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Pijper, A. 1949. Zur Frage der Bakterien-Geisseln. Schweiz. Zeitschr. Path. & Bakt., 12:681-691.

The question of bacterial flagellae

~~It is wholly untrue~~ that motile bacteria are motivated by flagellae, I have demonstrated to my satisfaction that the movement occurs through a spiral motion of the body of the bacterium and that the capsule of the bacteria consists of spiral filaments which in stained material resemble flagellae. (1946, 1947a, 1947b, 1947c, 1948b). I have been forced to espouse this theory after many years of observation of motile bacteria by my sunlight-darkfield method. [1]

Kauffmann (1948) in this journal has ventured to advance certain considerations concerning my theory which I hope to refute here — and at the same time I hope briefly to describe the observations, experiments, and deliberations which have culminated in this theory.

The Sunlight-darkfield Method

The difficulty with the serological diagnosis of Typhus-disease was impressed on me in 1928, when I was working with agglutination. The expression "flagellar agglutination" was current although nobody had actually seen flagellae in the Typhus organism! The only knowledge was

[1] I chose to exclude the large Spirilla, such as Spirillum volutans, from these observations. Their "flagellae" are of an origin and structure different from those of the usually motile bacteria, and were therefore kept distinct.

of the artificial product of the "Geisselfarbung" (flagellar-staining) and also whatever had been made visible through suspension of living bacteria in colloidal (usually gelatinous) suspensions by Reichert (1909), Neumann (1925, 1928), Neumuller (1927), and later Wei (1936) and Loveland (1933). In those suspensions, however (as I demonstrated, 1930, 1931/32), originates a precipitate of colloidal granules of bacterial-bodies and "flagellae", which flocculate into a solid sheath, thereby losing their natural behavior and motility; and resulting only in artifacts.

Use of the sunlight-darkfield method permitted me to see, photograph and film (1931/32, 1940) the natural behavior, and also to study and film (1938, 1941a, 1941b, 1942) their behavior during the agglutination process. I have usually worked with Typhus bacteria; my observations, however, also cover B. proteus, B. subtilis, B. megatherium, B. cereus, and B. caryophanum. In bouillon or water I have not seen (in the normal more or less linear movement) a single sheath of flagellae — which would be expected in the usually stained structures of the bacteria flagellated around the infundibulum. Thorough investigation revealed a tail (as in figure 1). The tail is not sharply defined; it can be of different lengths, as long as the bacterium itself, or, in the same bacterium, thick and tapering so that the distal end is always dim in photographs. Its appearance, the thickness and length depend on the pH and kind of nutrient. Peptone-water, for example, often gives a good motility but usually no tails. That the tail is a long drawn-out spiral was clear in slow-motion (figure 2). The tail can split in two (figure 3), then in three, and finally more quite thin spiral filaments, which usually group themselves around the bacterium (figure 4) and later detach themselves and disappear. It is obvious that this tail is the basis for the usually reported "successful flagellar-staining".[2] All reports of "flagellae" likewise spring from observations of what are called "tails" in this paper.

If the conception is correct that the unwinding of the "flagellae" drives the bacterium, then so does that of the tail, which it does. It is natural to assume that the tail works as a propellor; further experience, however, shows this assumption to be untenable.

The driving-force of the bacteria

Highly motile bacteria move so rapidly that the behavior of the bacterial bodies does not permit observations. Prolongation of the motion by reduction of temperature or by use of viscous media shows that the bacteria drive themselves spirally (figure 5). Rapidly swimming bacteria, photographed in "slow motion", show the same thing. The longer bacteria display a complete loop; the shorter bacteria describe a spiral; when they move their bodies are curved (figures 6 and 7). (One should remember that although the usually stained preparations of motile bacteria often retain an indication of this spiral shape, the dead-fixation does not entirely preserve the normal shape). The often mentioned "rocking" motion of the bacteria, which Reichert (1909) called "Trichter-bewegung," is a misinterpretation of spiral movement.

This new theory is of much consequence for the taxonomy and

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2 This and all other mentioned microscopic occurrences, including those associated with agglutination, are recorded on 16-mm movie film, preserved by the author.

and classification of bacteria, which we will not go into here.

The tail is not a propellor

The tail is not bound in any definite manner to any definite spot on the bacterium. During movement it seems to come sometimes from one side, sometimes from the other side of the hind end (figure 8 and 9).

From further recent work, carried out for the most part with an electron microscope, it appears that the bacteria possesss a thin, elastic membrane, which determines their shape and which has been called cell wall ("b" in figure 10). Within this cell wall lies the living protoplasm (cytoplasm), which next to the cell wall forms a more concentrated layer, the cytoplasmic membrane ("a" in figure 10). The cell wall is surrounded by a gelatinous layer, which is indicated as 'capsule' ("c" in figure 10), and, for example in Pneumococcus, is especially strongly developed.

The vital processes of the cell, naturally, occur within the cell wall. A cell wall cannot be considered 'alive'; nor can the capsule. ~~If one~~ Supposing that autonomous protoplasmic flagellae occur outside the cell wall, then the bacteria must carry on two lives, one inside and one outside the cell wall, if one does not, as it often appears he does, assume that the flagellae project through perforations in the wall. This "perforation theory", which recently has been supported by electron microscopy, postulates, first, that bacteria have the ability to ~~[illegible]~~ form new perforations (or else each part of the individual started with only half the customary flagellae), and, second, that the flagellae, as soon as they emerge, undergo a right-angle bend, and, plastered on the cell wall, run to the hind-end of the cell and enter into the spiral formation of the tail. Biologically and mechanically it is quite difficult to account for linear movement by this theory; anyway, the theory is untenable if one tries to explain by it what has been described as "salti mortale" (death jumps) or "plotzlicher Ruckgang" (sudden reversals). In the sunlight-darkfield microscope it can often be seen that a bacterium moving rapidly suddenly makes a "salto mortale" and yet proceeds in the original direction, whereas the tail does not 'cooperate', i.e., the bacterium turns itself around so that the froht end becomes the hind-end, but the tail shifts to the new hind-end (figures 11, 12, 13, 14). Also, when a bacterium suddenly shifts directions and then resumes its original direction, the tail stays at the rear end. Also in this "sudden reversal" which often occurs repeatedly at short intervals, often alternating with "salti mortali", the tail remains at the hind-end. When the bacterium reverses directions and swims back over its own tail, the tail shifts again to the hind-end (figures 15, 16, 17, 18). Both "salti mortali" and "plotzlicher Ruckgang" have been filmed repeatedly; for clarity we have constructed models and filmed the phenomena; the illustrations here are of those models.

The "perforation theory" and the conception of the tails ("flagellae") as organs of locomotion cannot explain the facts of salti mortali and sudden reversal. The assertion of Reicherts (1909) that a sudden reversal takes place rapidly in the terminally flagellated bacteria and very slowly in the bacteria flagellated around the infundibulum, so that the bacteria have time to reorient themselves, is simply not true. In the Typhus organism the movements here illus rated take place so fast that they almost cannot be filmed.

As was said, in certain nutrients in which good motility was observed, no tails are visible. One gathess that the bacteria do not need "flagellae," and that their motility is of another sort, unless it can be shown that the tails are present but too poorly developed to be visible. When one, however, takes a bouillon culture with strongly developed tails and shakes it vigorously 15 min., the tails almost all disappear, whereas in unshaken contro[illegible] controls they remain visible. The motility is quite the same in unshaken and shaken cultures. One can also pull off the tails without affecting the motili[illegible].

These observations and deliberations lead to the conclusion that the tails (and thus the "flagellae") cannot be organs of locomotion.

<u>The tails are the result of movement.</u>

As soon as one recognizes that the motive-force of bacteria does not lie in imaginary outer organs, but within the bacterial cell, the state of affairs becomes clear at once. The bacterial cell has the ability to drive itself in a spiral way by "waves" running through the body. The energy arises from the livi[illegible] protoplasm which the elastic cell wall permits to carry out the desired mov[illegible]ment. The movement of the bacteria, as also that of most aquatic ~~[illegible]~~ animalcules, as described by Breder (1926) and Hesse (1935), only occurs in bacteria in three dimensions, which in animalcules occurs in two dimensions. By this spiral locomotion the capsule flows to the hind-end of the bacterium, and when the material of the capsule skims itself ~~[illegible]~~ off, being optically refractive with water, it appears there as a tail. Whether it is only the spiral movement which produces the spiral filaments, or whether there already exists in the capsular material a tendency to a spiral structure, remains undetermined. Remarkably, Burton & Kohl (1946) published an electronmicrograph of a Pneumococcus, which showed a spiral structure, a discovery which Astbury (1945) has also called attention to as significant.

What is said about the sunlight-darkfield method above, finds a ~~single~~ simple explanation in the assumption that the spiral filaments of the flagellar-staining and of the electron-microscopy are not organs of locomotion, but only products of the capsule.

Objections to the theory

Kauffmann (1948) has prepared an H-serum with a non-motile culture of S. aberdeen. He does not say whether the culture displayed tails; in any case it makes no difference to my theory whether non-motile cultures can have H-serum. Further, Edwards, Moran & Brunner (1946) cite it; those authors had two "non-motile" Salmonellas, in which they succeeded in making visible "flagellae" with flagellar-staining. They did not add, however, that one of the Salmonellas later became "motile". I can yet add that also Hirsch (1947) had a "non-motile" Salmonella paratyphi, which H-agglutinated and which had stainabl "flagellae." From this apparent discovery of "non-motile" bacteria, which still showed stainable "flagellae", Kauffmann concludes that it cannot be movement which produces the flagellae. It depends a lot on what one means by "motile." Experience has taught me to be skeptical of statements about motility. This property changes with age of culture, with kind of nutrient, with pH; and even the putting on ~~of~~ a cover glass can paralyze the bacteria. The influence of the microscope lamp also stills the bacteria. Stuart, Wheeler, McGann & Howard (1946) often saw bacteria (including Shigella alcalescens and Salmonella typhi Phatnagar) which could pass as 'non-motile' and which became motile after repeated addition of watered agar. Similar observations were made by Weil and Slafkovsky (1948). The dividing line between motile and non-motile is thus not sharp. Perhaps one can distinguish between "motile" and "non-motile". Slightly rotating movement of the bacterial cells can account for the unwinding of the spiral filaments. Ruffer & Willmore (1909) described spiral movement in dysentery bacteria, which did not lead to locomotion, and Remlinger & Dumas (1915) found dysentery bacteria weakly motile and described the motion as rocking or trembling. That occasionally spiral filaments appear in "non-motile" bacteria with flagellar staining does not speak against my theory; Zettnow himself (1918), as he says, often had trouble distinguishing between "gelatinous filaments" and "flagellae." I can however bring to mind here that Johnson & Baker (1947) using electron microscopy, have found in Beggiatoa thin filaments seeming to unwind from the capsule; they could not tell whether there were flagellae or gelatinous filaments.

A further objection of Kauffmann is that O-forms are non-motile and have no H-antigen. He ascribes the absence of H-antigen to the absence of flagellae. I for one suppose that with the disappearance of the motility, the capsule material was also otherwise.

Kauffmann goes on that when one adds H-serum to motile bacteria, they are "suddently immobilized", and this is because the serum "paralyzes" the "flagellae". This is de facto untrue. As I have filmed and observed by the sunlight-darkfield method, bacteria first swim around freely for a while in the H-serum. Then fine particles from the bacterial body and the tail (eventually the sprial filaments) precipitate into the serum, and these mechanical impediments hinder the movement. A stage follows during which the bacteria take on the appearance as if they seek to be free of these hindrances by convulsive movements of the cells. The particles finally flow together into a solid sheath (as has been described above for bacteria in gelatine) and the bacteria quit convulsing and become as caricatures of themselves by random streaming (figure 19 and 20).

Random collision leads to agglutination. There is no evidence of "paralysis", but only of increased viscosity and mechanical hindering.

According to Kauffmann, in O-agglutination small clusters retain their motility, "while the flagellae beat on". Without sunlight, Kauffmann cannot see flagellae, and thus cannot possibly know whether they "beat on." With sunlight-darkfield one can see that the tails are flaccid and the bacterial cells remain motile in sofar as the close packing of the individuals permits.

Thus in the objections of Kauffmann, I can find nothing to refute my in-other-respects wellfounded theory.

Kauffmann refers further to a communication by Orskow, in which is described the use of the Burri-method of surface culture of Proteus bacteria. Orskow gives the impression that he had thereby seen flagellae which arrange India-ink particles in a halo around the bacteria to which they are attached. Using Orskow's method, I have seen this halo, but not the flagellae. Orskow & Kauffmann infer from this, without giving any explanation, that these haloes can be made visible also around bacteria which do not move. I can not understand how flagellae can be so vigorously "beating" that they, as Orskow, says, can disperse the India-ink particles in a large circle, without also moving their attached cells. Nor can I understand how, when the cells move only slightly or only revolves (which can hardly be discerned), the flagellae can be sufficiently in motion to set the tail(or the spiral filaments unwound therefrom) in sufficient motion to bring about the effect that Orskow describes. Logically, Orskow's experiment cannot gainsay my theory, and from what I have seen using his method, it augments my own assumptions very well.