METHYLCELLULOSE AND BACTERIAL MOTILITY

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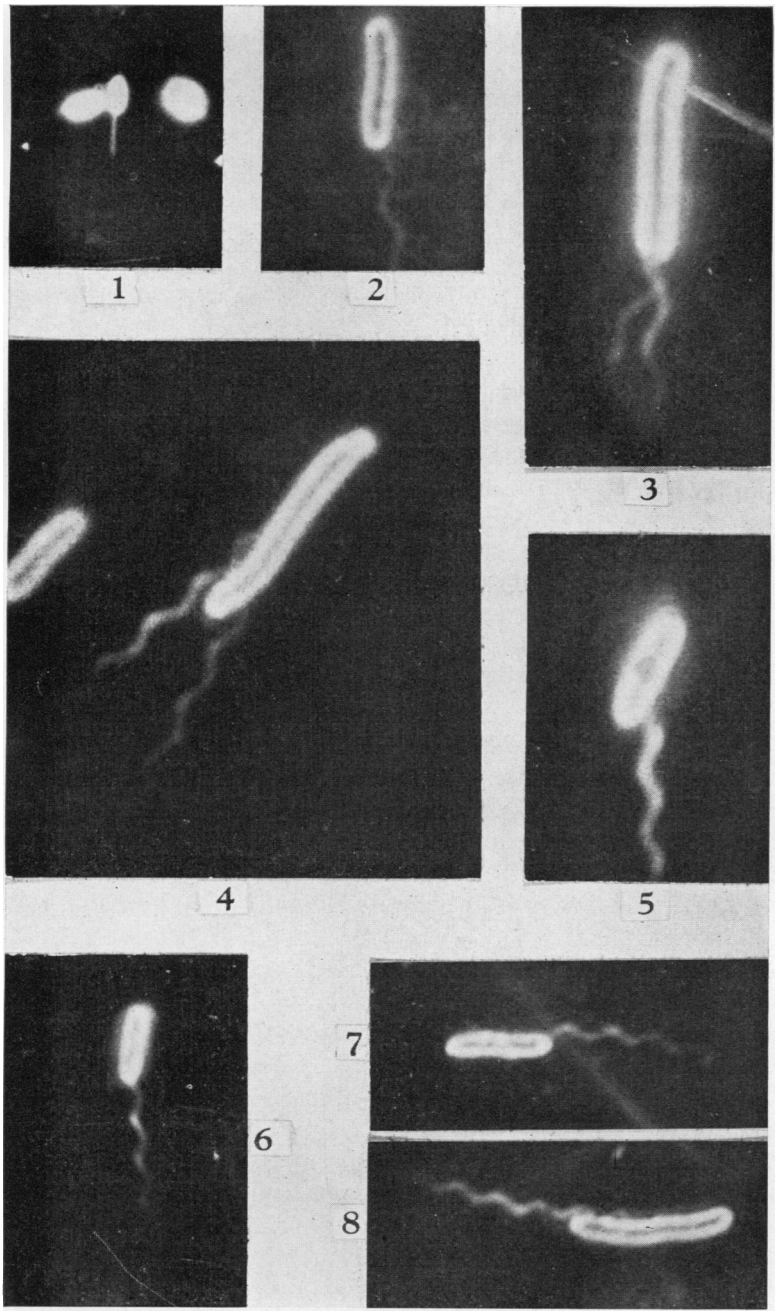
In a previous publication (1946) bacterial motility was shown to be due, not to activity of so-called "flagella," but to a gyrating and undulating movement of the bacterial body itself. "Flagella" are not motor organs but rather fortuitous appendages. The gyrating and undulating movement of the bacterial body originates in the protoplasm which lines the inner surface of the cell wall. It forces the cell wall into the shape of a moving spiral. As a result, the outer covering of the cell wall, which consists chiefly of polysaccharide material, is during fast movement, with the added effect of friction, mechanically twisted into a tail as in figure 1. This has been described, photographed, and filmed by me on previous occasions (1930, 1931–32, 1938, 1940, 1941*a*, 1941*b*, 1942). At other times this twisted material untwists into a varying number of wavy threads, which have so far gone under the name of "flagella." These twistings and untwistings have up till now only become visible with sunlight dark-ground microscopy (1938, 1940, 1941*a*).

All this not only fits in with modern conceptions of bacterial structure, as analyzed by Knaysi (1938, 1944) and Dubos (1945), but it supports them. A tough cell wall enclosing the protoplasm naturally bars this live substance from transmitting the required energy to supposititious "flagella" outside the cell wall. Even the electron microscope has not brought evidence that "flagella" pierce the cell wall, witness the comprehensive review of Mudd and Anderson (1944).

All protoplasmic energy must arise within the cell wall. Mechanically this energy reveals itself by throwing the cell wall into spirillar contortions, which result in movement in undulating and gyrating fashion. The outer covering of bacteria, referred to as the "slime layer" by Knaysi (1938, 1944), is largely polysaccharide material, and as such very plastic. It has long-chain molecules, with a tendency to form long micelles, and can be drawn out into threads several centimeters long (Knaysi, 1944). During rapid rectilinear movement it tapers automatically into a tail, as in figure 1. The tail may be very much longer than is shown in figure 1. Slowing down reveals its spiral nature, as in figure 2, as it is fashioned by the gyrating, undulating movement of the body. Occasionally it splits, as in figures 3 and 4, after which it may reunite again. Its general twisted structure becomes apparent on other occasions when I have seen it come apart in a number of fine wavy threads (1938). These are the supposed "flagella" for which so many staining processes have been invented, and which are now well known from electron microscope pictures, in which they lie as a tangled mass around the bacteria.

"Flagellum" both in bacteriology and zoology means a motor organ. As, however, both the tail and the wavy threads resulting from its untwisting owe



THE PHOTOMICROGRAPHS

Some of these have had to be made with sunlight, the others were made with a 100 CP pointolite lamp, but in no case do they illustrate more than what becomes visible when a 100 CP pointolite lamp is used, as stated in the text. It must be realized that overexposure of bacteria lying still or overprinting of negatives, which is sometimes necessary, leads to the appearance in the photomicrographs of thicker appendages than in reality exist.

FIG. 1. Bacterium with short straight tail. ×2,000.

FIG. 2. Bacterium slowing down, straight tail has become broadly wound coil. Note spiral shape of bacterium. ×4,000.

FIG. 3. Bacterium slowing down and splitting its broadly wound tail. Note spiral shape. ×5,000.

FIG. 4. Bacterium with split tail. Note spiral shape. ×5,000.

their origin to the motility of the body, their function must be regarded as entirely passive, and the term "flagellum" should no longer be applied to any of these structures. At best the tail acts as a passive rudder, contributing to the steadiness of the gyrating undulating movement of the body. But neither tail nor "flagella" possess energy; they do not represent a driving force. These things should be designated as "polysaccharide twirls," a term which adequately sums up and describes their nature and origin.

It also follows that motile bacteria should no longer be regarded and described as "rods." They may look rodlike in killed, fixed, and stained preparations, although even then curved shapes occur and have often been noticed. For systematic classification purposes, however, this rodlike shape should no longer be accepted as correct. Bacterial morphology should be based on the shape which becomes manifest during active life, which here means motility. "Motile bacteria" thus becomes a self-contradictory expression. When "bacteria" are motile, they move in undulating gyrating fashion and exhibit spiral shape, and not the appearance of rods, or "bacteria." This upsets bacterial classification, but facts must be recognized, and their recognition will eventually lead to clarification and simplification of taxonomy. In this paper the word "bacteria" is used without prejudice.

Different species of bacteria are credited with differences in the number and the attachment of their "flagella." Serology relies to a certain extent on differences in the physicochemical nature of the polysaccharide covering of bacteria. Such physicochemical differences may well express themselves in the number and situation of the polysaccharide twirls that become unwound, and have given rise to the terms, "peritrichic," "lophotrichic," etc.

POLYSACCHARIDE TWIRLS

Abolishing the word "flagella" in bacteriology and replacing it by a term like "polysaccharide twirls" does not rob the structures referred to of all importance. They remain curious morphological phenomena, and may even retain some taxonomic interest. Motility, however, is not dependent on their presence, nor development. Very motile bacteria need not exhibit any, as I have shown (1946). Their development depends on the production of a good "slime layer" of polysaccharide material. The haphazard results of staining methods for "flagella" here find their explanation. Very fast moving bacteria do not necessarily possess much polysaccharide coating.[1]

The action of H-agglutinating sera needs further revision. I have shown

[1] An illuminating story concerning Zettnow and "flagellar" staining in general is told by Levenson (1938). He was in a postgraduate class in which Zettnow taught his own method, but no member of the class succeeded in staining even a single "flagellum"!

FIG. 5. Bacterium with broadly wound coil attached to one side. ×4,500.

FIG. 6. Bacterium with tail in the shape of a broadly wound coil moving from the pole to the side. ×3,000.

FIG. 7. Bacterium with tail in shape of broadly wound coil definitely over to one side. ×3,000.

FIG. 8. Bacterium with tail in shape of broadly wound coil definitely attached to one side. ×3,000.

(1938, 1940) that such sera have no real agglutinating action. All they do is to cover "flagella" and body surface with a thick precipitate, presumably globulins, which stiffens the "flagella." This was confirmed with the electron microscope by Mudd and Anderson (1941). The stiffened "flagella" or "twirls" resemble corkscrews. This is the whole effect of the H-agglutinating serum. What then follows is *fortuitous* entanglement of the stiff twirls, caused by accidental currents and slight remnants of bacterial motility. "Flagellar agglutination," supposed to be the result of an H-agglutinating serum, does not exist; it is neither flagellar nor does the serum cause agglutination.

METHYLCELLULOSE

Methylcellulose is a water-soluble cellulose ether, produced and sold by the Dow Chemical Company of Midland, Michigan, as "methocel." According to the firm's booklet it forms colloid solutions, which are colorless, odorless, tasteless, and nontoxic. It is available in six viscosity types, ranging from 15 centipoises to 4,000 centipoises in a 2 per cent solution. Professor Robert Breed kindly supplied me with a quantity of methocel, with the suggestion that I should apply it to the study of bacterial motility, for which I here express my thanks. It was also through his kind help that, when I found it useful, the firm supplied me with larger quantities of the 15 cps and the 4,000 cps variety, for which I remain very grateful. Methocel has been of great assistance to me in elucidating the motility of bacteria.

EFFECT OF METHOCEL ON MOTILE BACTERIA

Most of my observations have been made on *Eberthella typhosa*, but control observations on *Proteus vulgaris*, *Bacillus megatherium*, *Bacillus cereus*, *Pseudomonas fluorescens*, and *Bacillus subtilis* confirmed that my conceptions of motility and "flagella" are applicable to most, if not all, motile bacteria.

The effect of methocel solutions on motile bacteria is twofold. Their viscosity slows down motility, and a slight precipitate which descends on the bacterial bodies and wavy appendages makes these last structures more easily visible.

Methocel solutions differ in their effect from the colloid solutions previously used by other authors for the purpose of slowing down motility through increasing viscosity (Neumann, 1925, 1928; Neumüller, 1927; Loveland, 1933; Wei, 1936). These authors used gelatin or gum, and the result was such a voluminous precipitate of these substances on the supposed "flagella" that they appeared as heavy corkscrews, the same as if they had been treated with an H-agglutinating serum, with similar end results (Pijper, 1930, 1931–32, 1938, 1941*a*). This precipitate was not noticed as such by the authors, and they regarded the thick wavy threads they saw attached to the bacterial bodies as plaits of otherwise normal "flagella." This misinterpretation added to the confusion. Such complete artifacts which have proved so misleading to previous authors do not usually occur in the weaker solutions of methocel, as employed by me, or if they do, they only occur after prolonged exposure.

Methocel solutions of suitable strength have the advantage of precipitating

just so much material onto bacterial appendages that their slight thickening makes them visible by ordinary dark-ground microscopy with a 100 CP pointolite lamp, without having to take recourse to sunlight. The "polysaccharide twirls," formerly called "flagella," become visible in full activity, in a condition which closely approximates normality. The slight coating of methocel scarcely affects their behavior and appearance. Figures 1 to 8 show early stages. Figures 9 to 15 show various more or less advanced stages of "untwisting." For demonstration purposes the methocel solution technique is bound to supersede the elaborate and often disappointing fixing and staining methods, which at best show a mass of entangled wavy threads, without any clue as to function.

The slowing down effect through viscosity is the feature which elucidates bacterial movement. In my previous studies of bacterial motility I used to aim at the brightest possible light, which was provided by the sun, and the fastest possible bacteria, in order to get a good view of their tails. As a result their bodies became just a blur, and the nature of their movement became hidden. Placing such high-speed bacteria in a suitable methocel solution and using less brilliant light in the form of a 100 CP pointolite lamp completely altered the picture. The bacteria now moved slowly with or without tails or other appendages. Their bodies exhibited graceful, regular undulating and gyrating movements, which propelled them through the fluid (figure 4). The slower they moved, the more pronounced the undulations became (figure 16). Readily visible in long forms, the same kind of movement also became quite evident in the shorter and even very short forms (figures 21 and 22). Very fast swimmers moved for a time as very elongated spirals (figure 17), but as a rule adopted a more leisurely, broadly undulating gyrating movement later on (figure 18). The whole spectacle can scarcely be watched without the thought dawning upon the spectator that in this undulating gyrating movement is the force which propels the bacterium, and that it is not in the tail, which follows somewhat limply behind. Every bacteriologist has occasionally watched bacteria move across the field with a curiously oscillating movement. This "waddle" has so far not been recognized for what it is, viz., a gyrating undulating movement propelling the body in spiral form. The spiral shape of the moving bacterium in methylcellulose solution is quite unmistakable, and it persists when the bacterium comes to rest, or dies (figure 19). It is more persistent after death in methocel solutions than in watery solutions. Dividing forms also show it (figures 20 to 22).

In not too viscous solutions, in which speed is not too much reduced, many bacteria still drag a straight tail along. Slowing down and more pronounced gyration and undulation cause a broadening of the coils of the tail (figure 2). With a return to higher speed the tail may stretch again. If, during periods of rest or slower speed, there is only one appendage, it is usually attached at one end; but it may then go somewhat to one side (figures 5 to 8). When the tail untwists in methocel solutions, it usually at first splits into two wavy appendages, rarely three (figure 14). In methocel solutions the untwisting sometimes proceeds further than this, and on occasion the twisted polysaccharide material

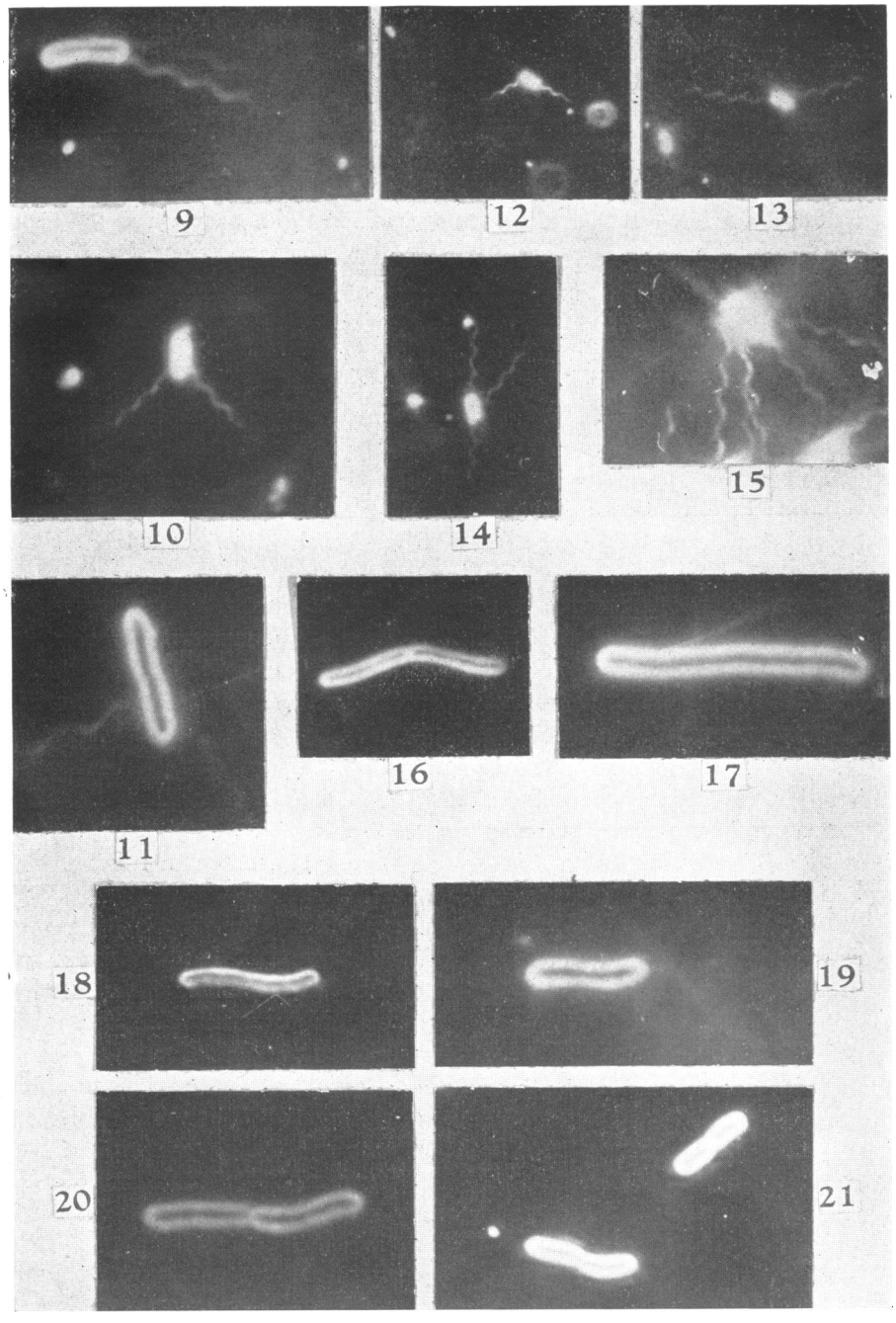


FIG. 9. Bacterium with tail definitely split into two wavy appendages. × 3,000.
FIG. 10. Bacterium with two symmetrically arranged wavy appendages. × 3,000.
FIG. 11. Bacterium with two irregularly arranged wavy appendages. × 4,000.
FIGS. 12 and 13. Two bacteria, each with two irregularly arranged wavy appendages. × 1,500.
FIG. 14. Bacterium with three wavy appendages. × 1,500.
FIG. 15. One bacterium of which the body is badly blurred in order to show at least 5 wavy appendages. × 4,000.
FIG. 16. Two bacteria which have just divided, both showing spiral shape and together forming a larger spiral. × 2,000.
FIG. 17. Long bacterium showing drawn-out spiral shape. × 3,500.
FIG. 18. Bacterium showing perfect spiral shape. × 2,000.
FIG. 19. Dead bacterium still showing spiral shape. × 3,000.
FIGS. 20 and 21. See facing page for legends.

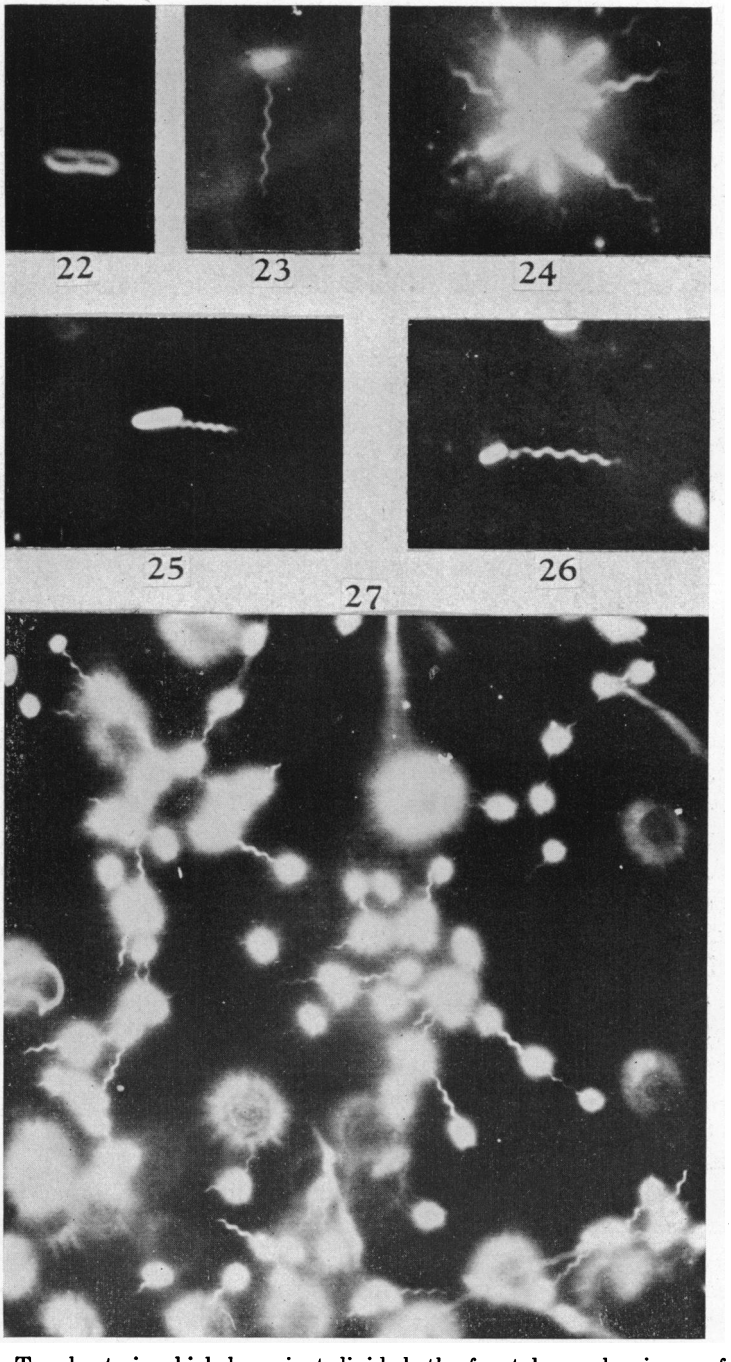


FIG. 20. Two bacteria which have just divided, the frontal one showing perfect spiral shape. × 3,000.

FIG. 21. Two sets of bacteria which have just divided. Note spiral shape of both sets. × 3,000.

FIG. 22. Short bacterium which has just divided and both the new individuals and the combination show spiral shape. × 3,000.

FIG. 23. Bacterium with one wavy appendage which ends in indistinct mass around bacterium. × 2,000.

FIG. 24. Clump of bacteria with wavy appendages sticking out. × 2,000.

FIGS. 25 and 26. Two bacteria which have been in methylcellulose solution too long, with marked thickening of wavy appendages. × 2,000.

FIG. 27. Large number of bacteria, most of them slowing down or stopping suddenly and showing broadly coiled tails. × 2,000.

comes apart into several fine wavy threads, as in figure 15. I have, however, never seen in methocel solutions the very large number of wavy threads that I have seen in watery solutions, in which it may reach the number of 20 or thereabouts. The explanation probably is that the thin coating of methocel prevents complete deployment.

When the tail splits into just two appendages, which is shown in various stages in figures 4, 9, and 10, they may be arranged symmetrically, but often there is no regularity about their attachment (figures 11, 12, and 13).

In many instances the appendages stop short a little distance from the bacterial body, indicating that they really belong to the outer covering (figure 23).

In methocel solutions bacteria show a tendency to stick to one another once contact has been made accidentally. This leads to formation of small groups, the bodies becoming attached, while wavy threads stick out (figure 24).

TECHNIQUE AND DETAILED RESULTS OF OBSERVING MOTILE BACTERIA IN METHOCEL SOLUTIONS

Methocel solutions are best made by weighing out the required quantity, pouring on half the required saline at boiling temperature, allowing the material to soak for some minutes, and then adding the rest of the saline. The mixture, in a corked flask, is then placed in a refrigerator and left there until solution is complete. For the more concentrated solutions it is advantageous to invert the flasks from time to time. The solutions will be found to be perfectly clear to the naked eye, but for dark-ground work, where every blemish shows up, it is advisable to subject the thicker solutions to rapid centrifugalization, and the thinner ones to filtration through a Seitz filter. In a refrigerator solutions made under sterile conditions keep very well. I have occasionally noticed fungal growth; it is better to discard such flasks. In methocel solutions viscosity increases with a rise in temperature, and so at room temperature the viscosity is higher than in the refrigerator. It is probably further increased by the heat rays in the focus of the dark-ground condenser, but to what extent it is impossible to say. The amount of precipitation on bodies and appendages is probably also affected by the temperature, apart from the concentration of the solution. Then there is the time factor. As time proceeds the amount of precipitate always increases, and a bacterium that looks like figure 1 or 2 in a suitable solution of methocel may keep this appearance for several hours, but eventually it will look like figure 25 or 26, although 24 hours or more may be required to reach this stage. Taking all these uncontrollable factors into account, it is hardly feasible to prescribe the exact strength and kind of solution required for a particular effect.

Microscope preparations are made by placing a large loopful of the methocel solution on a slide and rubbing a very small loopful of broth culture gently into it. A cover slip is dropped on and sufficient time given for the drop to spread out to the edges. The cover slip is sealed down on two sides. An enclosed small air bubble is helpful for supplying oxygen; in its neighborhood bacteria live longer and remain more active.

A 100 CP pointolite lamp is sufficient to see everything that is illustrated in my photomicrographs. A small 5 amp. arc lamp does the same. I use a Zeiss cardioid condenser, but other good glass dark-ground condensers answer well. As an objective I like the 60× Zeiss, with iris, and a fairly strong ocular, say 15× or even 20× is indicated. Binoculars can be used, and when they are arranged to give a stereoscopic effect, the undulating gyrating movement becomes particularly obvious. All binocular attachments, however, decrease the amount of light reaching the eye, and finer details may become invisible this way unless the lamp is very bright.

It is essential to work with bacteria possessing a high degree of motility, and this can usually be achieved by making them grow in several passages through soft agar, either in a petri dish or through a U tube.

For the benefit of those wanting to repeat the experiments I shall now briefly give the results obtained in various methocel solutions, with the reservation that, for the reasons mentioned above, it is difficult to duplicate results. The notes refer to typhoid bacilli, strain Ty 901.

METHOCEL VISCOSITY TYPE FIFTEEN CPS

With *0.25 per cent solution*, absolute viscosity about 2.5 cps. Motility very good, with numbers of very long and thin tails, much longer than appears in figure 1. After half an hour some tails become broadly wound coils, as in figure 2 or 27, but motility remains good. An occasional one untwists and becomes like figure 9. Movement remains too fast to show undulation and gyration. After two hours motility decreases, and, although some very long and straight tails are left, many bacteria now show two appendages. These sometimes come together again and form one tail, which then again splits, thus illustrating the reversibility of the change.

With *0.5 per cent solution*, absolute viscosity about 3.5 cps. Motility very good, with numbers of long tails. Occasionally slight gyrating and undulating movement is visible, but on the whole movement is too fast. Many suddenly slow down with tail becoming a broad coil, as in figure 2 or 27, and then again return to high speed and a long thin tail. Collisions rather more frequent than is normal. Untwisting and retwisting of tails quite common. After a few hours: motility decreased, some swimming with broadly coiled tail; undulating gyrating movement visible here and there, but not pronounced.

With *1 per cent solution*, absolute viscosity about 6 cps. Motility at first quite good, with many long thin tails, and very little undulation and gyration. After some minutes broadly wound tails appear as in figure 2 or 27, which, however, often return to the elongated shape. Many bacteria in their straightforward course suddenly reverse frontal and caudal pole, while the tail remains in place. This means that they can turn a half somersault within their coat of polysaccharide combined with methocel material. Bumping against one another is not uncommon. After one hour: still many normal tails, although generally motility is much less and many broadly wound tails and double appendages have appeared. Many bacteria start swimming in narrow circles,

swinging their tails around. After a few hours: little motility left, many broadly wound tails, moving and still. Some splitting of tails still occurs. No obvious undulation and gyration.

With *1.25 per cent solution*, absolute viscosity about 7.5 cps. Motility at first very good, but slows down fairly soon, and then undulating gyrating movement begins to show up (figure 4). Tails become broader spirals rather soon. Bumping occurs, and the individuals often stick. Appendages thicken visibly after a few hours and motility comes to an end.

With *1.5 per cent solution*, absolute viscosity about 9.5 cps. After initial high velocity with long tails, many sudden stoppages and reverses take place. Indications of undulating and gyrating movement start early (figures 16, 17, and 18). Circular movement with broadly wound tails fairly common. Tails split and reunite often. Clumps form easily, with wavy appendages sticking out, sometimes revolving erratically, probably as a result of tension in the material (figure 24).

With *2 per cent solution*, absolute viscosity about 15 cps. Very much the same as in the 1.5 per cent solution. Sudden "reverses" quite common.

With *2.5 per cent solution*, absolute viscosity about 24 cps. At first quite normal long tails, but the bigger bacteria begin to show undulating gyrating movement quite clearly, while the smaller ones often swim in narrow circles. Tails rapidly become broader coils and splitting is common. Thickening of appendages not more marked than in previous less viscous solutions.

With *3 per cent solution*, absolute viscosity about 37 cps. On the whole the same as in the 2.5 per cent solution, but there is more undulating gyrating movement. Motility stops rather soon. Thickening of appendages not more than in previous solutions.

With *3.5 per cent solution*, absolute viscosity about 54 cps. At first good motility with typical long tails, but undulating gyrating movement starts quickly and becomes quite manifest, while the tails rapidly become broad coils.

With *4 per cent solution*, absolute viscosity about 80 cps. After usual initial high velocity with long thin tails, undulation and gyration become quite marked and motility endures, some bacteria undulating and gyrating with long thin tails and others with broadly wound tails.

With *4.5 per cent solution*, absolute viscosity about 113 cps. Motility very poor except near air bubbles. Those that move show fair undulation and gyration with long tails that rapidly become broad coils.

With *5 per cent solution*, absolute viscosity about 160 cps. Scarcely any motility.

METHOCEL VISCOSITY TYPE FOUR THOUSAND CPS

With *0.1 per cent solution*, absolute viscosity about 7 cps. Very good motility with faintly visible but very long and straight tails.

With *0.25 per cent solution*, absolute viscosity about 20 cps. Very good motility, tails becoming more easily visible. Occasionally a tail becomes a broad coil. Sudden reversals in direction also become visible, with the tail

sometimes left behind, and the bacterium then swims over its own tail, making it obvious that the body directs the tail and not the tail the body. Motility decreases with time, and broadly wound tails appear. There is then little gyrating undulating movement.

With *0.5 per cent solution*, absolute viscosity about 50 cps. Very good motility at first, with thin long tails, which rapidly become more easily visible. The longer forms begin to show gyration and undulation, but the curves of the bodies remain fairly flat, the speed being still fairly high.

With *0.75 per cent solution*, absolute viscosity about 140 cps. Motility is now much less from the start, and there is a good deal of undulation and gyration. Tails easily visible, sometimes still as long, straight structures, sometimes as broad coils. Tails are often left behind at sudden reversals in direction, and then appear again at the other end. Tails often extremely long. This medium is very good for showing definite gyration and undulation with tails attached. Bacteria lying still usually show definite spiral shapes.

With *1 per cent solution*, absolute viscosity about 300 cps. Motility is slow, there is very marked undulation and gyration, tails usually are broad coils. A few rapidly moving bacteria still show elongated, very straight tails. Undulation and gyration become quite universal after an hour.

With *1.5 per cent solution*, absolute viscosity about 1,200 cps. Undulation and gyration very common from the start, with slow motility, while tails gradually change from the elongated form to broad coils. Many bacteria reverse suddenly their direction and then swim over their tails, which appear again at the other end. After half an hour many clumps are formed, with coiled appendages sticking out.

With *2 per cent solution*, absolute viscosity 4,000 cps. Motility very slow, with very marked undulation and gyration, sometimes with a long, thin tail, which is often left behind during sudden reversals of direction. Motility stops fairly soon, but even after an hour there are still individual bacteria showing very clear undulation and gyration, with elongated tails.

With *2.5 per cent solution*, absolute viscosity 11,000 cps. Motility very limited and slow, but this is accompanied by very good undulation and gyration.

SUMMARY

Solutions of methylcellulose (sold as "methocel" by the Dow Company) provided a particularly suitable medium for the study of bacterial motility. Such solutions possess sufficient viscosity to slow down the movement of otherwise fast-moving bacteria and thus supply conditions for detailed observation of movement, which appears as if it were in "slow motion." The special advantage of methocel over the solutions of gelatin and gum used hitherto for this purpose is that the precipitation of the colloid material on bacterial bodies and appendages is minimal, so that motility is not hindered by this thickening. With gelatin and gum the amount precipitated is always so large that the bacteria, with enormously thickened appendages, appear as caricatures of themselves, and no valid conclusions can be drawn from such artifacts. With

methocel this precipitation and thickening is a very slow process and solutions can be prepared in which for a long time the amount precipitated on the bacteria is just enough to make the fine appendages ("flagella") visible by ordinary dark-ground methods with ordinary lamps, without having to take recourse to the brilliancy of sunlight and dark-ground techniques. Methocel therefore places the dark-ground study of bacterial motility and bacterial appendages during life within the scope of ordinary microscopic technique.

A study of motile bacterial under these conditions leaves no doubt that such bacteria propel themselves by means of undulating gyrating movement of their bodies, in which "flagella" play no active part. It becomes obvious that the wavy appendages which appear under these conditions and which used to be called "flagella" are just the product of motility and not its cause. They consist of the outer covering of bacteria, which is mainly polysaccharide, and through the undulating gyrating movement of the body they are twisted off in the shape of long spiral tails or more or less numerous thin, wavy threads.

This new conception of bacterial motility and of the nature of the so-called "flagella" has already been put forward, with all the evidence on which it is based, in a previous paper (Pijper, 1946). The present paper describes in detail the technique and the results achieved with various methocel solutions. On the whole, methocel of the 15 cps variety is more suitable for making the conduct of appendages visible, and methocel of the 4,000 cps variety brings out the undulation and gyration more readily, but both phenomena can be observed in suitable solutions of either variety.

Motile bacteria representing several groups have been examined, and they all exhibit these undulating gyrating or "spirillar" movements. It therefore appears that motile bacteria can no longer be regarded as rods, or "bacteria," but must be classed as something like spirilla. The term "flagella," which would indicate motor organs, will have to be dropped and replaced by an expression such as "polysaccharide twirls" or "mucous twirls."

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