MICROCINEMATOGRAPHY OF THE AGGLUTINATION OF TYPHOID BACILLI

ADRIANUS PIJPER

Pretoria, South Africa

Received for publication February 5, 1941

This paper describes how microcinematographic pictures were made of the agglutination process of typhoid bacilli (both H and O), and what the resulting 16 mm. films showed.

TECHNIQUE

The technique used was described in detail in a previous paper on the microcinematography of the motile organs of typhoid bacilli (1940). Here the following chief points may suffice.

Sunlight was taken as the source of light. It was brought direct from a heliostat mirror through a 2-meter focus collector lens to the dark ground condenser of the microscope. No further mirrors, nor coolers, nor light filters were employed. This had been proved to be the only way to get sufficient light onto the photographic film.

The darkground condenser was the Siedentopf cardioid. Of great help were the combined glass-mica microscope slides, invented by Zeiss, which provide a perfectly clean surface by splitting off a layer of mica. With glass slides it is very difficult to get a perfectly dark background.[1]

The objective used was the Zeiss oil-immersion apochromat, 35 ×, N. A. 0.85. In combination with the Zeiss photographic eye-piece 4 ×, and a distance of 15 cm. between microscope and cinema film, a magnification of a little over 50 was reached. This proved ample for all details, and low enough to send sufficient light to the photographic film.

[1] These glass-mica slides can now also be obtained from George T. Gurr, 136, New King's Road, Fulham, London, S.W. 6.

It can hardly be sufficiently emphasized that in microcinematography of this kind the supreme difficulty is to get enough light to the photographic film. The Movikon film camera had to run at 12 pictures per second, with the shutter at the maximum opening of 180°. The exposure thus became one twenty-fifth of a second. With Kodak Super XX film this was just sufficient to get the fainter details. It is regrettable, but unavoidable, that in order to get these fainter details, the more brilliant parts of the picture suffer from over-exposure. This resulted in blurring of the outlines of the bacillary bodies.

A Zeiss beamsplitter was employed which gave sufficient extra-magnification to view everything that was filmed, in comfort.

The agglutination reactions were made to take place in a thin film of fluid between microscope slide and coverslip. As a rule a drop of fresh broth was placed on the clean mica surface, and lightly touched with a small loopful of a young broth culture of typhoid bacilli. For O-agglutination the strain Ty 901 was used, and for H-agglutination the strains Ty 901 and Ty 2, both from Dr. Felix of the Lister Institute. They were both used alive, and care was taken to have them fully motile. The drop was then covered with a very clean, new, 0.17 mm. coverslip. Vaseline was applied round the edges, leaving open one corner. When such a preparation looked satisfactory under the microscope, a drop of diluted agglutinating serum was placed at the non-vaselined corner. By varying the dilution of the serum, the speed of the reaction could be controlled. For instant action it was found better to place a loopful of bacilli in a drop of agglutinating serum on the slide instead of in broth. The agglutinating serums came from inoculated rabbits, the H-serum being an H- and O-serum from which the O-element had been absorbed, the O-serum having been made by injections of steamed bacilli.

The thickness of the fluid film between slide and coverglass was found to affect the process of agglutination. A thick film, resulting from a large drop of broth, allowed more motility, and the formation of definitely three-dimensional clumps. It necessitated more painstaking adjustments of both dark ground con-

denser and objective, and there was more blurring from the thicker clumps. Thin films did away with these difficulties, but had the disadvantage of providing less natural conditions for the formation of clumps which had to become nearly two-dimensional. The thickness of the film had to be adapted to the feature to be filmed.

It follows that the microcinematographic pictures of agglutination could not be taken in the short space of time and in the same order in which they appear on the projected film. In O-agglutination it was sometimes possible to take a series of shots from one and the same preparation. The more involved happenings in H-agglutination necessitated frequent changes of preparations before an orderly sequence of all agglutination events had been secured. Good sunlight is only available during a limited number of hours, even on the best days. This precluded prolonged continuous observation of any particular preparation, and later stages of a process could sometimes only be observed by preparing slides some hours before they were used.

Editing the films proved unexpectedly difficult. This was not just due to the fact that the final film had to be pieced together from a large number of short bits which had to be picked from the usual 100-foot lengths, and that there was, from the difficult nature of the work, very considerable wastage. Other considerations entered into the problem, and I mention them with some hesitation. My impression of microcinematography is that a sequence of frames, when projected as a cinema film on a screen, can give the observer quite a definite idea of a particular happening, while the individual frames when viewed in the usual film editing apparatus, standing still, seem to be lacking the features the screen had led one to expect. The human eye seems to be capable of linking up a series of pictures, of which many are more or less defective, into a perfect whole, bridging the gaps which become sometimes painfully manifest when individual frames are inspected. These considerations make the selection of suitable shots particularly difficult. Also, the impression which the finished film makes as a whole, cannot be properly judged from the

few individual frames which accompany this paper by way of illustrations. They are enlarged prints of the 16 mm. film, and the total magnification reached is about 600 ×.

PREVIOUS WORK

Typhoid bacilli, according to my previous observations (1930, 1931–32, 1938) propel themselves by means of a long tail. The tail is a long drawn out spiral structure, and is in continuous spiral motion, rotating the bacillus round its long axis, and propelling it at the same time. It is a screw-like motion. Slowing down is accompanied by a widening of the spiral. The spiral structure, as can be seen sometimes when the motion stops,

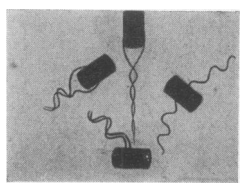

FIG. 1

consists of two flagella, attached to the sides of the bacillus, and twisted round one another at the rear. At rest the "tail" can unwind or swing over to one side. All this is diagrammatically illustrated in figure 1.

Still pictures of different phases of H- and O-agglutination were published on a previous occasion (1938). The present films confirm the observations of that time, and allow the observer to watch the agglutination processes step by step for himself.

THE PRESENT FILMS

(1) *O-agglutination.* The O-agglutination film begins by showing typhoid bacilli swimming about very rapidly in a drop of broth, and showing their tails. It is curious that notwithstanding their high and blind speed they never collide. An acceptable explanation for this would be that they are all negatively charged.

It is then shown how a drop of agglutinating serum is placed at the free corner of the coverslip. This is followed by a shot of the currents set up by the diffusion of the serum into the broth.

After a while more quiet conditions are seen to return. Then the serum takes effect, and a bacillus is seen to make straight for another one, bumps into it, and remains attached (fig. 2). More bacilli follow the example of the first one, and throw themselves headlong at the growing clump (figs. 3 and 4). They always become attached at one of their ends, polar fashion, not side to side. In this way the clump is seen to grow steadily. It sometimes happens that a bacillus swims straight at the clump, pauses there for a moment, even touches it, and then makes off again. Many others steer quite clear of the clump. There is no difference in appearance between those that become attached and those that do not. It requires a certain amount of luck to witness the beginnings of a clump, as most small clumps keep on moving.

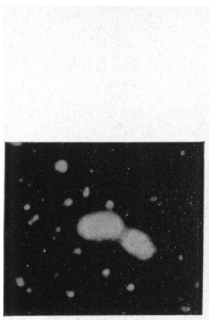

FIG. 2

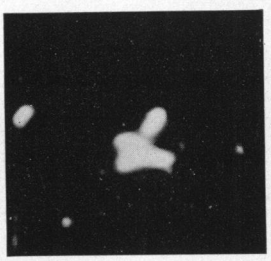

FIG. 3

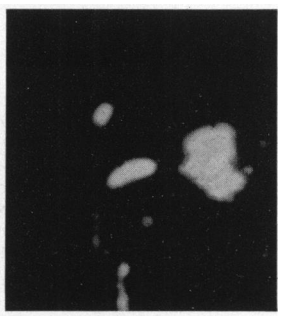

FIG. 4

A series of small clumps, swimming about somewhat erratically, is shown by the following shots. A typical example is figure 5.

The film continues by showing a small clump lying still (fig. 6). This is then seen to grow into figure 7 and finally into figure 8. The process of growing is the same all the time. A fast-swimming bacillus will make straight for the clump, often from quite a distance, and become attached head foremost. Many of these show their tails, but as these were always difficult to focus, and attention was centered on the clump, which had to be kept in focus, no effort was made to bring out the tails. As soon as the clump is reached, the tails crumple up, as shown before (1938).

Occasionally attachment takes place in a somewhat hesitant manner. It also happens that a bacillus after touching a clump,

makes off again and joins a neighboring clump. Very occasionally a bacillus which has apparently become definitely attached to a clump, and has been quiescent for quite a while, is seen to leave the clump and take to a free existence again.

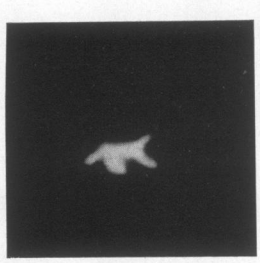

FIG. 5

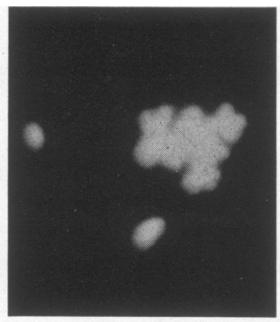

FIG. 6

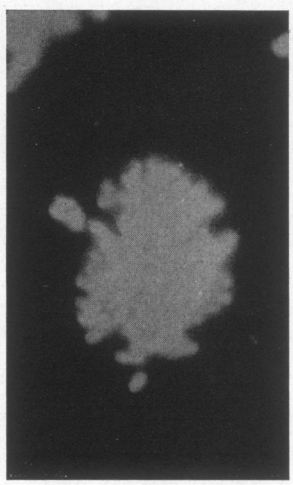

FIG. 7

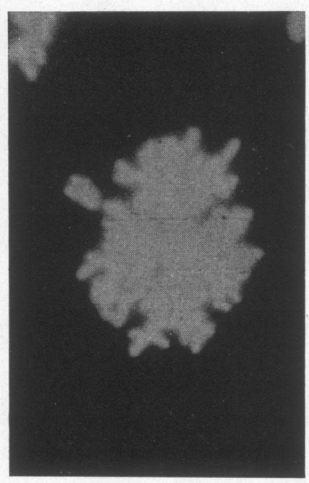

FIG. 8

All these eventualities are illustrated in the film.

By virtue of the way in which the bacilli join up, the architecture of the clumps is simple and orderly. As more and more bacilli join clumps, the field gradually clears, and instead of free bacilli, a number of clumps are left. In a thin film of fluid, which

forces the clumps to be formed in what may be regarded as one plane, their shape strongly suggests crystal formations (figs. 9 and 10). Figure 9 especially resembles frost crystals on windows. In the larger three-dimensional clumps which form the final pictures of the film, this crystal structure is less obvious on account of the thickness of the clumps and the glare produced by so many bacillary bodies all on top of one another. The film makes a rather successful effort to overcome this by presenting such clumps as they appear when subjected to a slow change of

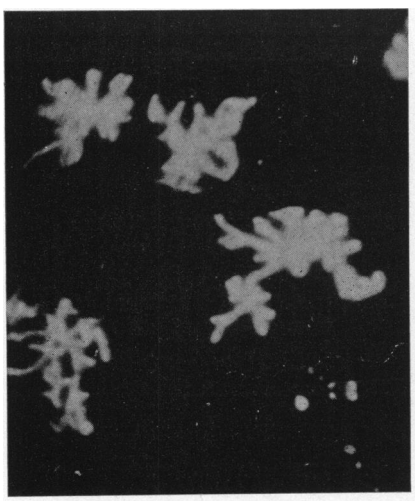

FIG. 9

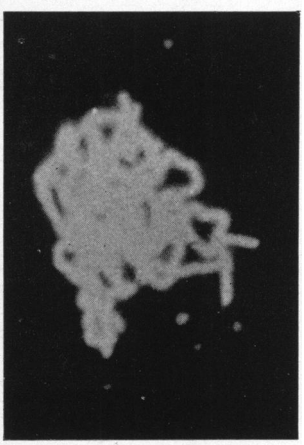

FIG. 10

focus brought about by gently turning the fine adjustment screw (fig. 11).

The whole film leaves no doubt that in O-agglutination the original repulsion which kept the bacilli apart and allowed them to follow courses of their own, disappears. It is seen to be replaced by mutual attraction, which acts in the direction of their long axis. The nature of this change is hidden, but it is tempting to think of changes in electrical charge.

The film is about 375 feet long.

(2) *H-agglutination.* This is a much more involved process, and as the film shows, not really specific agglutination at all.

The film opens in the same way as the O-agglutination film, by showing freely motile bacilli in broth. As however in this case the motile organs are of particular importance, more attention is paid to them. Some bacilli are shown which in slowing down their forward movement, exhibit much broader windings of their tails. Others are shown which have come to rest completely, and exhibit a broadly wound spiral attached to the side of the body. One bacillus was caught in the act of untwisting its tail into the two constituent flagella, one on each side of the body. These motile structures are all very thin, although of course it

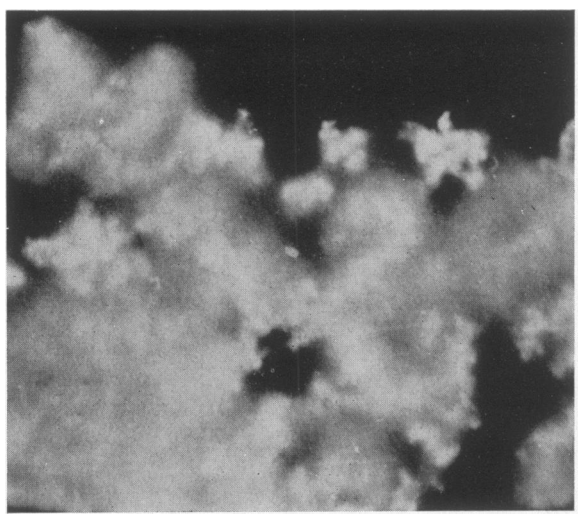

FIG. 11

must be remembered that their apparent thickness is dependent on the brightness of the darkfield at the particular moment.

After thus making the spectator familiar with the normal appearance of the motile organs (one tail or two flagella) (fig. 1) a drop of H-serum is seen to be added to the preparation. Wild currents are caused by its diffusion, as in the O-agglutination film, the bacilli being swept in various directions, some of them swimming against the storm. When calm has been restored, quite a different spectacle follows. Something seems to affect the tails of a whole series of bacilli shown. In these cases, the

tail, instead of pushing the bacillus forward, seems suddenly capable of convulsive spiral movements only, which makes the bacillus turn round in a jerky spasmodic manner. Closer inspection reveals that this sudden change is accompanied by a deposit of small granules on the tail (fig. 12). This is becoming more and more evident as the film proceeds. The tails (and also the bodies, but that seems to be of less practical importance) become covered with smaller and larger granules which come so close together that they surround the tail like a sheath. This makes the tails very much thicker, and impedes movement. In

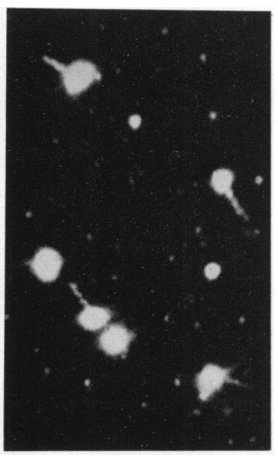

FIG. 12

this way gradually all the tails take on the appearance of thick broad spirals, and they become stiffer and stiffer, and in the end closely resemble corkscrews (figs. 13 and 14). Occasionally it happens that the change overtakes a bacillus which has just untwisted the tail into its two flagella, and such bacilli appear with a "corkscrew" on either side of their body (fig. 15). The granular nature of the thickening is specially demonstrated by a series of pictures, like figure 16. It also happens that the thickening does not extend over the whole of the tail, and this is shown by shots like figure 17.

The thick "corkscrews" have not completely lost the power

of moving in spiral fashion and pushing the bacillus along. But their forces are weakening, and as the process goes on, the field becomes more and more filled with inert bacilli with corkscrews attached. This is the end and the sum total of the specific effect of the H-serum.

It is therefore obvious that the H-serum as such does not produce agglutination. If the field could be kept perfectly quiet,

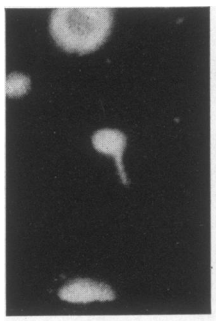

FIG. 13

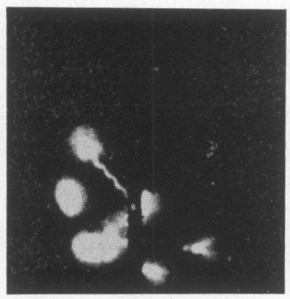

FIG. 14

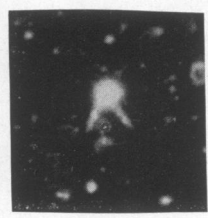

FIG. 15

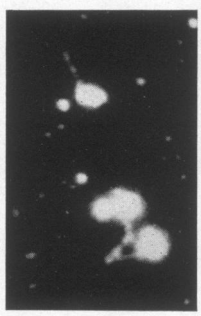

FIG. 16

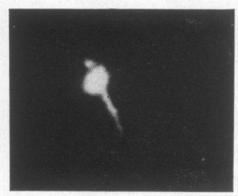

FIG. 17

nothing further would happen, and there would be no agglutination at all. Some movement however is always seen to persist. Part of it is merely Brownian movement. Part of it is caused by differences in temperature at different places, which are enhanced by the heat of the condenser focus. Part of it also is due to some faint motility left in the thickened motile organs.

And so bacilli, all complete with "corkscrews" are seen danc-

ing round one another for long stretches of time without anything further happening. It would seem that the stiff corkscrew-shaped motile organs would provide good opportunities for bacilli becoming entangled with one another, but it is surprising how long they can dance about in close proximity to one another without this happening. It is obvious that, contrary to what takes place in O-agglutination, there is no mutual attraction.

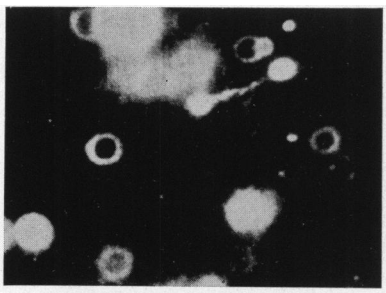

FIG. 18

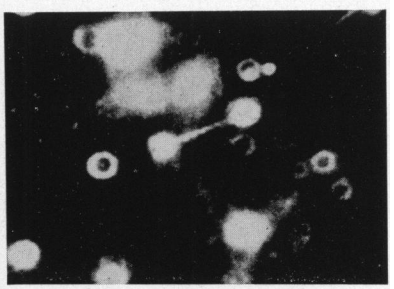

FIG. 19

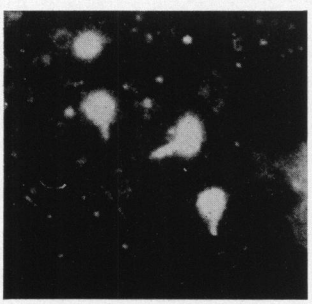

FIG. 20

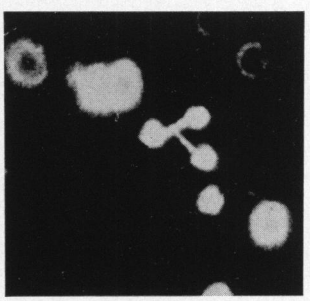

FIG. 21

One has to be extremely lucky to witness the actual entanglement of the corkscrews of two or more bacilli. The film shows several of such lucky shots. Figure 18 shows two bacilli whose corkscrews have very nearly linked up. In figure 19 the linking up has actually taken place. The three bacilli of figure 20 a few seconds later did come together as shown in figure 21. But the film makes it very clear that such linking up is completely fortuitous. It appears from the film that the coming together

of two or more bacilli is purely a matter of chance. The serum has created the possibility for entanglement, but it is very slowly made use of.

Further shots show several small groups, the individual bacilli linked together by means of their thickened tails or flagella, some of them still exhibiting feeble swimming movements. Sometimes one more active bacillus is seen to pull the rest along.

Further agglutination, i.e. the formation of larger clumps, is also shown to be entirely dependent on chance. Slight currents, some of them set up artificially by putting a drop of saline

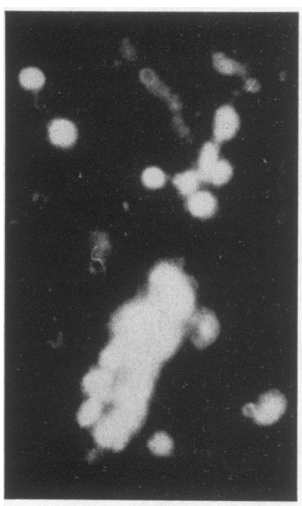

FIG. 22

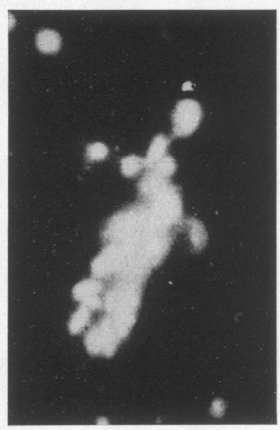

FIG. 23

at the free corner of the coverglass, others arising by themselves, are seen to bring together small clumps. In this way the small group at the top of figure 22 is washed up against the larger group below. In figure 23 they are seen to have come together for good. In this way everywhere groups of bacilli hanging together by means of their thickened motile organs, looking like twisted thick cables, are seen to float around. They attach themselves to groups they happen to meet, either lying still or themselves also in slight motion.

The attachment is seen to be brought about by entanglements of "corkscrews" and bodies. Clumps grow in this way under

one's eye in a purely haphazard manner. There is no design about their structure, in contrast to the O-agglutination film with its orderly architecture of clumps.

Towards the end of the film some rather large readymade clumps lying in one plane (their actual formation would take too long) are shown. They had to be in one plane to show their structure (fig. 24).

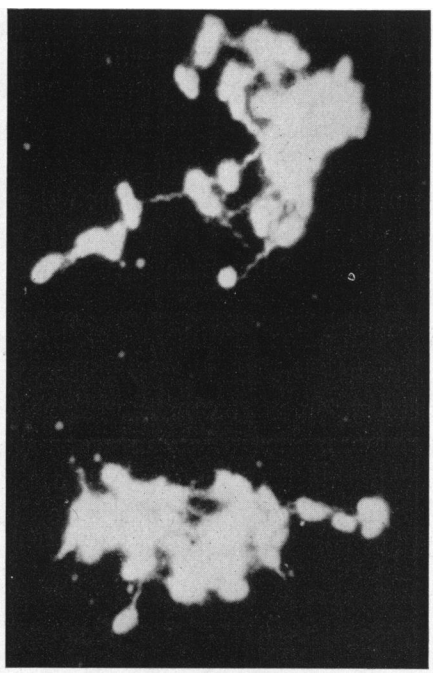

FIG. 24

That the thickening of the tails and flagella in H-agglutination is granular in origin is brought home again by the end of the film which shows the gradual breaking up again of the sheath round tails and flagella into small granules.

The film is 600 feet long.

SUMMARY

A technique is described of making 16 mm. motion pictures of the motility of typhoid bacilli and their agglutination by means

of a sunlight dark-ground method. Sunlight had to be used as being the only source of light powerful enough for the purpose. Even then the magnification had to be kept low, and the exposure time as long as possible. Kodak Super XX film was used, and a Zeiss cardioid dark-ground condenser.

H- and O-agglutination are dealt with in separate films. It is first shown that typhoid bacilli are propelled by a very elongated spiral tail, consisting of two spiral flagella.

Addition of O-serum is seen to result in a direct formation of clumps which is brought about by the bacteria attracting one another instead of avoiding one another as they were seen to do before the serum was added. The force of attraction obviously acts in the direction of the long axis of the bacilli, and this leads to orderly patterns in the clumps, of which the growth can easily be followed in the film.

Addition of H-serum is shown to cover the motile organs with small granules which coalesce and form a sheath. The resulting thickening visibly impedes motility, and changes the originally thin motile organs into thick, stiff, broadly wound spiral structures. This is the specific effect of the H-serum. The film goes on to show how these thick stiff spiral structures may become entangled with one another and so produce agglutination. But it is made abundantly clear that there is no question of any attraction. All linking up is purely fortuitous. Larger clumps are seen to be formed by smaller clumps being washed up against one another as a result of accidental currents or slight motility. From the film there is no doubt that in H-agglutination the specific action ends with the thickening of the motile organs and that the actual agglutination is merely a mechanical after-effect.

Making these films is a continuation of my investigations on the microscopic aspects of bacterial H- and O-agglutination for which I have received a grant from the Research Grant Board of the Union of South Africa.

NOTE

Copies of these two 16 mm. films on typhoid agglutination (O-agglutination, 375 feet, and H-agglutination, 600 feet) can be

had on request at the cost of duplication. Also the 16 mm. film described before (1940), which dealt with microscopic technique and the motility of typhoid bacilli (300 feet).

REFERENCES

PIJPER, A. 1930 Begeiszelung von Typhus- und Proteusbazillen. Zentr. Bakt. Parasitenk., I, Orig., **118**, 113–121.

PIJPER, A. 1931–32 Nochmals über die Begeiszelung von Typhus- und Proteusbazillen. Zentr. Bakt. Parasitenk., I, Orig., **123**, 195–201.

PIJPER, A. 1938 Darkground studies of the flagellar and somatic agglutination of *B. typhosus*. J. Path. Bact., **47**, 1–17.

PIJPER, A. 1940 Microcinematography of the motile organs of the typhoid bacillus. J. Biol. Phot. Assoc., **8**, 158–164.