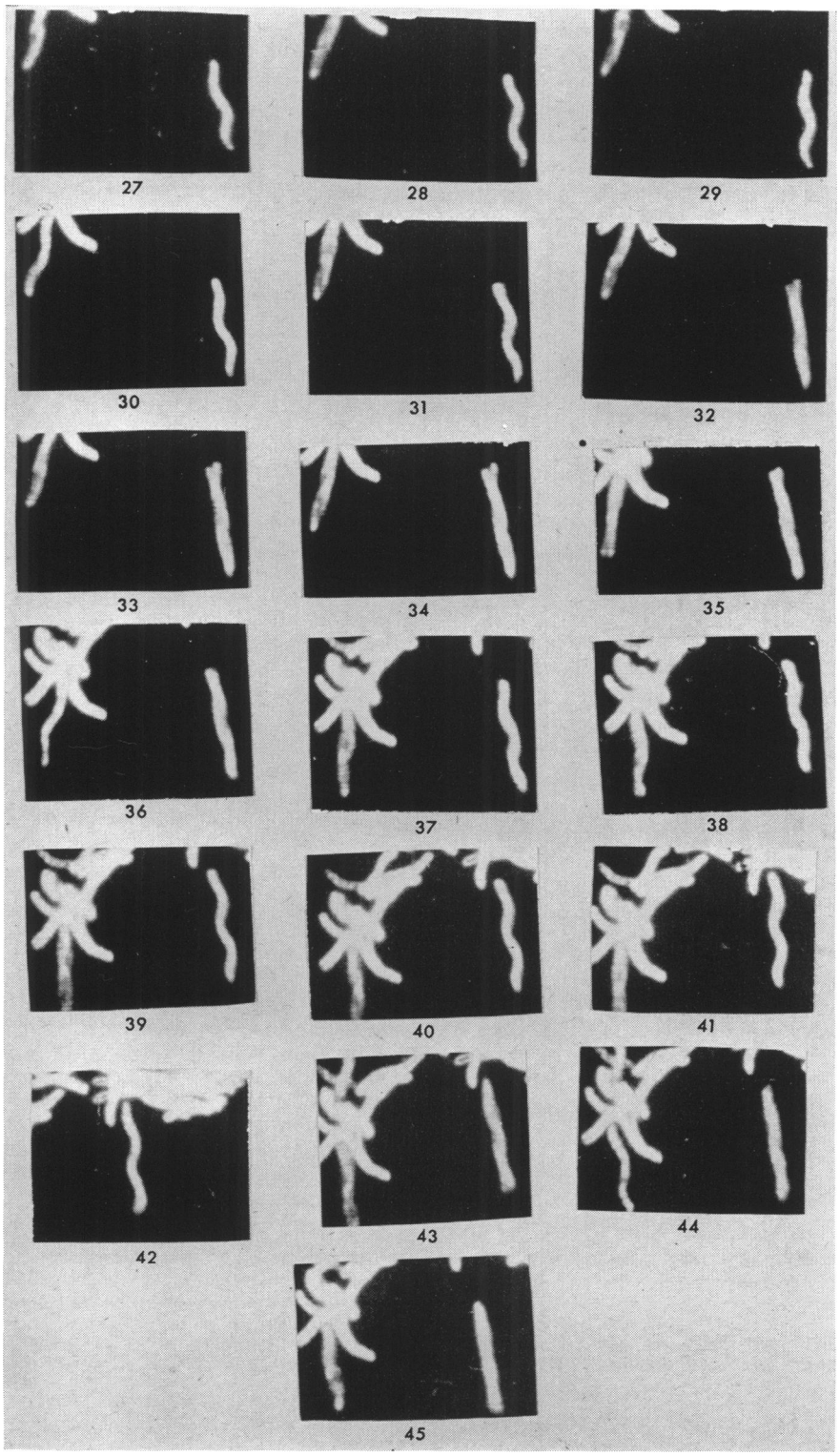


Figures 18 to 25. Diagrams illustrating origin of artifacts in electron micrography.

MOTILITY OF SPIRILLUM SERPENS

S. serpens is a very motile microbe and its positive phototaxis facilitated dark ground investigation. As the flagellum did not come off by shaking, probably through being wound too tightly round the body, this way of showing the independence of motility from the activity of flagella, which was successful with *S. volutans* (Pijper, 1949*a*), was not open to us.

S. serpens, during motility continually stretching or shortening its coils, proved itself a very elastic and flexuous spiral, which made it difficult



Figures 27 to 45. Copies from 16 mm film of *Spirillum serpens* showing changes in shape and size of moving spirillum. 850 ×.

to imagine that all these changes took place through the activity of a flagellum attached at a pole. They rather suggested the activity of inner forces in the spirillum which made it change its shape in such a fashion that propelling forces resulted. Cinematographic records supported this. Figures 27 to 45 are a continuous copy of such a record, with the exception of two frames between figure 41 and figure 42, taken out because no change took place. They illustrate what happened to one spirillum in one and a quarter second, the camera running at 16 frames a second. In figures 27 to 31 the spirillum lay still, as can be checked from the surroundings. In figure 32 it suddenly stretched itself and became correspondingly narrower. The next moment it darted forward at a very high speed towards the clump. Too slow a camera speed made it look like two spirilla twisted together. Notwithstanding this photographic imperfection, movement obviously started with stretching of the body. In figure 36 a slowing down had set in, and this continued in figures 37 to 42. In the meantime the spirillum had reached the clump. During the decrease in speed the spirillum went back to its more curved shape but maintained its increased length as compared with the similar shape in figures 27 to 31. After bumping against the clump, motion was reversed as shown in figures 43 to 45 where the body again straightened and narrowed. These pictures are typical examples of how *S. serpens* moved.

Figure 26 shows a clump of spirilla, practically all at rest and exhibiting the curved shape associated with that condition. Outside the clump at least four spirilla were rushing past at high speed, all looking as straight as darts.

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SUMMARY

The flagellum and motility of *Spirillum serpens* were investigated, chiefly by sunlight dark ground microscopy.

S. serpens was found to be monotrichate or amphitrichate, not lophotrichate. Its flagellum differed markedly from the flagella of *Salmonella*. It appeared to be a continuation of the cell wall, the two structures being connected by a narrow stem at the polar end of the cell. It did not pierce the cell wall, and previous evidence to this effect is explained differently.

S. serpens appeared to move by changing the shape of its body which was found not to be rigid but very flexuous and elastic. Normal motility appeared to be independent of the flagellum.

The observations support the senior author's view that bacterial flagella are not homologous and that the flagella of each genus should be investigated in the live state and regarded as structures *sui generis*.

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