ELECTRON MICROSCOPICAL OBSERVATIONS ON BACTERIAL CYTOLOGY

II. A STUDY ON FLAGELLATION

by

A. L. HOUWINK AND WOUTERA VAN ITERSON*

Institute for Electron Microscopy, Delft (Netherlands), the R.C.A. Laboratories, Princeton, N.J. (U.S.A.) and Laboratory for Microbiology, Technische Hogeschool, Delft (Netherlands)

I. INTRODUCTION

The present study is the outcome of investigations independently performed by the authors in two different countries.

The work of the first author was made possible by a grant of the "Nederlandse Organisatie voor Zuiver Wetenschappelijk Onderzoek" to the Institute for Electron Microscopy. The author wishes to thank Prof. Dr A. J. KLUYVER for his generous help and advice and Ir J. B. LE POOLE and Ir A. J. A. NIEUWENHUIJS for their assistance in the electron microscopical part of the work.

The second author has had the great privilege to receive a Junior Research Fellowship of the National Institute of Health, Bethesda (U.S.A.), which enabled her later to work in Dr JAMES HILLIER's laboratory at the R.C.A. Research Laboratories Division at Princeton, N.J. A highly appreciated collaboration with Dr JAMES HILLIER, Dr STUART MUDD and Dr CARL ROBINOW is to a large extent responsible for her results. The author is indebted to these colleagues for their constant help and advice and their generous permission to publish many data used in the preparation of this paper. She wishes to express here her sincere gratitude to the National Institute of Health for the awarding of the fellowship and to the R.C.A. Laboratories Division Princeton, N.J. for the hospitality granted.

Electron micrographs were made at Delft with the electron microscope as developed by J. B. LE POOLE, and at Princeton with Dr J. HILLIER's instrument of the R.C.A. type E.M.U.

The motility of those organisms, which we now call bacteria, was discovered almost as early as their very existence by ANTHONY VAN LEEUWENHOEK[1], but it was nearly two centuries later before the actual study of their propelling organs was undertaken.

* National Institute of Health Junior Research Fellow.

References p. 43.

Ever since EHRENBERG[2] observed a "threadlike trunk" (fadenförmiger Rüssel) on *Ophidiomonas* (= *Thiospirillum*) *jenensis* which could serve as an organ of locomotion and which seemed to produce currents in the medium, the problem of bacterial appendages has stirred the minds of bacteriologists. A perusal of literature reveals that opinions have remained equally divided on their significance as "organs of locomotion" (most authors) or "useless appendages" (VAN TIEGHEM[3], DE BARY[4], HUEPPE[5], KURTH[6] and recently PIJPER[7, 8, 9]). Descriptions are given of "filamentous flagella" sometimes occurring in large numbers (most authors), whereas according to other authors never more than a few flagella or tails are present on a single organism (PIETSCHMANN[10, 11], PIJPER[7, 8, 9]).

Neither is there any agreement on such problems as modes of insertion, origin etc. VAN TIEGHEM[3], BÜTSCHLI[12], BABES[13], BUNGE[14], ZETTNOW[15], HINTERBERGER AND REITMAN[16], MIGULA[17], PIJPER[7, 8, 9] and others are of opinion that flagella somehow originate from the superficial layers, whereas on the other hand TRENKMANN[18, 19], FISCHER[20], ELLIS[21], FUHRMANN[22], MEYER[23], REICHERT[24], YAMAMOTO[25], PRENANT[26], LASSEUR AND VERNIER[27] are amongst those who claim a protoplasmic origin.

MUDD, POLEVITSKI AND ANDERSON[28], KNAYSI[29] and VAN ITERSON[30] are inclined to conclude from electron micrographs that flagella are connected with the interior of the cell.

Bacterial flagella were first described by COHN[31], DALLINGER AND DRYSDALE[32], and WARMING[33]. KOCH[34], being the first to tackle the problem by photography and staining, put their actual existence beyond any doubt. Since 1888 development of adequate mordanting and staining techniques by LOEFFLER[35], ZETTNOW[15] and others led to numerous data on the flagellation of bacteria.

Such fixed and stained preparations showed that some bacterial species bear filamentous flagella exclusively at one or both poles, whereas in others they seem to be distributed at random all over the surface. Moreover, it was found that related bacteria are often characterized by the same mode of flagellation, so that this character seemed to be of significance for bacterial classification.

The appellations introduced by MESSEA[36], *viz.*, "*Monotricha*", "*Lophotricha*", "*Amphitricha*" and "*Peritricha*" are still in use.

With a few exceptions, investigation of flagella had, up to the moment that REICHERT[24] applied darkfield microscopy in his investigations, been limited to the study of dead and stained material. The new technique made it possible to obtain an impression of the bacterial appendages in the actually swimming organisms. Also FUHRMANN[22], MEYER[23], NEUMANN[37, 38], NEUMÜLLER[39], LOVELAND[40], WEI[41], PIJPER[7, 8, 9], PIETSCHMANN[10, 11] and KINGMA BOLTJES[42] made contributions to darkfield research. The number of flagella on a living bacterium appeared often considerably reduced as compared to the number of those shown by the stained preparations. To REICHERT it was obvious that during motion *Spirillum* combined its flagella into a bundle (in German: Zopf). NEUMANN came to the same conclusion for peritrichous bacteria. He saw the "flagella" appear and disappear, which meant to him a winding and unwinding of the elementary flagella, since these (except for *Vibrio* and *Spirillum*) are too thin to be individually discerned in the darkfield of the microscope.

Some of these students of flagellation set more value upon the observation of living bacteria in darkfield, than upon the evidence which can be obtained after fixing and

References p. 43.

staining. Differences between the outcome of the two modes of observation are ascribed to artefacts, due to manipulation of specimens (suspending, fixing and drying). PIJPER as well as PIETSCHMANN, both far more radical than NEUMANN, deny the very existence of peritrichy, since they never observed more than one, two or seldom three "tails" or "flagella", all close to the rear of the bacterium. PIETSCHMANN tends to accept only one type of flagellation, *viz.*, subpolar implantation. Seeming exceptions, when more "flagella" in long rods like *Proteus* produce a peritrichous appearance, are explained on the basis that such an individual represents an undivided row of several cells. This is a weak argument, since many rods are equipped with such a large number of flagella that it is hardly feasible to attribute not more than two of them to each individual cell.

PIJPER[9] goes even further than PIETSCHMANN. Unable to observe the attachment of the flagella to the cell he insists that they are not inserted at all. His ideas are best rendered by the following quotation:

"The very viscous anisotropic polysaccharide material of the bacterial slime layer consists of long-chain molecules which readily combine into longer micellae. The gyratory undulating movement of their bodies which propels bacteria subjects this material to a twisting influence. The slimy material hangs loosely round the body and is not firmly attached to the cell wall. The effect of rotation combined with forward movement is that the slime layer is drawn out into the tail described".

In other places PIJPER refers to this tail as a "flagellum", whilst he refuses to apply this term to the "wavy threads" which he sometimes encountered when "tails" disintegrated. With a view to the general use of the word "flagella" for the structures observed in stained preparations this seems unacceptable.

In order to avoid confusion we shall maintain the use of the term "flagellum" for these elementary structures, whilst referring to those seen in the darkfield as "tails".

For PIJPER the "wavy threads" are mere artefacts resulting from death and maltreatment. This conception is not new, since SWELLENGREBEL[43] already ascribed the disintegration of "flagella"—tails according to our nomenclature—into "fibers" to the crude treatment during the staining procedure.

It is clear that PIJPER's views, to which much significance is attached in recent German and American textbooks, cannot be reconciled with the almost general belief that some bacterial species are always cephalotrichous, whereas other species are constantly peritrichous. If flagella are mere artefacts, caused by fixing and drying, it is impossible to explain the reported constantly recurring differences in the mode of flagellation of different species.

In this connection it should be remembered, however, that PIETSCHMANN disputes the correctness of the current interpretation of the microscopical observations on stained preparations, thus indirectly supporting PIJPER's theory.

It will not need elucidation that a decision in this controversy is of the utmost importance, especially with a view to the fact that the mode of flagellation is widely used as a diagnostic character.

If PIJPER's ideas would prove to be true this would obviously cause a revolution in bacterial taxonomy.

This being the state of affairs, the authors, realising the urgent need for new and basic facts, decided to apply the electron microscope in a study of the following problems:

1. Can convincing evidence be obtained for the occurrence amongst bacteria of

References p. 43.

definite types of flagellation, more especially, is there reason to distinguish cephalotrichous and peritrichous bacteria?

2. Can a study of the genesis of flagella provide an answer to the question whether the threadlike flagella have to be considered as separate morphological entities like stained preparations suggest? Or are they merely secondary products of an irreversible untwisting of "tails"?

3. Do the "tails" as observed in the darkfield of the light microscope represent agglomerates of elementary flagella?

4. Is the motility of bacteria indispensable for the generation of flagella?

5. Can anything be established regarding the cytological nature of flagella?

On examining our preparations, filamentous structures in addition to flagella were sometimes seen. These filaments radiate from the bacterial surface. Being clearly distinct from flagella, they will be discussed in the Appendix.

II. PREPARATION TECHNIQUES

The following methods have been used in order to obtain suitable preparations of flagellated bacteria.

1. Agar grown bacteria are carefully suspended in distilled water. Flagella are least injured when a little water is dripped on a well grown young culture in a petri dish. Immediately hereafter a droplet is transferred to a collodion coated object holder by dipping the latter into the suspension. Excess water is drained off with filter paper.

2. A culture of bacteria in a liquid medium is poured on to peptone agar in a petri dish. By turning the plate upside down in slanting position most of the liquid is drained off and only a thin layer of the fluid medium with a suitable number of bacteria remains on the agar.

In some experiments these bacteria were given a subsequent period of growth (method 2a). In other instances, however, growth was stopped with formaldehyde vapour in order to fix the bacteria and their flagella such as they are when grown in a liquid medium (method 2b).

During the drying process all dissolved substances diffuse into the agar, leaving the bacteria as clean as when suspended in distilled water. Drying completed, a 0.5% solution of collodion in amylacetate is poured on to the agar. The plate is turned over and tilted once more. After evaporation of the amylacetate the collodion membrane is cut into small pieces. The dish is then carefully immersed in water. The collodion membrane floats off in little squares on to the surface. Each of these can be mounted as usual, rinsed with distilled water and dried. Many, even sometimes all, bacteria adhere to the collodion. Success depends on the properties of the species and on the moistness of the agar. It is, of course, not necessary to use the whole of the culture in the petri dish to make such a "strip-off" preparation. Sometimes some square cm of agar were cut out and treated in the described way.

3. Some species of bacteria, when cultivated in a liquid medium, tend to attach themselves to solid substances. This has been taken advantage of by offering these bacteria the opportunity to settle on a floating collodion membrane*. One or two days, depending on the behaviour of the species and the concentration of nutrient substances in the medium, suffice to get the desired number of bacteria on the lower side of the membrane. The latter can be torn to pieces, each of which can be mounted as usual, rinsed with distilled water and dried.

4. Though methods 1, 2 and 3 have been used occasionally by the second author too, she mainly used the technique as developed by HILLIER, KNAYSI AND BAKER[47]. Bacteria are grown on top of a collodion membrane spread over nutrient agar in a petri dish. Small drops of a bacterial suspension are pipetted on the membrane. Addition of 0.02% tryptone to the suspension lowers the surface tension and promotes smooth spreading over the collodion membrane. By sucking off, with a small pipette, so much of the suspension is removed as to leave the collodion surface just wetted. To deprive the bacteria from surrounding water the petri dish is left open during the last period of the incubation. Small circular areas of bacterial growth are thus obtained. Once development is considered to be

* This is only CHOLODNY's technique adapted to the demands of the electron microscope. CHOLODNY[44] deposited slides into the soil and found that many bacteria settled on the glass surface. Later on this technique has been used by several investigators *e.g.* by HENRICI[45] for the study of fresh water flora and by ZOBELL AND ALLEN[46] in work on marine bacteria.

References p. 43.

sufficient, parts of the membrane bearing promising areas are selected with the light microscope, cut out with the agar and floated off on water. After mounting on large pieces of copper wire mesh the preparation is examined once more, whereupon the chosen section is cut out so as to suit the object holder.

The method of HILLIER *et al.* has advantage over the stripping-off technique (method 2) since in the former the bacteria are situated on top of the collodion, and thus ought to remain fixed in the original position of development, whereas in the latter displacements may occur during the floating-off on water. Washing, undesirable of course, can be omitted, since on examination in the electron microscope these preparations turn out to be sufficiently clean.

In Delft the preparations were shadowed with an alloy of gold and manganin, whereas chromium was used in Princeton.

III. RESULTS

1. *Is it possible to distinguish definite types in bacterial flagellation?*

To answer this question various bacteria belonging to different species and genera were prepared according to one of the methods described in the preceding chapter. From the numerous electron micrographs made, Fig. 1–7 are considered to portray best the characteristics of flagellation.

Very large numbers of flagella have been found on *Proteus mirabilis* (Fig. 1) and *Pr. vulgaris* (Fig. 2). The former was cultivated in a liquid medium and stripped off from agar according to method 2b. The cell of Fig. 2 is a typical swarmer cell grown on nutrient agar. Before centrifugation in distilled water the culture had been fixed in formalin. This explains why the cell in question has flattened much less than the cells reproduced in the other figures.

Even if—as several authors have suggested—these very long rods should represent undivided chains of several cells, each individual cell must be equiped with a considerable number of flagella. It seems, therefore, to us that already these figures leave scarcely any doubt as to the existence of peritrichous flagellation in the two species.

Both cells bear a number of lateral flagella and are therefore unmistakably peritrichous according to current nomenclature.

Fig. 1, 2 and 3 remind one strongly of the photographs of stained preparations. With these they have in common that the flagella cannot be traced further than the periphery of the cell, leaving undecided which flagella end underneath, and which on the upper side of the cell.

In other more favourable preparations, *e.g.*, that of *Eravinia* spec. reproduced in Fig. 4, the surface structure was well brought out by the shadowing. Since in these cells the surfaces are neither veiled by slime nor in any other way, it is possible to trace several of the flagella up to the points were they emerge from the cells.

An objection often raised by those who defend the occurrence of only a restricted number of flagella at each cell is that by suspending the bacteria in water the "fibrils" might detach from the cells and on drying the preparation secondarily might reattach to the periphery of other cells.

In our opinion the distribution of the flagella, as shown in the micrographs, cannot possibly be explained on such a basis, as these preparations showed hardly any loose flagella. Moreover, the criticism in question can certainly not apply to the stripped-off preparations.

Still more reliable and therefore very convincing results were obtained by the method of HILLIER *et al.* *Proteus vulgaris* (Fig. 5) and *E. coli* (Fig. 6) were cultivated for

References p. 43.

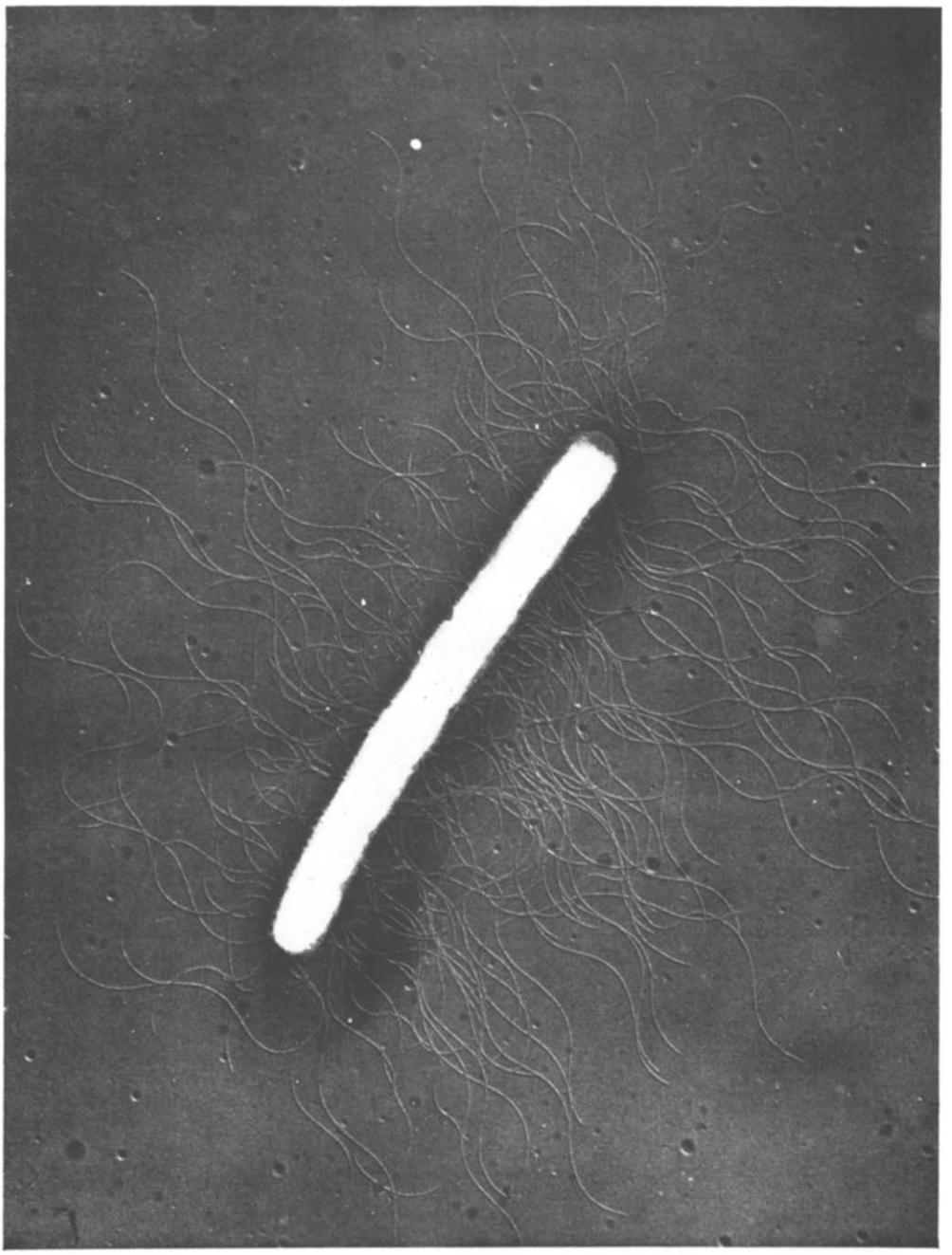

Fig. 1. *Proteus mirabilis*, grown in peptone water, stripped off (method 2b). Cell from a 20 hrs old culture with many peritrichous flagella. (10 000 ×; E.M. Delft)

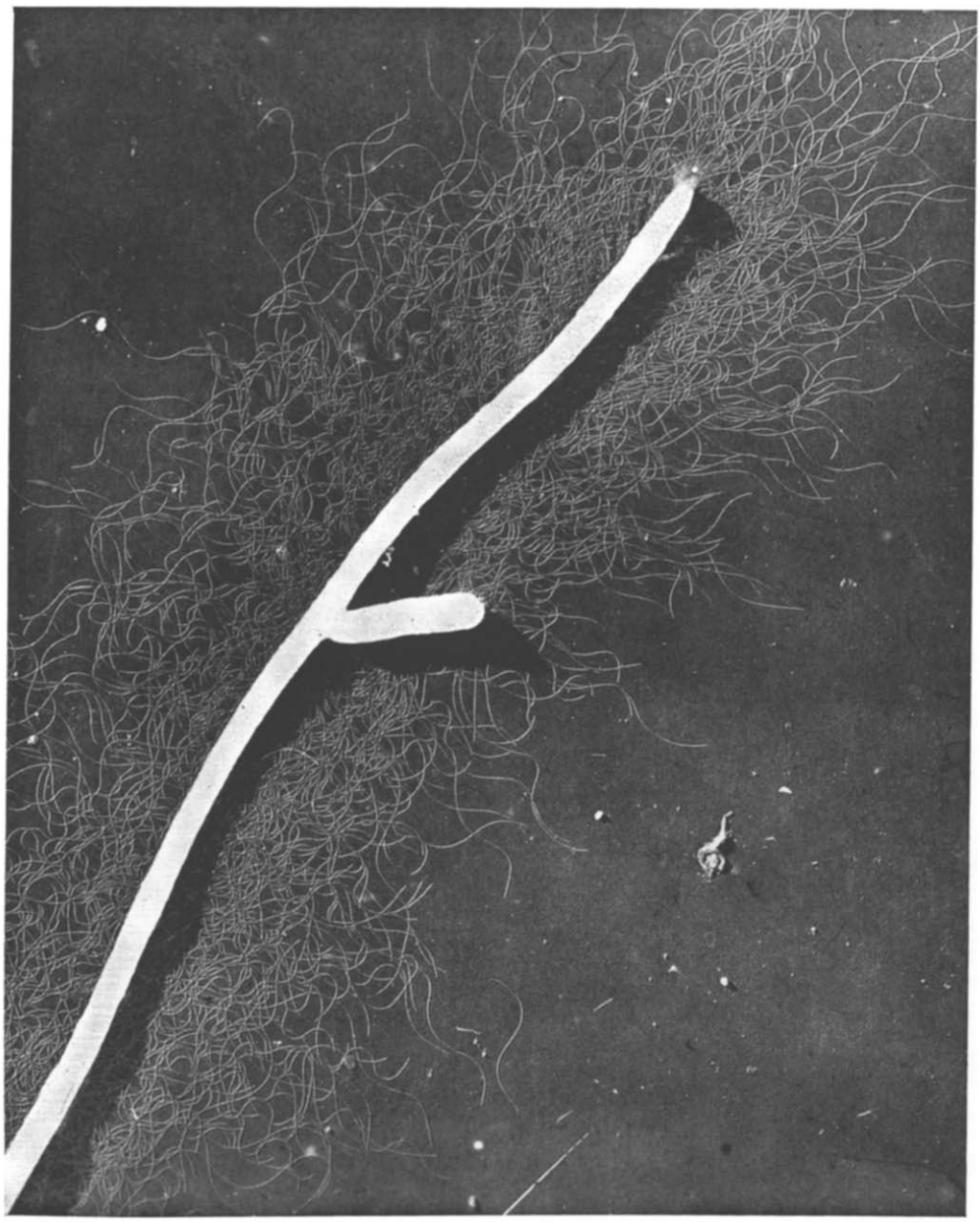

Fig. 2. *Proteus vulgaris*. Preparation Dr C. F. ROBINOW and Dr J. HILLIER. Swarmer cell with an abundance of flagella. Fixed in formalin. (16 000 ×; R.C.A. Lab.)

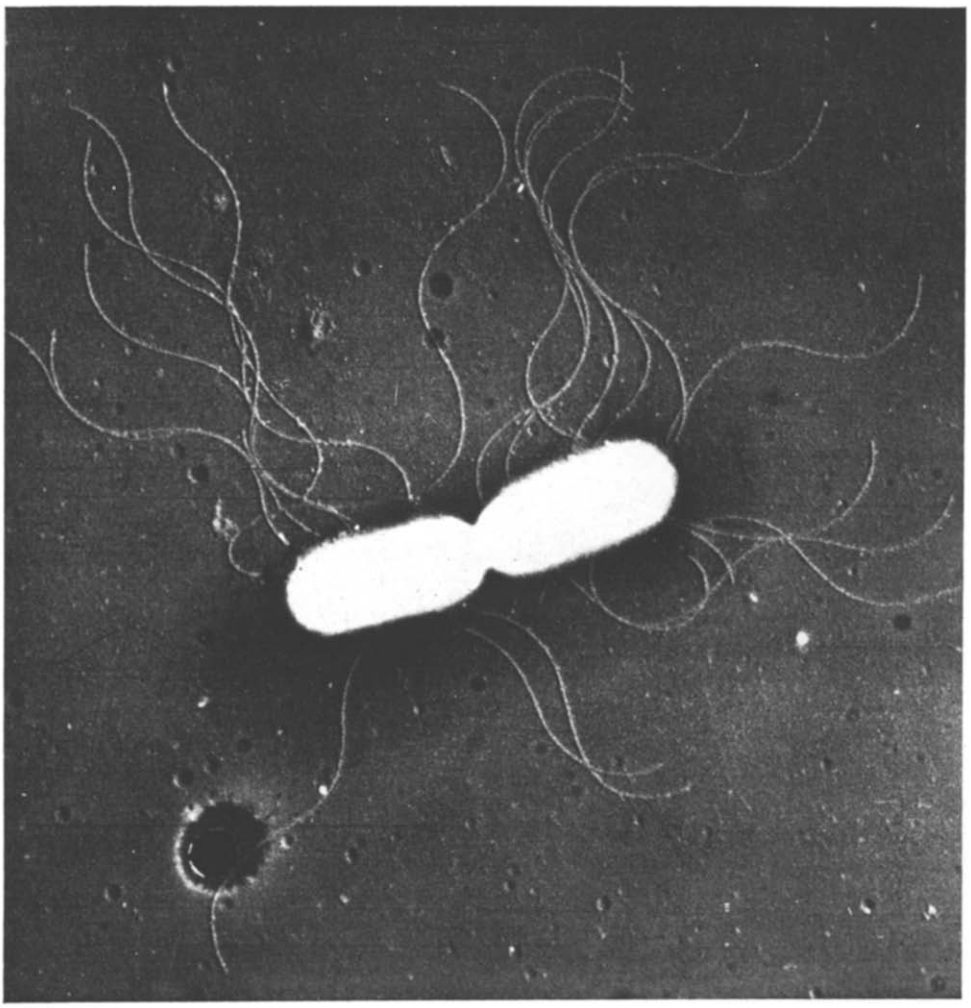

Fig. 3. *Bacterium herbicola*. A normally shaped bacterium with peritrichious flagella. Grown in peptone water and subsequently stripped off from agar. (11 000 ×; E.M. Delft).

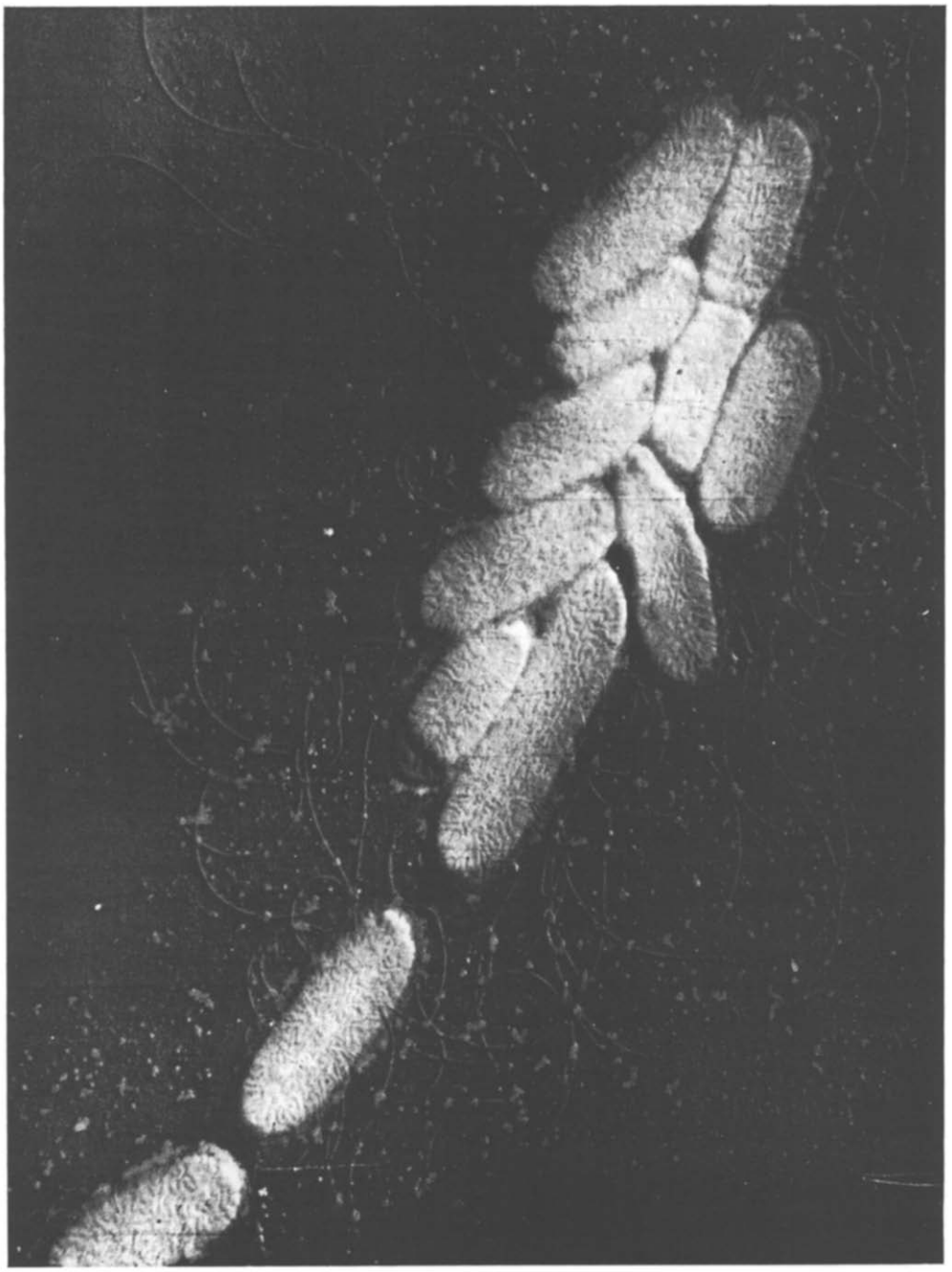

Fig. 4. *Erwinia* spec., grown on agar, suspended in distilled water. The surface structure of the bacteria is probably caused by the shrinkage of the contents during the drying. Several flagella can be traced up to the points where they emerge from the cells (19 000 ×; E.M. Delft).

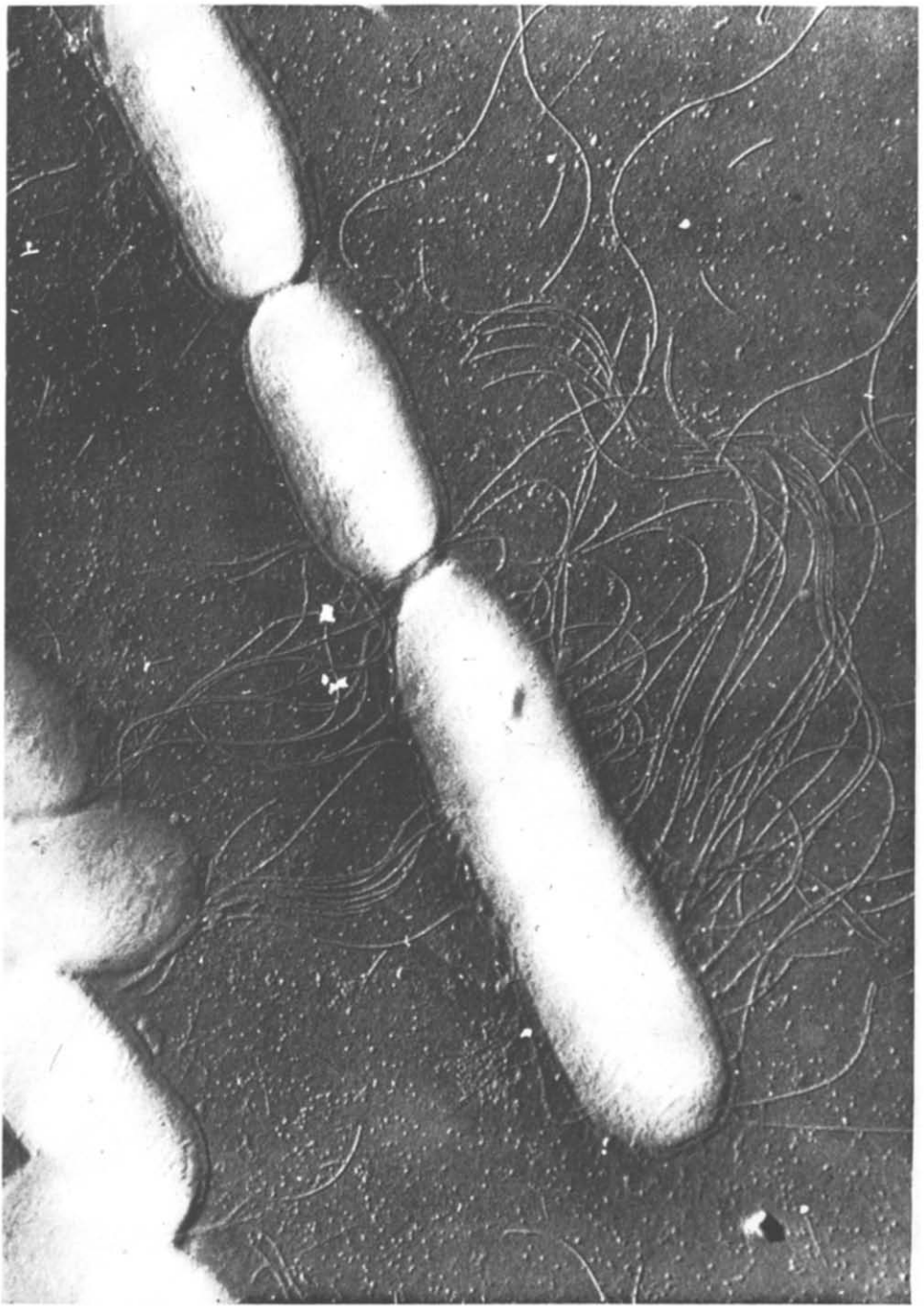

Fig. 5. *Proteus vulgaris*. The cells developed on the collodion membrane which, during the time of incubation, was overlying nutrient agar. The lower cell, which likely never had any opportunity of moving about, still produced peritrichous flagella. (17 500 ×; R.C.A. Lab.)

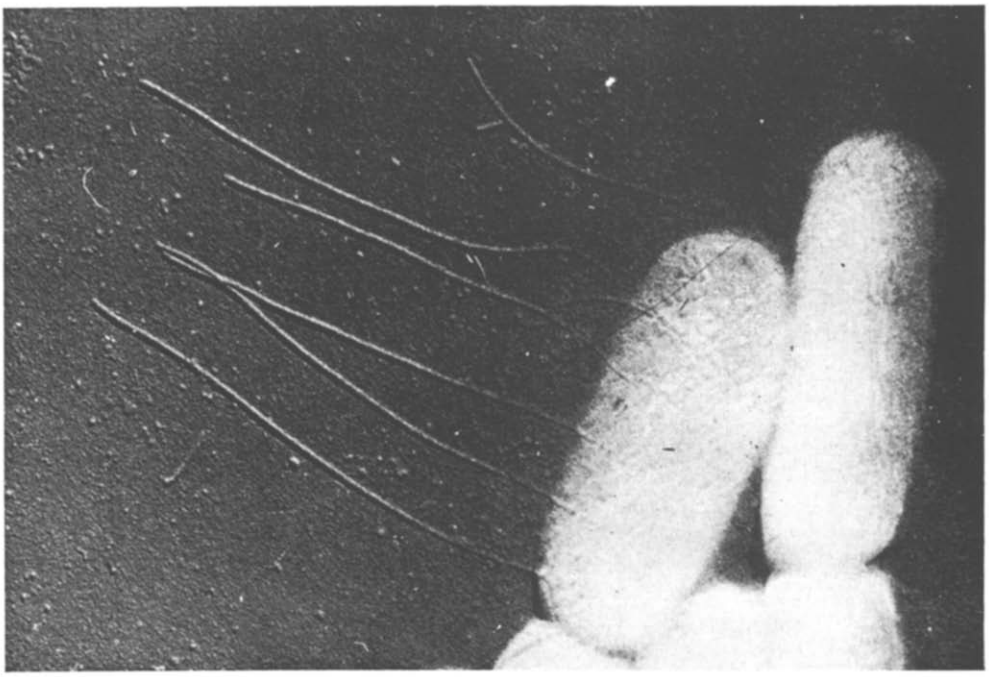

Fig. 6. *Escherichia coli*, 4 hrs'culture on a collodion membrane overlying nutrient agar. Flagella, very straight and short, are probably in the process of out-growth (25 000 ×; R.C.A. Lab.).

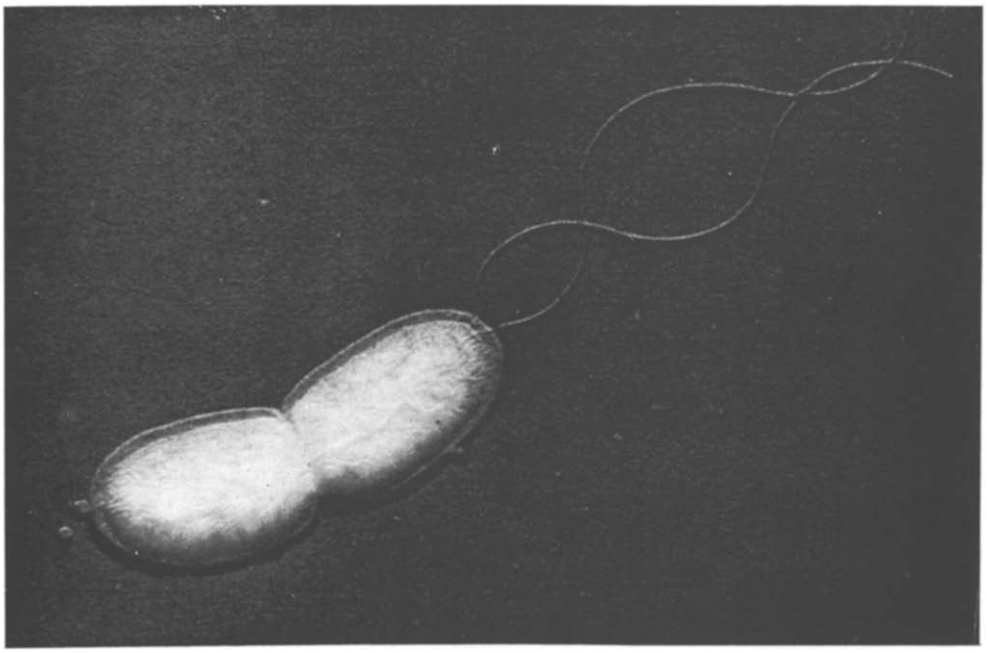

Fig. 7. *Pseudomonas fluorescens*, grown on agar, suspended in water. Example of a cephalotrichous bacterium. (29 000 ×; E.M. Delft).

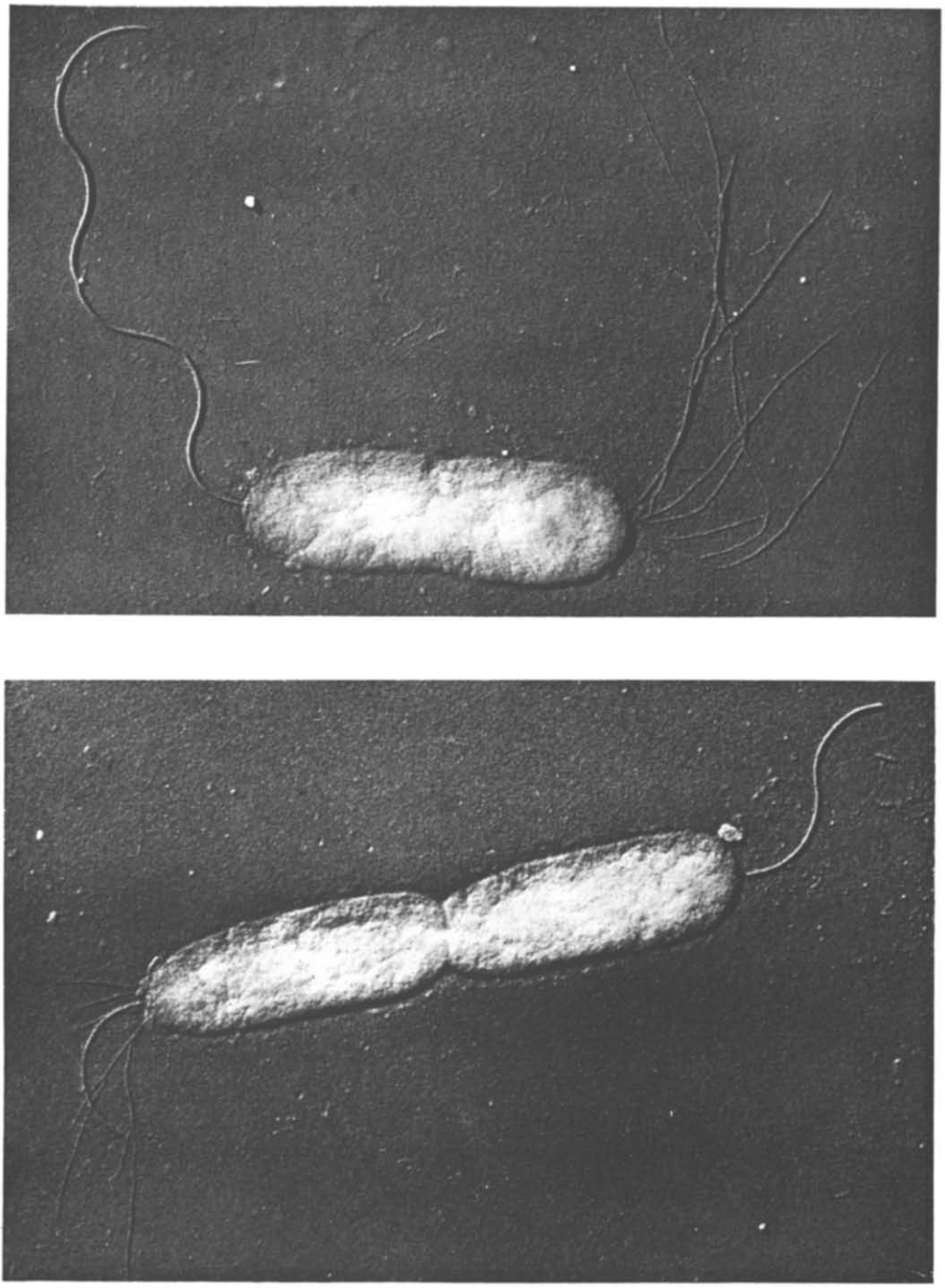

Fig. 8 a. and b. *Pseudomonas pyocyanea*, 4 hrs'culture, stripped off from agar. Note the shortness of the flagella in b. which are probably in the course of development. In both figures "filaments" in addition to flagella are shown, which are discussed in the Appendix. Wide shadows along some filaments in a. may be fissures in the collodion membrane. (20 000 ×; E.M. Delft)

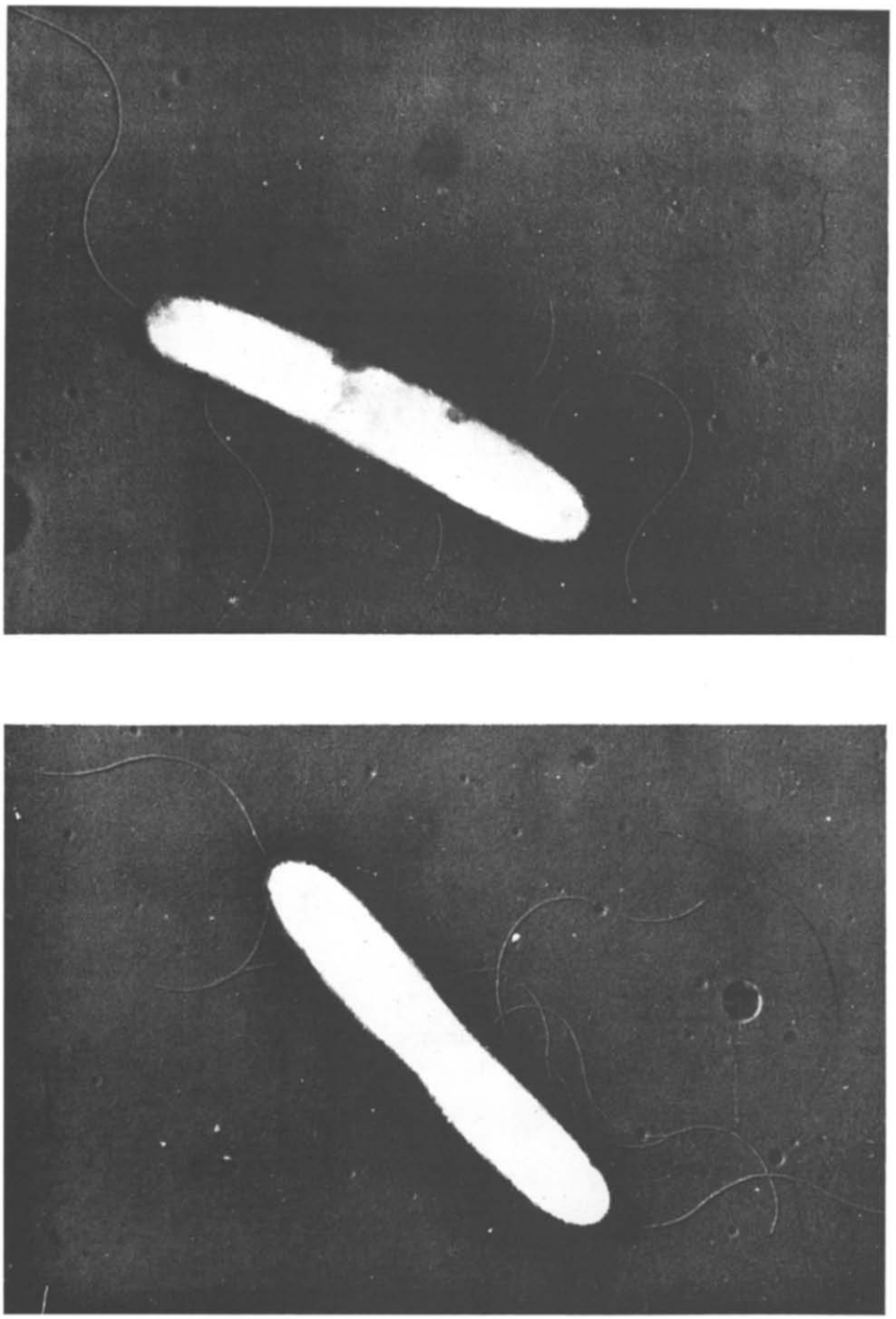

Fig. 9. a. and b. *Bacterium* of Mr E. GRAY, grown in dilute peptone water with 0.1% glucose, stripped off (method 2b). This organism possesses some lateral and one slightly thicker polar flagellum. (12 500 ×; E.M. Delft)

about 4 hours on a collodion membrane with so little moisture at their disposal that for the greater part of the individual cells the conditions for free swimming must have been extremely restricted. We wish especially to draw attention to Fig. 6 where a typical cell clearly shows flagella in development, although this cell is still attached to a natural conglomerate which seems to exclude the possibility of any active swimming. The flagella are arranged in a peritrichous manner. The youth of the cells in Fig. 5 and 6 will be responsible for the small number of flagella present. Actually the cell with the 5 or 6 unusually short flagella in Fig. 6 was one of the very few in this preparation showing flagella by that time. These do not yet exhibit the usual undulation (compare Fig. 20). We, therefore, feel that these juvenile flagella must have been caught in the very act of outgrowth. They obviously emerge from the upper side of the cell. It may be excluded that these cells should have possessed a flagella-bundle, which disintegrated and dispersed during the drying process.

Micrographs of *Salmonella typhosa*, *Alcaligenes faecalis*, *Serratia marcescens*, *Aerobacter levanicum*, *Agrobacterium radiobacter*, *Agrobacterium tumefaciens*, *Bacillus subtilis* and *Bacillus mesentericus* leave no doubt that also these species are peritrichous.

Let us now consider the situation in the case of organisms belonging to the genera: *Spirillum*, *Vibrio* and *Pseudomonas*. Examples of polar flagella-bundles in *Spirillum serpens* and of a single polar flagellum in *Vibrio metschnikovii* have previously been published by one of us ([30] Fig. 10, 11, 9). Micrographs of *Pseudomonas fluorescens* with two elegantly waved, apparently separately inserted, flagella at one pole (Fig. 7) and of *Ps. pyocyanea* (Fig. 8, a and b) with a single flagellum are presented here*. All these, as well as other examined species of *Pseudomonas* can rightly be called cephalotrichous.

As yet we are not certain whether cephalotrichy should be subdivided in mono-, lopho- and amphitrichy, as MESSEA[36] first suggested. In some species, generally accepted to be monotrichous, *e.g. Vibrio metschnikovii*, sometimes two flagella have been found. From irregularly shaped cells in old or abnormal cultures of this species flagella may even be seen to arise from arbitrary spots instead of from the poles.

A probably new type of flagellation was found in an unidentified bacterium sent to Delft by the courtesy of Mr E. GRAY, M.R.C.V.S., Cambridge (England). This organism bears a flagellum at one or at both poles, and remarkably also four lateral flagella (Fig. 9, a and b). The polar flagella, when carefully examined, turn out to be somewhat thicker than the others. Such a type of flagellation—a combination of peritrichy with cephalotrichy—has, as far as we know, never been described before.

2. *The genesis of the elementary flagella.*

The presence of flagella as normal appendages of bacterial cells having been placed beyond dispute, our next task will be to arrive at a conception of the possible relation between these flagella and the "tails" observed on moving organisms.

In this connection we will first direct our attention to the genesis of flagella.

For PIJPER's conception of this genesis, according to which the flagella are merely "products of motility", we refer the reader to the Introduction.

It will suffice to recall that for PIJPER flagella generate from the mucus surrounding the bacterial cell, and in this respect the following observations are of special interest.

* The finer threadlike appendages of *Ps. pyocyanea* will be discussed in the Appendix.

References p. 43.

In Fig. 10 a 3 hours old culture of *Bac. mesentericus* is shown, which was obtained by inoculation of a sporesuspension on a collodion membrane spread over a nutrient agar (method 4). Flagella as described in the preceding paragraph can be clearly distinguished from the simultaneously present slime layer. The flagella with their sharp contours disappear here and there into a diffusely spread mucus. Alongside the cells the slime has accumulated forming little dikes. In the right upper side of the picture they even stretch beyond the ends of the cells, which will be due to contraction of the cells during drying. It seems extremely unlikely that the flagella in this picture did originate by a twisting of the slime layer.

A positive contribution to our insight into the genesis of threadlike flagella can be derived from Fig. 6 (*E. coli*) which shows the presence of juvenile flagella. Here all evidence is in favour of an individual outgrowth of the flagella.

Another case which proves that in young actively growing cultures many cells are characterized by the scantiness and shortness of their flagella is reproduced in Fig. 11, which should be compared with Fig. 12 and 3. *Aerobacter herbicola* was inoculated on peptone agar (method 2). At the time of inoculation the bacteria, which had been grown in peptone water, had many long flagella (Fig. 3). After 3 hours only few are present, unequal in length and partly very short (Fig. 11). Apparently in these fast growing and multiplying cells most of the flagella are only in the beginning of their development. In an analogous 11 hours old culture cells were found to possess much longer flagella, though still unequal in length (Fig. 12).

Fig. 8a and 8b offer another example of developing flagella, this time in cephalotrichous *Pseudomonas pyocyanea* from a 4 hrs old culture on agar. One should compare the beautifully waved flagellum in Fig. 8a with the short organs in Fig. 8b. The lower flagellum in Fig. 8b has not yet quite reached a full wave-length, whereas the upper one has not even half that length.

Observations on *Proteus vulgaris* and *E. coli* grown on a collodion membrane showed after inoculation first an increase of cell size and a rapid division, whilst only after about four hours the first newly flagellated cells could be noticed with certainty.

3. *On the formation of flagella bundles.*

The evidence in the preceding section is against the idea that flagella are secondary splitting products of a primarily developed tail. On the other hand in several of our preparations we came across structures which strongly suggest to represent bundles of flagella. A few electron micrographs will be given which in our opinion show that flagella possess a tendency to unite into bundles.

A striking feature of Fig. 13, representing a 3 hours old *Bac. mesentericus* grown from spores on a collodion membrane, is the presence of a few bundles of tightly arranged fibers besides the thin peritrichous flagella. Like Fig. 10 this picture is veiled by mucus. The short bundle to the left may well be in the beginning of its outgrowth.

Another phenomenon with which one is often faced when *Proteus vulgaris* develops in row-like cell complexes on a collodion surface is demonstrated in Fig. 14. Numerous flagella are arranged side by side, often so close together that the individual entities are not well resolved by the electron microscope. Such bundles are usually seen to emerge from the narrow corners between neighbouring cells. They are probably forced to do so for want of moisture and for lack of space. It is usually not clear from which cell or cells they originate.

References p. 43.

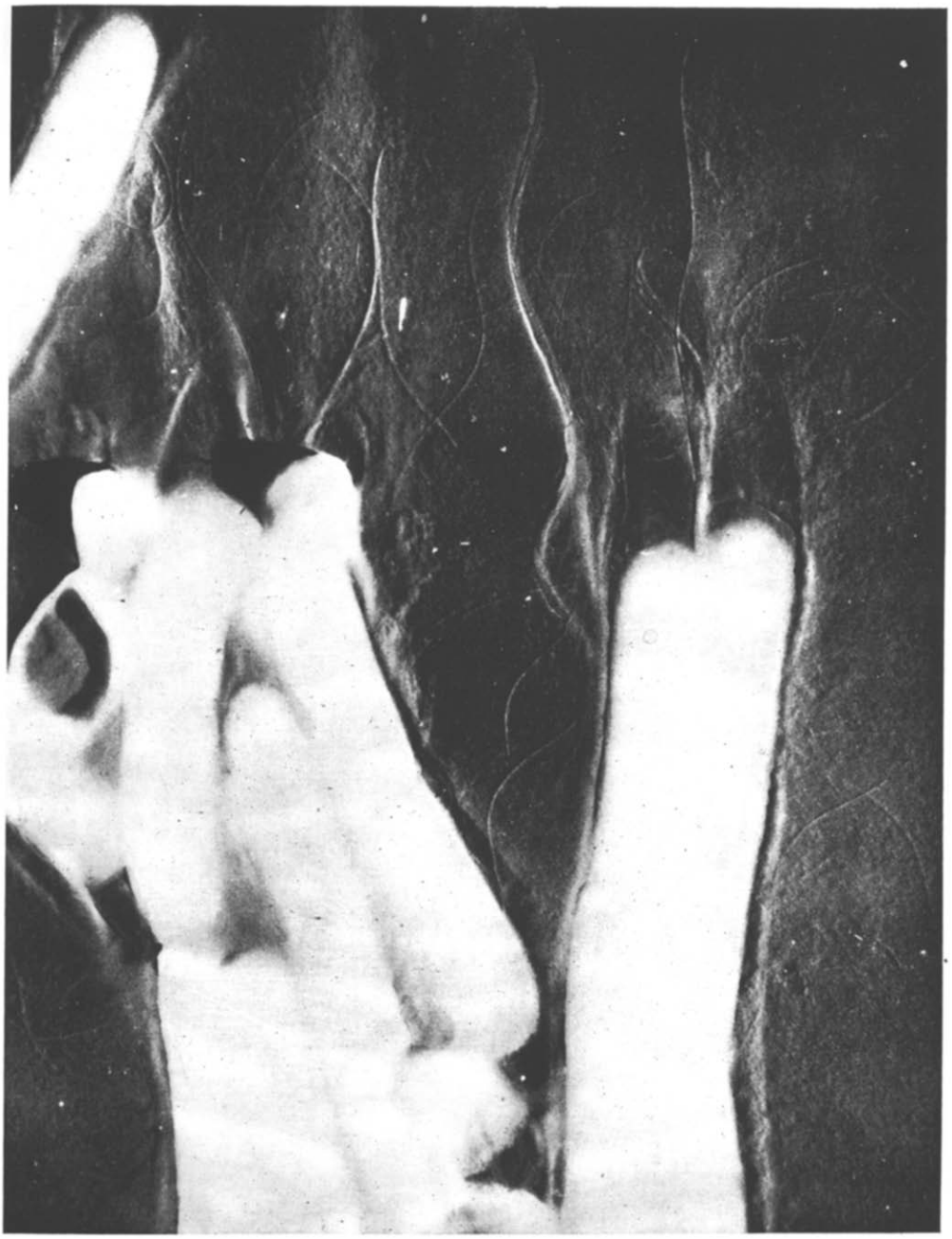

Fig. 10. *Bacillus mesentericus*, germinated from spores on the collodion membrane. The micrograph shows the difference between the slime which accumulated alongside the cells, and the flagella. (16 000 ×; R.C.A. Lab.)

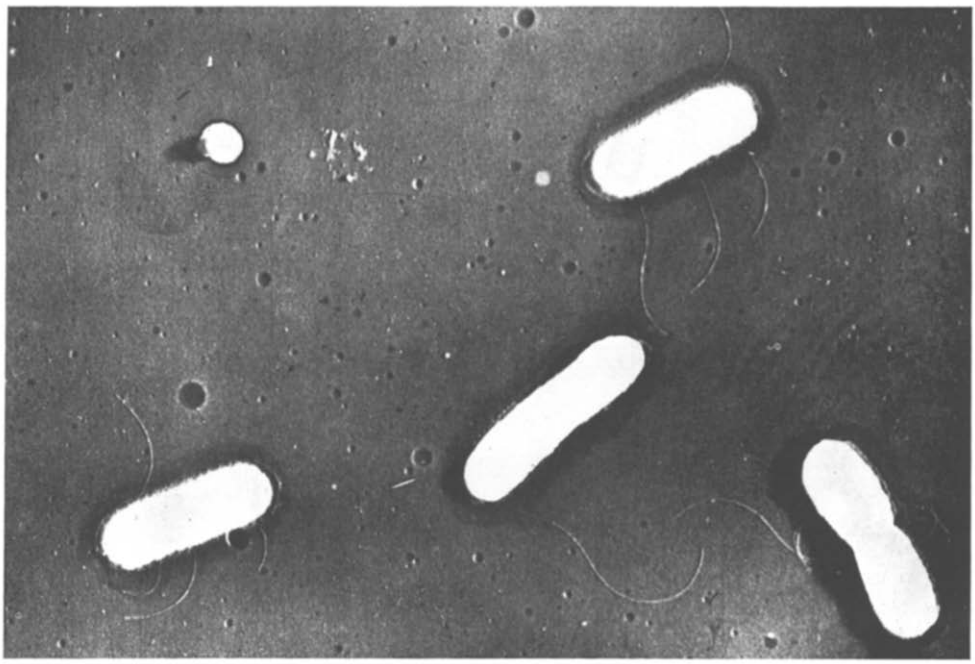

Fig. 11. *Bacterium herbicola*. In this 3 hrs old culture on agar flagella are few and very short. (8000 ×; E.M. Delft)

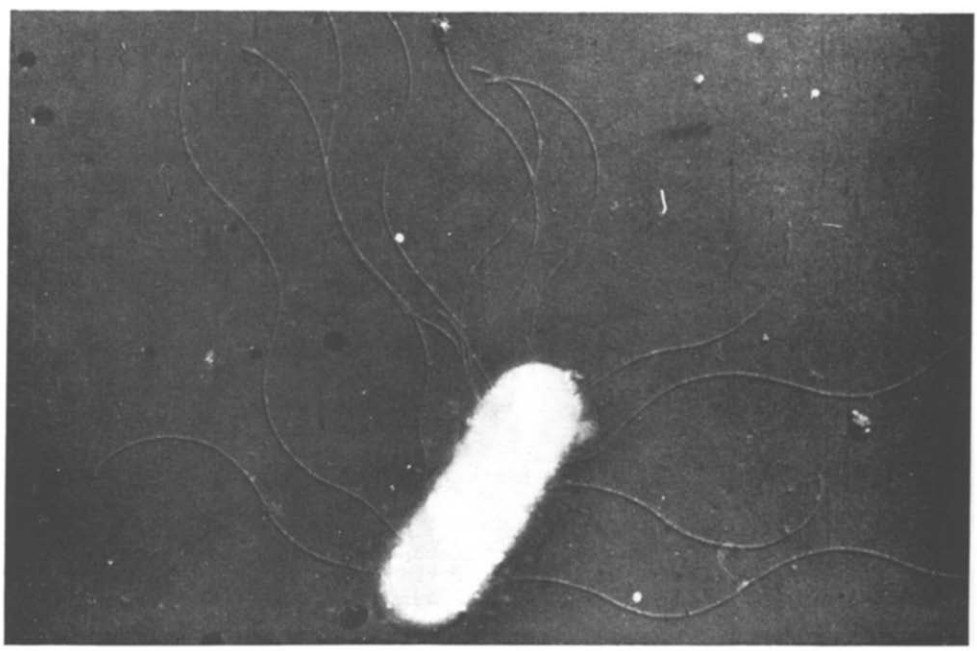

Fig. 12. *Bacterium herbicola*. After 11 hrs incubation the flagella are longer but still differ in length (14 000 ×; E.M. Delft)

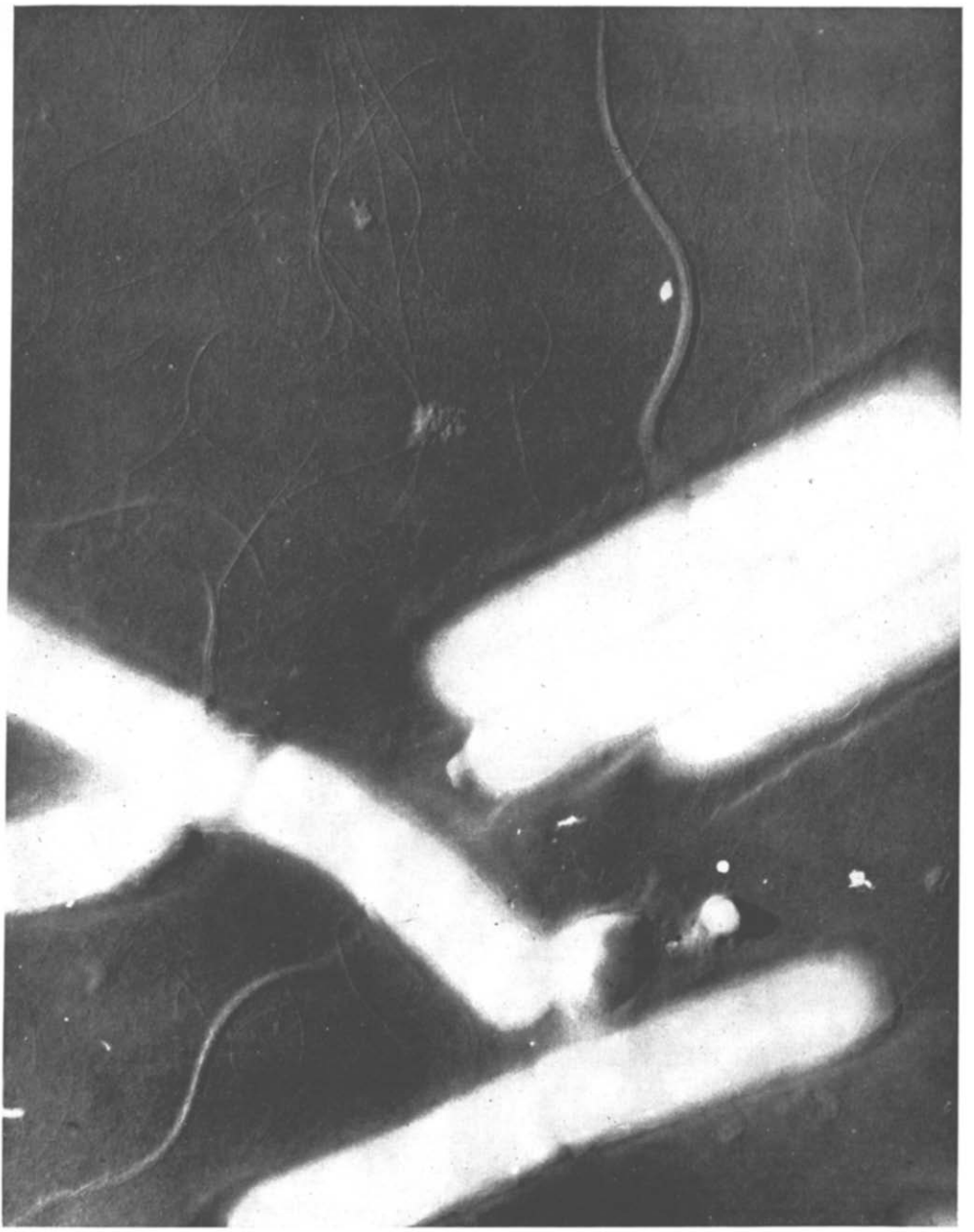

Fig. 13. *Bacillus mesentericus*, germinated from spores on the collodion membrane. Note remarkable structures which apparently are bundles of flagella. (16 000 ×; R.C.A. Lab.)

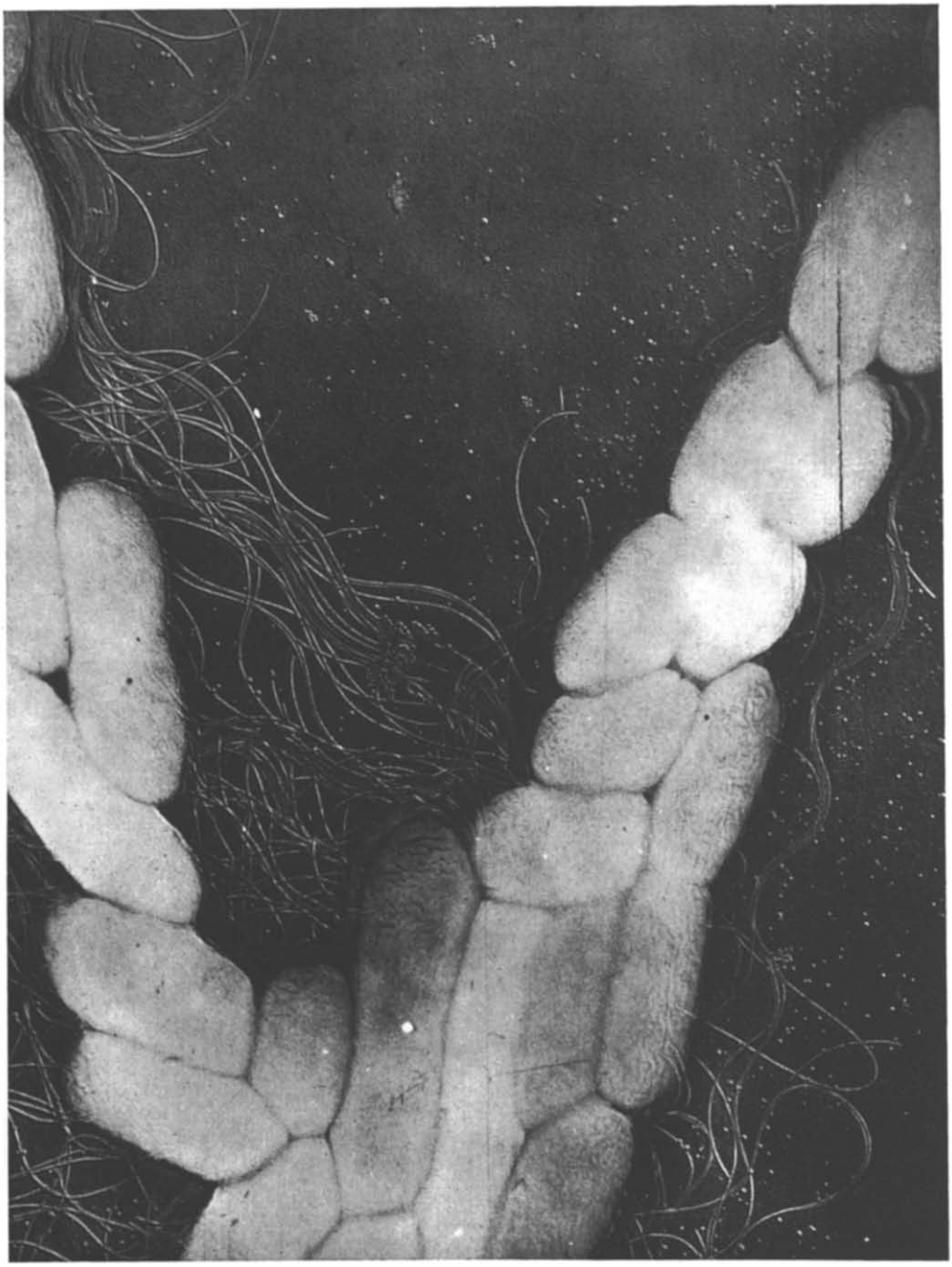

Fig. 14. *Proteus vulgaris*, grown on the collodion membrane. Many of the flagella are so closely packed as to be no more distinguishable as individual entities (16 000 ×; R.C.A. Lab.)

In the foregoing cases the possibility that these flagella aggregations owe their origin to a "twisting" during the act of swimming can be practically excluded.

Another example of the remarkable tendency of flagella to aggregate is the huge flagella bundle represented in Fig. 15 which even in the dead state is relatively stable. We came across it in an overnight culture of *E. coli*, fixed with 5% formalin. Such structures have been described in German literature as "Riesenzöpfe". The most current explanation is that for some reason flagella bundles of individual living bacteria become entangled and are torn off. As can be deduced from the micrograph by comparing the size of the bundle with that of the adjacent bacteria, such conglomerates can assume really enormous dimensions.

Yet our micrograph cannot be well reconciled with the idea that only an entanglement of elementary flagella is responsible for the origin of the "Zopf". If this would hold, one could not expect the orderly arrangement of the elementary filaments shown in the picture.

For an explanation of the phenomenon the fact that in our best flagella-pictures faint indications are to be found for a cross-structure in these organs may possibly be of significance. We shall comment upon this point in the Discussion. The examples of bundle formation discussed in this section seem to us instructive in so far as they point to an affinity between the individual flagella.

4. *Flagella formed by a sessile bacterium.*

In the preceding sections we have repeatedly stressed that flagella cannot be considered to be "products of motility".

In our opinion the evidence which has been supplied by our observations on the genesis of flagella (*vide* Fig. 5 and 6) is sufficient to justify our opinion. We can add one more argument to strengthen our conclusion.

A species of *Caulobacter**, recently isolated at Delft by one of the authors, is represented in Fig. 16. This bacterium, which has a strong tendency to attach itself to solid structures and then develops a stalk, multiplies by ordinary fission. After this, one cell stays with the stalk, whereas the other becomes a free *Vibrio*-like bacterium. Later on this cell also attaches itself to some surface, growing a new stalk at the flagellated side. At first the free end of the cell is without a flagellum. Only shortly before fission a polar flagellum grows out, which apparently will serve the new cell as an organ of locomotion.

In our trend of thought the important fact is, that the polar flagellum grows out during the non-motile, stalked stage, shortly before cell division.

5. *The place of origin of the flagella.*

In section 2 we concluded that flagella can be distinguished from external mucus (Fig. 10). We also stressed that since they develop on non-motile bacteria, they cannot be mere twisted slime twirls. On the contrary we observed flagella which seemed to disappear into the cell body (Fig. 4).

In a preceding paper[30] a connection has been established between the flagellum of *Vibrio metschnikovii* ([30] Fig. 9) and its cell content, the base of the flagellum sometimes

* This organism may be identical with, or at least closely related to the bacterium which Miss JONES[48] isolated from water at Chicago, and to *Bacterium flagellatum*, described by OMELIANSKI[49]. Besides the presence of stalks, a tendency to form rosettes is a common characteristic. The name *Caulobacter* for the genus has been proposed by HENRICI and JOHNSON[50].

References p. 43.

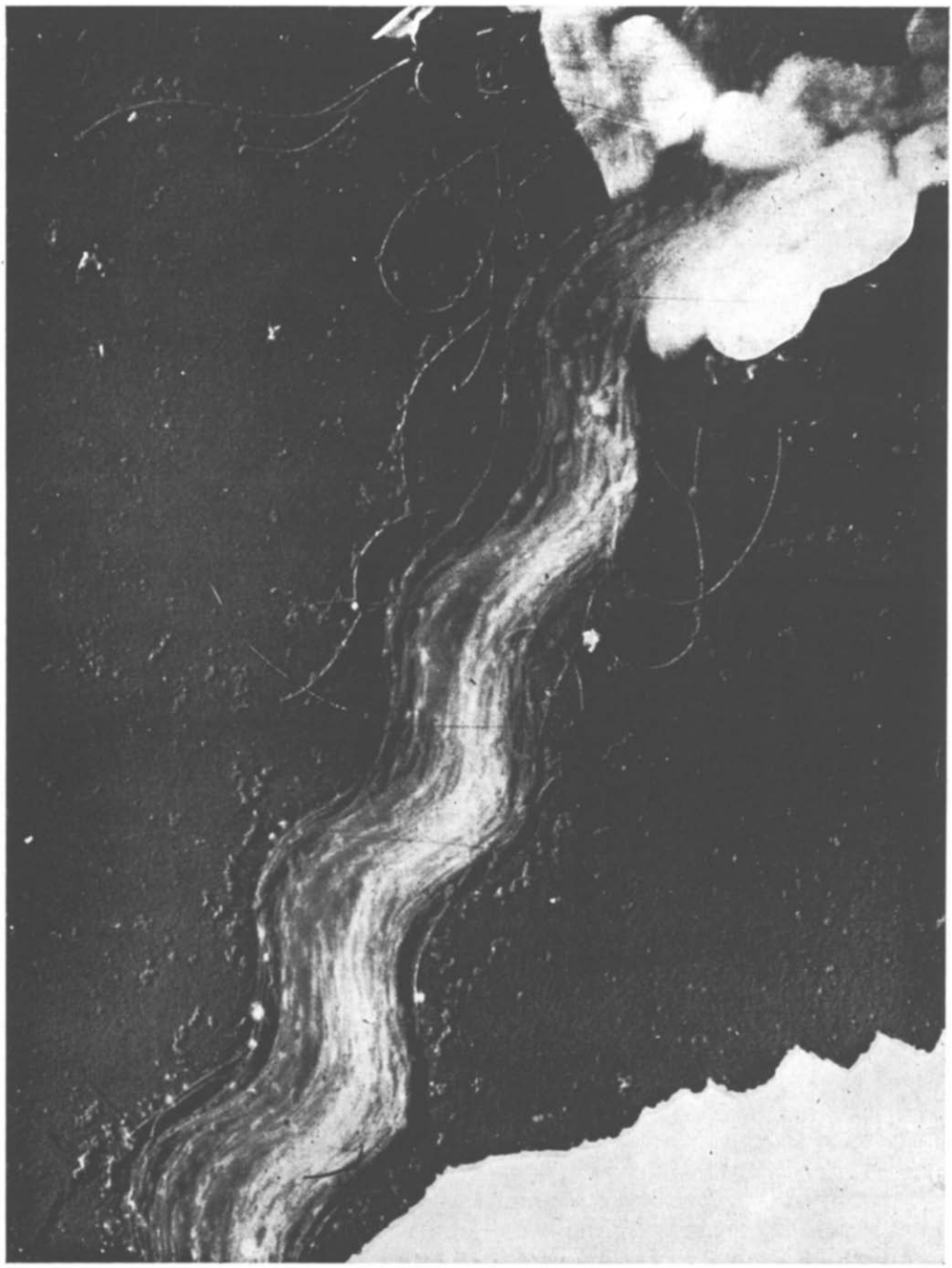

Fig. 15. *Escherichia coli*. Huge agglomeration of flagella encountered in a 17 hrs' culture on agar fixed in formalin. The flagella of this "Riesenzopf" are not twisted together but show parallel arrangement. (16 000 ×; R.C.A. Lab.)

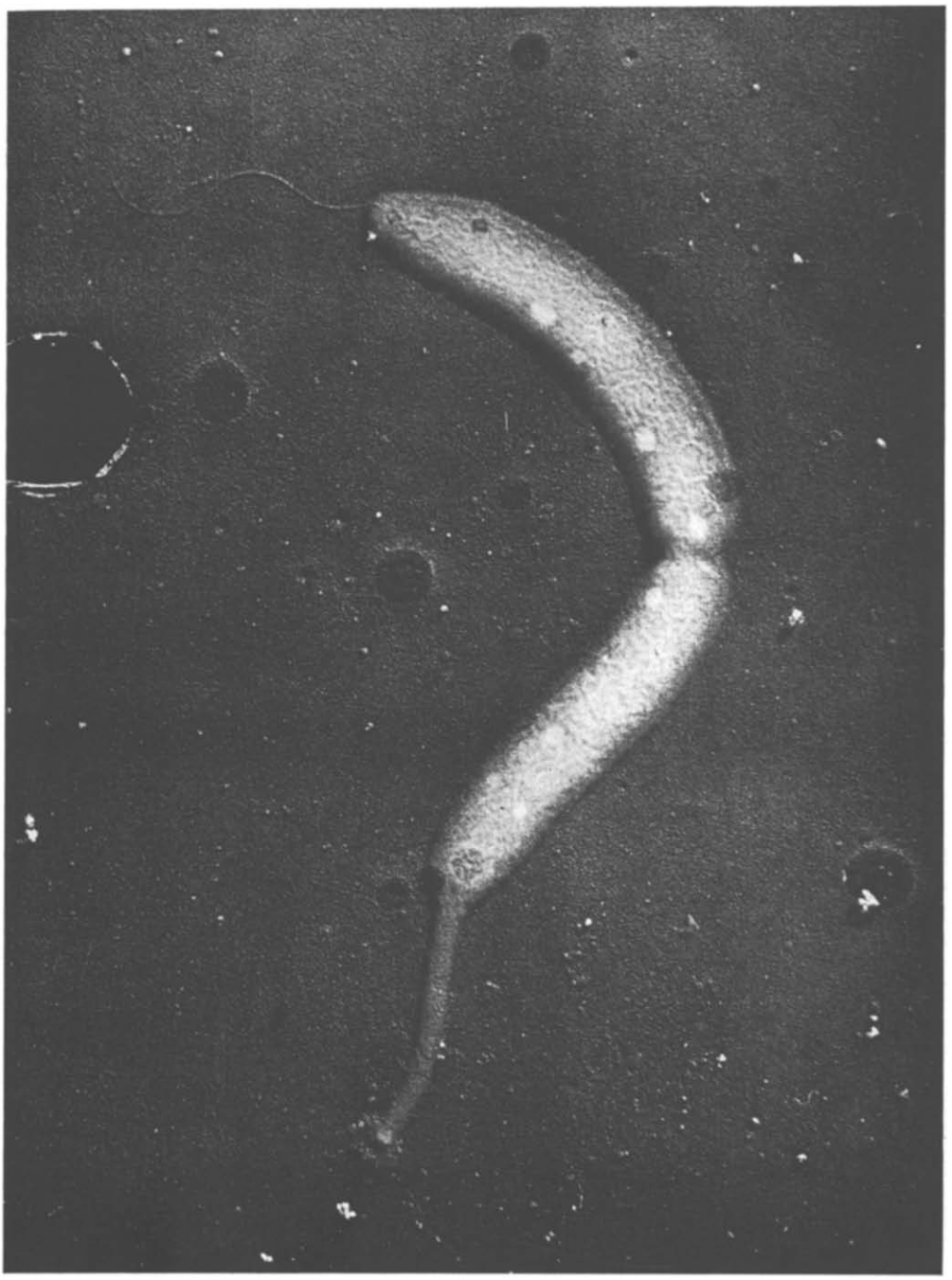

Fig. 16. *Caulobacter* spec. This organism has attached itself to a collodion membrane floating on dilute peptone water and has developed a stalk. It is about to divide and is now forming a flagellum at the upper end. (23 000 ×; E.M. Delft)

appearing as a protrusion of the protoplasm. Also in *Spirillum serpens* ([30] Fig. 11) flagella seem to be connected to the protoplast by small rhizoid extensions. Though in the latter picture it is not clear where exactly the flagella pierce the cell wall, the important fact is that the existence of the connection in question can hardly be denied.

Interesting evidence in favour of the conception of the basal part of flagella being situated inside the cell wall is supplied by Fig. 17. This represents a preparation taken from the upper layer of a liquid medium culture of *Agrobacterium radiobacter*. Cast off flagella were abundantly present. The striking feature is the presence of a little hook at one end of several flagella. Since we know from other micrographs of the same species that such hooks never occur at the free end of a flagellum, we feel justified to assume that each hook is the basal end, and that it is normally situated inside the bacterial body.

In order to examine the basal parts of the flagella unhindered by the presence of surrounding protoplasm, Dr CARL ROBINOW devised a special technique. A 6 hours old culture of *Proteus vulgaris* swarming on nutrient agar was stored for 36 hours at 5° C. After 5% formalin fixation and repeated washing by centrifugation, the cells were mounted in distilled water. The preparation now showed a number of very transparent autolysed cells. Fig. 18 and 19 are most elucidating in so far as they clearly demonstrate that the peritrichous flagella rise from little spheres within the cells. These spheres are of a remarkable constant diameter, being very close to 100 mμ. In the upper part of the cell of Fig. 18 nothing of the original cell contents is left, except for these particles. In the cell in Fig. 19 the remains of the protoplasm still extend over the whole length of the cell, but are bordered by a definite line parallel to the cell wall. Some authors are inclined to call this border the cytoplasmic membrane. It is, however, perhaps premature to attribute definite membrane properties to the boundary layer of the protoplasm.

In the picture some particles near the edge of the cell lie between the protoplasmic borderline and the cell wall, so that one could conclude that these spherical bodies are situated outside the protoplasm in a living bacterium as well. To us it seems much more probable that in this case the spheres, being the basal granules of the flagella, have been torn off from the shrinking protoplasm. In the live bacterium these organs may well be imbedded in the protoplasm.

DISCUSSION

As set forth in the "Introduction" the aim of this study has been to elucidate some problems connected with bacterial flagellation which have been discussed for a century. There is as much difference of opinion concerning this subject now as ever was. In the last decade it has even been doubted whether the threadlike structures which are seen in stained preparations may righteously be called organs of the bacterial cell. PIJPER especially maintains against current opinion that these threads are artefacts.

Our results are altogether incompatible with PIETSCHMANN and PIJPER's conceptions. Our micrographs clearly disprove the former's thesis that all flagellation is of a "subpolar" nature. As for PIJPER's views our results have shown that flagella cannot be identified with slime. Dried slime and flagella could clearly be distinguished, slime being amorphous and flagella having a definite linear structure. (Fig. 10) The micrographs also prove that motility is not indispensable for the formation of flagella (Fig. 5, 6, 16). Arguments against PIJPER's product-of-motility theory have also been forwarded by KINGMA BOLTJES[42], ØRSKOV[51], KAUFMANN[52] and others, who studied living bacteria.

References p. 43.

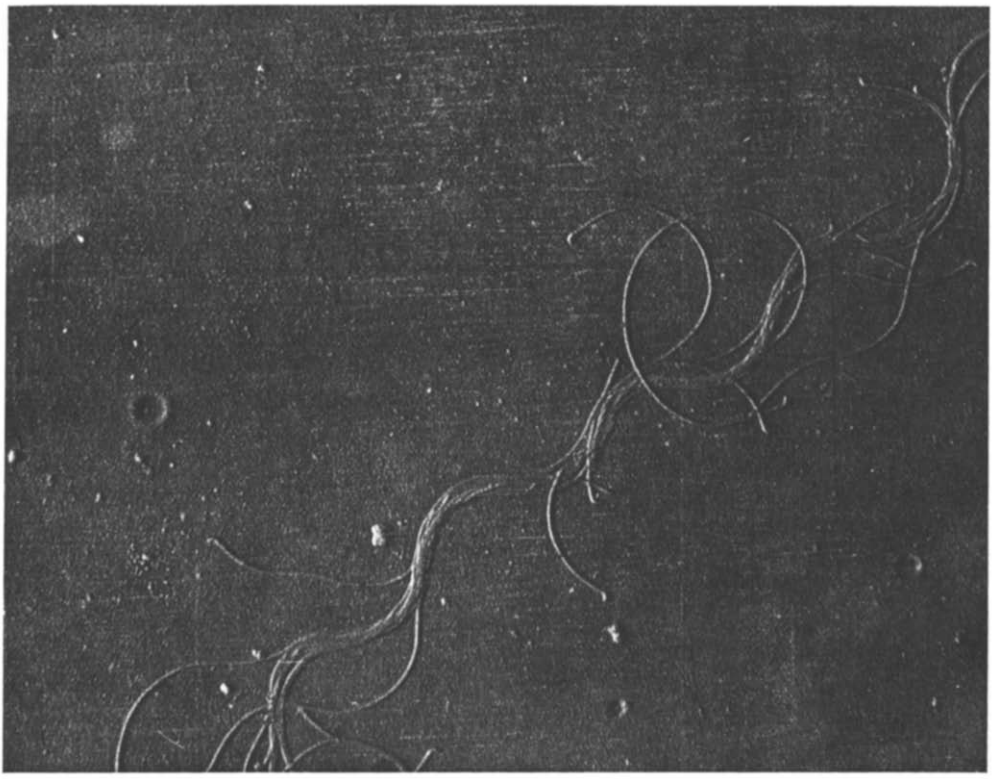

Fig. 17. *Agrobacterium radiobacter*. Detached flagella which were caught floating on the surface of a liquid medium. The little hooks at one end have never been observed on inserted flagella, and are therefore probably normally situated inside the cells. (20 000 ×; E.M. Delft)

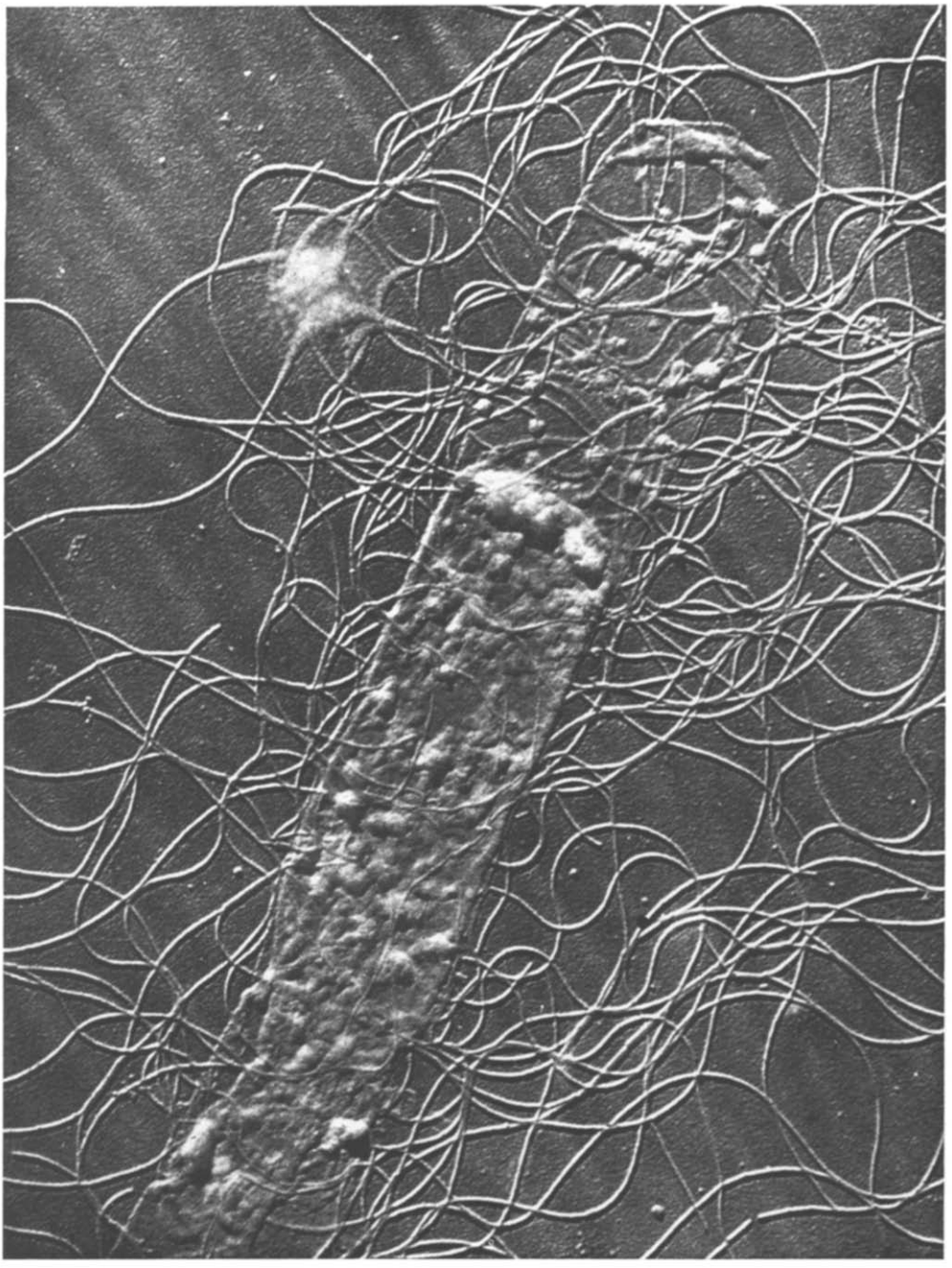

Fig. 18. *Proteus vulgaris*. Preparation Dr C. F. ROBINOW. In the upper part of the autolysed swarmer cell nothing of the protoplast is left except for the granules at the base of the flagella. (25 000 ×; R.C.A. Lab.)

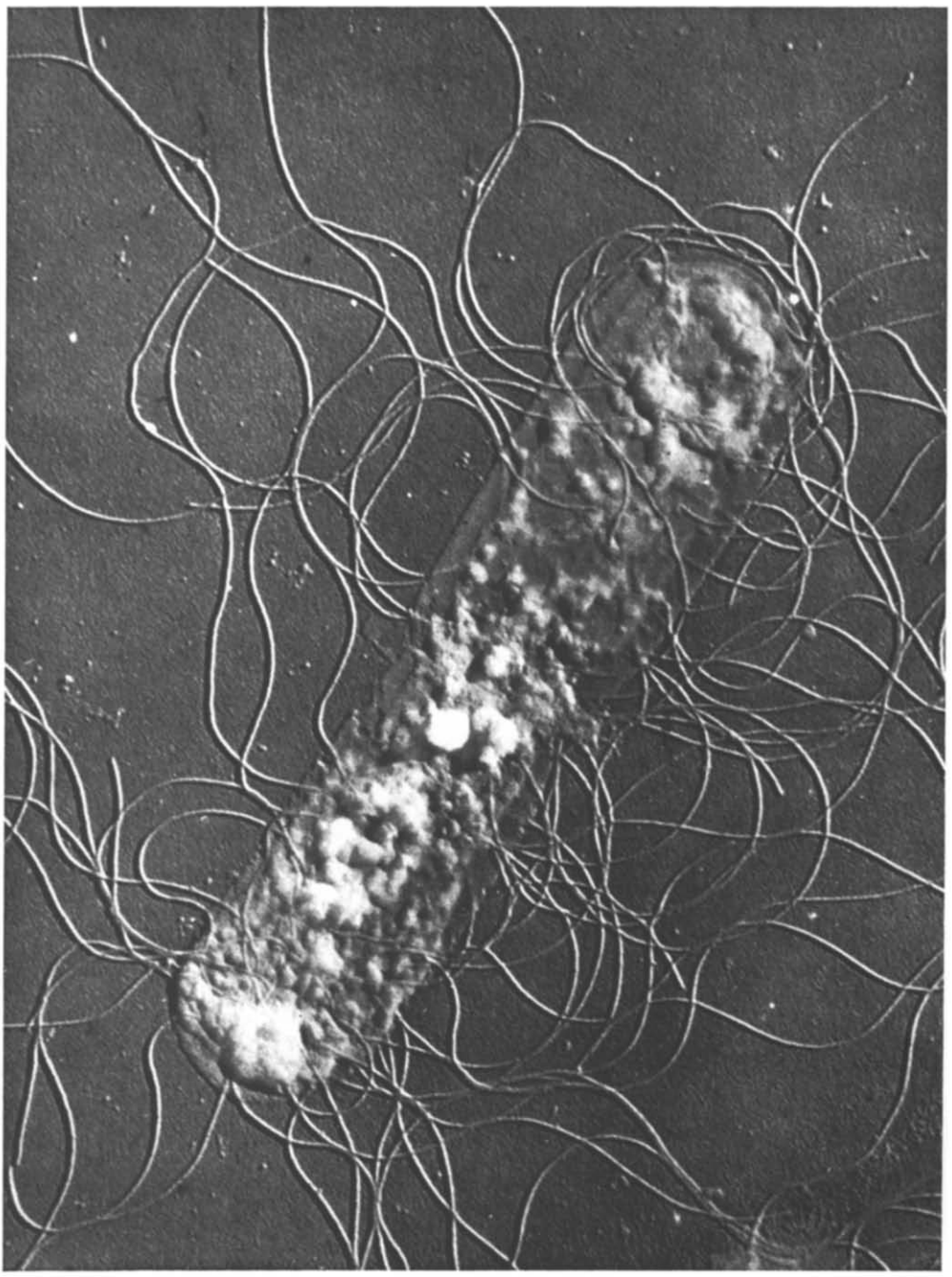

Fig. 19. *Proteus vulgaris*. Incompletely autolysed cell. Some of the basal granules are seen between the cell wall and the border of the receded protoplast. (25 000 ×; R.C.A. Lab.)

The inadequacy of PIJPER's conception of the nature of flagella is also apparent from our observations on the genesis of these bacterial appendages. As the electron microscope is not apt for the study of living material, we had to content ourselves with "snap-shots" of bacteria from very young cultures. Although in these experiments, made according to method 4, the bacteria were hardly motile, still flagella had grown out (Fig. 5, 6). The mean length of the flagella seems to increase with the age of the culture (Fig. 8, 11, 12). On a fresh medium the outgrowth of flagella can obviously lag behind compared with general cell growth. We did not yet undertake the challenging task of correlating growth curve with flagella development.

Observations on the genesis of flagella are comparatively scarce and incomplete. Several data found in the publications of FISCHER[20], ELLIS[21], FUHRMANN[22], REICHERT[24], NEUMANN[37, 38] and LEIFSON[53] fit in well with our findings. The last named author examined the development of flagella on the vegetative rods obtained by germination of spores of 3 species of *Bacillus*. These flagella appeared one by one and lengthened gradually. Motility did not occur with the first appearance of flagella, but only after a certain development.

It has long been surmised that the base of a flagellum is situated inside the bacterial cell. The fact that a granule had been found at the base of the Protozoan flagellum was a reason to look for a similar structure in bacteria. In these, so-called basal granules have been recorded by TRENKMAN[18, 19], FISCHER[20], FUHRMANN[22], REICHERT[24], YAMAMOTO[25], and PRENANT[26]. The importance of these observations can be easily overestimated since the minuteness of the observed structures makes these statements rather assailable. Moreover, one can cite as many opposite conclusions from other investigators.

With the aid of the electron microscope it has been possible to establish the presence of spherical bodies at the base of the flagella of *Proteus vulgaris* (Fig. 18, 19). This remarkable result is due to the application of a special technique which has been devised by Dr C. F. ROBINOW to bring about autolysis of swarming cells. The basal granules have a diameter of about 100 mμ, and for this reason they could not have been observed with a light microscope. It therefore remains uncertain whether these spherical bodies are homologous with the structures which have been reported by the authors mentioned above.

It cannot be denied that on actively moving bacteria "large flagella" or "tails" can be observed in the light microscope in the darkfield, as well as with the phase-contrast technique. Therefore, the relationship between these tails and the fibrillar flagella as shown by many microphotographs of stained preparations and clearly revealed by the electron microscope, remained to be established. Earlier evidence is in favour of the idea that the elementary flagella join to form a tail during swimming (REICHERT[24] and NEUMANN[37, 38]). An investigation of bacterial movements being outside the scope of this morphological study we had to draw our conclusions from electron micrographs in which flagella were seen to have somehow combined into bundles. We found that flagella demonstrate an interesting tendency to cohere (*vide* section 3, Fig. 13, 14, 17).

In this connection we want to draw attention to the following. Aggregates of submicroscopical fibers in parallel arrangement are well known from electron-microscopical preparations of collagen, myosin, fibrinogen *etc.*, which all represent fibrous proteins characterised by the presence of periodic cross-structures. It therefore would

References p. 43.

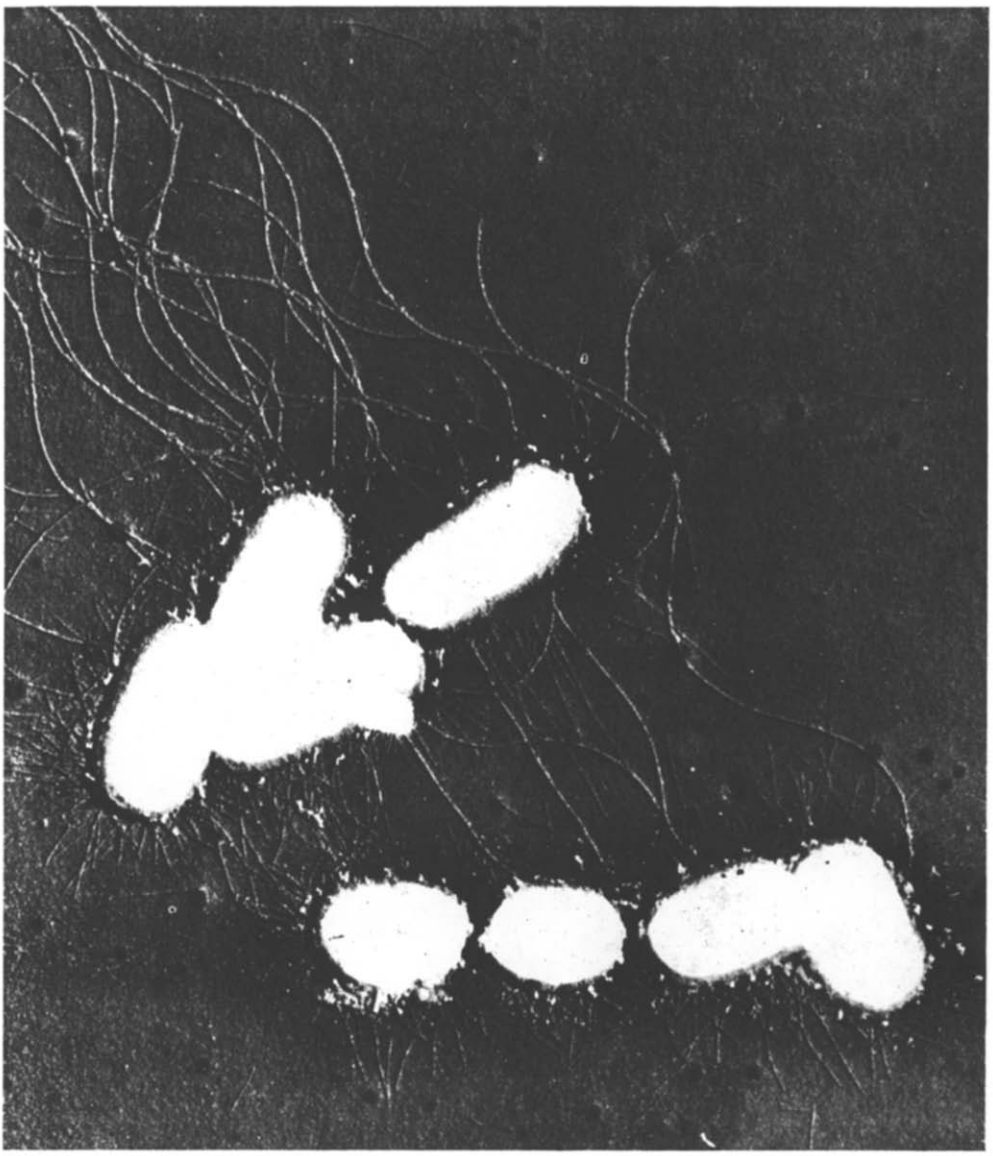

Fig. 20. *Escherichia coli*, grown in dilute peptone water (0.1 %). These cells have attached themselves to a floating collodion membrane. Flagella are present but may have been formed before the attachment occurred. Surface-growing filaments radiate to all sides on the membrane. (26 000 ×; E.M. Delft)

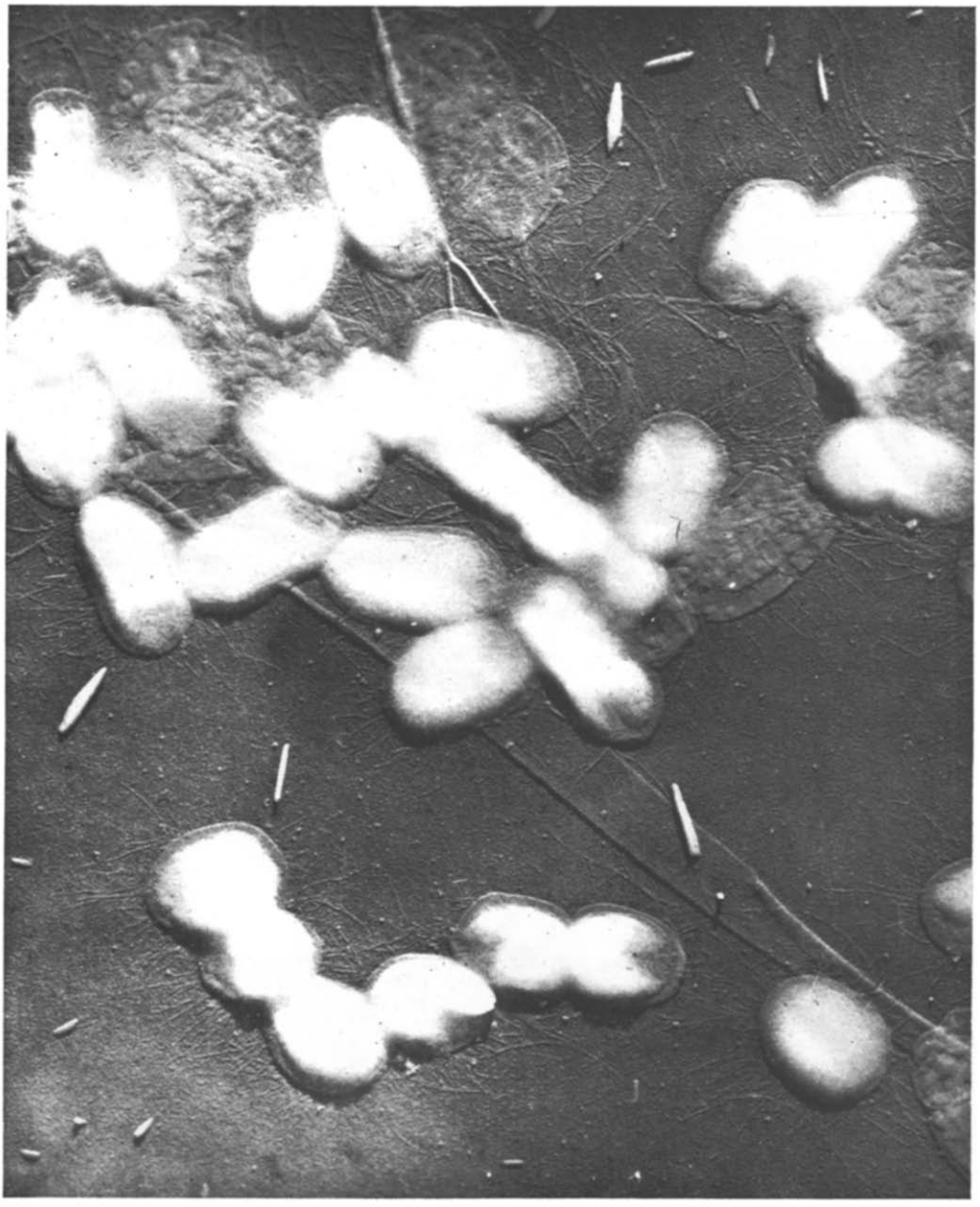

Fig. 21. *Escherichia coli*. These cells from a motile strain have been floated off from the agar surface by means of distilled water (method 1). In this case, as in many other experiments where filaments are present, flagella are missing. (16 000 ×; R.C.N. Lab.)

be important to establish with certainty whether the cross-structure we observed in our best flagella pictures is real.

Already MIGULA[17] observed the digestibility of flagella in pepsin. Recently WEIBULL[54] established by chemical methods the protein character of flagella. Further ASTBURY AND WEIBULL[55] found by x-ray diffraction that the diagrams of highly purified flagella preparations conform in the main to the α-type characteristic of the great family of elastic fibrous proteins which is called the keratin-myosin-epidermis-fibrinogen group. According to the authors flagella of *Proteus* may even be described as "monomolecular hairs or muscles".

The latter statement will most likely prove of importance in a future analysis of the function of flagella, in which also the part played by the basal granules will have to be considered. At present, however, we can only hint at a possible analogy between the property of some members of the keratin-myosin-epidermis-fibrinogen group to form relatively stable aggregates and the property of flagella to combine reversibly into bundles. The observation of REICHERT and others that the formation of a tail is promoted by special electrolytes and that it disintegrates in distilled water will also have a bearing on the phenomenon under discussion.

Special attention has also been given to the mode of flagellation; we wanted to make the existence of peritrichy quite certain. The results described in the first section of the preceding chapter are not new to students of the old school who made use of stained preparations. Our observations confirm in full the data obtained by means of an instrument with far inferior resolving power.

We believe, however, that our methods of preparation guarantee more reliable results as compared with the crude treatment to which stained flagella preparations are submitted. Especially the technique in which bacteria are grown on a collodion membrane spread over nutrient agar seems to us very mild. Here the only treatment to which the bacteria have been submitted is desiccation, which—when preparation is well performed—does not cause any change in their arrangement.

It seems to us that our results yield a solid basis for the prevalent, but in later years often fanatically contended, view that bacterial species are characterized by the manner in which single flagella are inserted. The classic differentiation between peritrichous and cephalotrichous (lopho- and monotrichous) bacteria has appeared to be well founded, and thus the type of flagellation may well be a useful diagnostic in bacterial classification. CONN AND ELROD who subscribe to the same opinion remark "that one must use considerable caution in describing any organism as either peritrichic or monotrichic, since there seem to be species in which the peritrichic flagellation is of a degenerate type, so that strains with only one flagellum to the cell can be found". The term "peritrichy" implies that flagella can be inserted all around the bacterial cell. In many of our micrographs representing bacteria from young cultures (compare Fig. 11) flagella are missing at the poles. This has also been mentioned by FRACHE[56] and may well be the consequence of frequent division. However, the polar flagellum of GRAY's bacterium was present even in young cultures. This fact, in addition to the already discussed difference in width, might offer an argument for the idea that this polar flagellum is a special feature and not just another peritrichous flagellum. This new type of flagellation may well serve as a characteristic in the description of the species.

As has already been observed by careful workers in the field, flagella show differences in width and undulation between different species. For example: the flagella of

References p. 43.

Bacterium herbicola, *Serratia marcescens* and *Agrobacterium radiobacter* are very thin, but a strain of *Alcaligenes faecalis* was found to possess much more substantial ones. The flagella of *Spirillum serpens* are very thick and do not show as many undulations as for instance those of the two *Proteus* species. They are also altogether different from those of *Pseudomonas* species and even from the thick flagellum of *Vibrio metschnikovii*.

We now feel confident that elementary flagella represent definite bacterial organs which are inserted inside the bacterial cell at least in the case of *Proteus* by means of basal granules. They demonstrate characteristic differences and analogies in modes of implantation and in appearances, and they may well be of use in the classification and description of species.

APPENDIX

In the course of the foregoing study we were struck by the occasional presence of thin apparently fragile filaments in addition to flagella. These filaments are easily distinguishable from the latter by their irregular structure and smaller diameter. That they do not represent young flagella can be derived from the manner in which flagella grow out; they also lack the smooth undulation of flagella. Their length may attain several microns.

In several species these structures have been found to occur when the cells are grown on nutrient agar (Fig. 8, 21), or on collodion (method 4). They can also be abundant on bacteria which have attached themselves to a floating collodion membrane (method 3). *Escherichia coli* in Fig. 20 offers evidence that in the living state the filaments cling to the membrane, whereas the flagella probably dangle freely in the liquid, since during desiccation the latter have apparently all been dragged in one direction by the evaporating water.

In cephalotrichous *Ps. pyocyanea* (Fig. 8) the thin filaments radiate from one or both of the polar ends of the bacterium. The flagellum may be located at the same or at the opposite end. In *E. coli* (Fig. 20, 21), however, the filaments are arranged in a peritrichous manner. This agreement in the position of flagella and thin filaments does not always hold, since in *Photobacterium splendidum* with its polar flagellum we found ample evidence for the presence of peritrichously arranged filaments.

It is our impression that these filaments are formed on bacteria which are in contact with a solid surface as for example nutrient agar. We did not find them on free swimming bacteria (Fig. 1, 3). It should be mentioned, however, that *Proteus mirabilis* was found to develop filaments at the surface of a liquid medium, even in the absence of a collodion membrane. Perhaps other species may do so as well.

Although there are in literature several reports on bacterial appendages other than flagella, a survey of these articles has given us the conviction that they deal with formations quite different from the filaments we described above, so that they need not be discussed here. In this respect we wish to make one exception. In 1901 HINTERBERGER[57] published microphotographs of a stained preparation made from an old culture of *Bac. anthracis* with innumerable fine threads. Three years later HINTERBERGER AND REITMAN[16] found the same phenomenon with *Ps. pyocyanea*. These threads were called "Myzel" and were abundant on a comparatively dry medium; flagella on the

References p. 43.

other hand were present in larger number on moist agar. The authors suggested that the "Myzel" may serve the uptake of nutrient substances.

It is true that HINTERBERGER's microphotographs do not show much resemblance to our electron micrographs Fig. 8, 20 and 21, but one should bear in mind that his cultures were very much older, *i.e.*, some weeks. Therefore we will not altogether reject the possibility that our thin filaments are identical with HINTERBERGER's "Myzel".

HINTERBERGER's idea that some bacteria might develop a food-gathering "mycelium" is undoubtedly attractive, especially in connection with ZOBELL's theory[58] that the tendency of many species to settle on glass and other solids is connected with the presence hereon of adsorbed organic substances. We also wish to mention the observation of HEUKELEKIAN AND HELLER[59], who found that the lower the concentration of nutrient substances in the medium, the higher the percentage of cells in an *E. coli* culture which attach themselves to glass.

The filaments might, however, also serve quite different purposes, as for instance to fasten the organisms to the substrate, or they might be mere excretion products.

We wish to state here explicitly that as yet we do not know anything about the function, if any, of the filaments described.

SUMMARY

An electron-microscopical study has been undertaken in the hope of arriving at a final solution of some basic problems concerning bacterial flagellation.

1. The question whether in the bacterial kingdom definite types of flagellation exist has of late become a subject of controversy, and a decision in this matter may be deemed most desirable with a view to the general application of this characteristic in bacterial classification. Ample evidence has been obtained that, next to the already often clearly demonstrated cases of cephalotrichy in certain bacterial species, also true peritrichy undoubtedly exists. In addition a new type of flagellation has been encountered in which certain aspects of cephalotrichy and peritrichy are combined.

2. A study of the genesis of flagella led to the conclusion that they grow out individually. A comparison of the flagella on bacteria in cultures of different age left no doubt that they gradually increase both in length and in number. This result is incompatible with PIJPER's theory according to which elementary flagella represent secondary products of an irreversible untwisting of a mucous tail. In preparations of *Bacillus mesentericus* it was even possible to differentiate clearly between flagella having a distinct linear structure and simultaneously present amorphous slime.

3. In connection with the occurrence of "tails" on fast moving bacteria, as observed in the darkfield of the light microscope, attention has been given to the question whether the elementary flagella tend to agglomerate to bundles. Several instances of such a bundle formation have been encountered which are suggestive of the existence of a mutual affinity between the individual flagella.

4. At variance with PIJPER's theory it has been found that mobility of a bacterium is not an essential condition for flagella formation. Firstly flagella were demonstrated on cells grown on a collodion membrane under conditions which did not allow swimming of the cells, and secondly in the case of a *Caulobacter* species flagella were found to have developed in the sessile stage of this bacterium.

5. Following a suggestion of C. F. ROBINOW a study was made of the flagellation of swarmer cells of *Proteus vulgaris* after subjecting them to autolysis. The remarkable observation was made that in this bacterium flagella arise from little spheres of about 100 mμ diameter which evidently under normal conditions are embedded in the protoplasm.

6. The foregoing warrants the conclusion that elementary flagella represent definite organs of the bacteria and may therefore well be applied in the diagnosis of bacterial species and in their classification.

7. In the Appendix filamentous appendages of bacterial cells are described which can occur in some species and which are easily distinguishable from flagella. When in these species cells are attached to a surface, be it the surface of the medium or of a solid substance, filaments are seen to radiate from these cells.

References p. 43.

RÉSUMÉ

Nous avons entrepris une étude au microscope électronique afin d'obtenir une solution définitive de certains problèmes fondamentaux concernant la flagellation chez les bactéries.

1. L'existence chez les bactéries de types définis de flagellation est devenue, ces derniers temps, un sujet de controverses et une décision dans cette matière semble très désirable afin de permettre l'application de la flagellation comme caractéristique dans la classification bactérienne. Nous avons obtenu plusieurs preuves de l'existence chez certaines espèces bactériennes de péritrichie vraie à côté de la céphalotrichie qui a déjà été clairement démontrée plusieurs fois. De plus, nous avons rencontré un nouveau type de flagellation dans lequel certains aspects de la céphalotrichie et de la péritrichie sont combinés.

2. Une étude de la genèse des flagelles nous a amenés à la conclusion qu'ils se développent individuellement. Une comparaison des flagelles de bactéries dans des cultures d'âge différent ne laisse aucun doute sur le fait qu'ils augmentent graduellement en longueur et en nombre. Le résultat est incompatible avec la théorie de PIJPER selon laquelle les flagelles élémentaires représenteraient des produits secondaires d'une "queue" muqueuse qui se défait de façon irréversible. Il nous a même été possible de différencier clairement, dans des préparations de *Bacillus mesentericus*, des flagelles de structure linéaire distincte et un mucus amorphe présent simultanément.

3. L'apparition de "queues" sur des bactéries à mouvement rapide observée dans le champ obscure du microscope optique a attiré notre attention sur la question si les flagelles élémentaires ont la tendance de s'agglomérer en faisseaux. Nous avons rencontré plusieurs exemples d'une telle formation de faisceaux. Elle suggère l'existence d'une affinité mutuelle entre les flagelles individuels.

4. En désaccord avec la théorie de PIJPER nous avons trouvé que la mobilité d'une bactérie n'est pas toujours une condition essentielle de la formation de flagelles. En premier lieu, des flagelles ont été démontrés sur des cellules croissant sur une membrane de collodion dans des conditions qui ne permettent pas aux cellules de nager, en second lieu, nous avons trouvé dans le cas de *Caulobacter* spec. que des flagelles s'étaient développés dans la phase sessile de la bactérie.

5. Sur l'instigation de C. F. ROBINOW nous avons entrepris l'étude de la flagellation chez les cellules de *Proteus vulgaris* soumises à l'autolyse. Il est remarquable que dans cette bactérie les flagelles aboutissent à de petites sphères d'environ 100 mμ de diamètre qui, dans des conditions normales, se trouvent évidemment enfoncées dans le protoplasme.

6. Les faits susnommés permettent de conclure que les flagelles élémentaires représentent des organes définis des bactéries et peuvent donc servir à la diagnose et à la classification des espèces bactériennes.

7. Dans un supplement nous décrivons des appendices de cellules bactériennes rencontrés dans certaines espèces et que l'on distingue facilement des flagelles. Si des cellules de ces espèces sont attachées à la surface du milieu nutritif ou d'une substance solide on voit des filaments rayonner de ces cellules.

ZUSAMMENFASSUNG

Eine elektronenmikroskopische Untersuchung wurde in der Hoffnung unternommen für einige grundlegende, die Geisselbildung bei den Bakterien betreffende Probleme eine endgültige Lösung zu finden.

1. In letzter Zeit ist die Existenz verschiedener Arten von Geisselbildung im Bakterienreiche viel bestritten worden und eine Entscheidung dieser Frage wäre sehr erwünscht. Sie würde die Anwendung der Geisselbildung als Merkmal bei der Klassifikation der Bakterien ermöglichen. Wir fanden zahlreiche Hinweise auf wahre Peritrichie bei einzelnen Bakterienarten, neben der schon früher in vielen Fällen bewiesenen Cephalotrichie. Ausserdem fanden wir einen neuen Typ von Geisselbildung der gewisse Aspekte der Cephalotrichie und der Peritrichie vereinigt.

2. Eine Untersuchung der Geisselbildung führte zu dem Schluss, dass diese einzeln wachsen. Ein Vergleich der Geisseln von Bakterienkulturen verschiedenen Alters liess keinen Zweifel darüber, dass sie allmählich an Länge und Zahl zunehmen. Dieses Ergebnis ist unvereinbar mit der Theorie von PIJPER, nach der die einzelnen Geisseln Nebenprodukte einer irreversibelen Umbildung eines Schleimanhanges seien. Es war sogar möglich, in Präparaten von *Bacillus mesentericus* zwischen Geisseln mit deutlich linearer Struktur und einem gleichzeitig auftretenden amorphen Schleim zu unterscheiden.

3. Im Anschluss an die Beobachtung von "Schwänzen" an rasch sich bewegenden Bakterien im Dunkelfeld des Mikroskops wurde untersucht, ob die einzelnen Geisseln zur Vereinigung in Bündeln neigen. In der Tat wurde eine derartige Bündelbildung mehrmals beobachtet. Sie weist auf eine gegenseitige Affinität der Geisseln hin.

4. Abweichend von der PIJPER'schen Theorie fanden wir, dass Beweglichkeit einer Bakterie keine notwendige Bedingung der Geisselbildung darstellt. Erstens beobachteten wir Geisseln an Zellen, die unter Bedingungen welche kein Schwimmen erlauben auf einer Kollodiummembrane

References p. 43.

wuchsen und zweitens fanden wir Geisseln die sich bei *Caulobacter* spec. im ruhenden Stadium entwickelt hatten.

5. Einer Anregung von C. F. ROBINOW folgend, untersuchten wir autolysierte Schwärmerzellen von *Proteus vulgaris*. Bemerkenswerter Weise wachsen hier die Geisseln aus kleinen Kugeln von ungefähr 100 mμ Durchmesser heraus, die offensichtlich unter normalen Bedingungen in das Protoplasma versenkt sind.

6. Die obenerwähnten Tatsachen erlauben den Schluss, dass die Geisseln bestimmte Organe der Bakterien darstellen und deshalb zur Diagnose und Klassifikation der Bakterienarten verwendet werden können.

7. Im Appendix werden fadenförmige Fortsätze der Bakterienzelle beschrieben, die in einigen Arten vorkommen und leicht von den Geisseln unterschieden werden können. Wenn in diesen Arten Zellen an der Oberfläche des Mediums oder einer festen Substanz haften, dann strahlen Fäden von diesen Zellen aus.

REFERENCES

[1] A. VAN LEEUWENHOEK, *Arcana naturae detecta*, 1683.
[2] CH. G. EHRENBERG, *Die Infusionstierchen als vollkommene Organismen*, Leipzig, 1838.
[3] PH. VAN TIEGHEM, *Bull. soc. bot. France*, 26 (1879).
[4] F. DE BARY, *Vorlesungen über Bakterien*, Leipzig, 1887.
[5] F. HUEPPE, *Naturwissenschaftliche Einführung in die Bakteriologie*, Wiesbaden, 1896.
[6] KURTH, *Botan. Zt.*, 1883.
[7] A. PIJPER, *Zentr. Bakt., Parasitenk. Abt. I*, 118 (1930) 113.
[8] A. PIJPER, *J. Path. Bact.*, 47 (1938) 1.
[9] A. PIJPER, *J. Path. Bact.*, 58 (1946) 325.
[10] K. PIETSCHMANN, *Arch. Mikrobiol.*, 10 (1939) 133.
[11] K. PIETSCHMANN, *Arch. Mikrobiol.* 12 (1942) 377.
[12] O. BÜTSCHLI, *Über den Bau der Bakterien*, Leipzig, 1890.
[13] V. BABES, *Z. Hyg. Infektionskrankh.*, 20 (1895) 429.
[14] R. BUNGE, *Centr. Bakt. Parasitenk.*, 17 (1895) 102.
[15] E. ZETTNOW, *Z. Hyg. Infektionskrankh.*, 30 (1899).
[16] A. HINTERBERGER UND C. REITMAN, *Centr. Bakt., Parasitenk. Abt. I*, 37 (1904) 169.
[17] W. MIGULA, *System der Bakterien*, I, Jena, 1897.
[18] TRENKMANN, *Centr. Bakt. Parasitenk.*, 6 (1889) 434.
[19] TRENKMANN, *Centr. Bakt. Parasitenk.*, 8 (1890) 385.
[20] A. FISCHER, *Vorlesung ü. Bakterien*, Jena, 1897.
[21] D. ELLIS, *Centr. Bakt. Parasitenk. Abt. I*, 33 (1902-1903) 1, 81, 161.
[22] F. FUHRMANN, *Vorlesungen ü. Technische Mykologie*, Jena, 1913.
[23] A. MEYER, *Die Zelle der Bakterien*, Jena, 1912.
[24] K. REICHERT *Centr. Bakt. Parasitenk. Abt. I*, 51 (1909) 14.
[25] YAMAMOTO, *Centr. Bakt., Parasitenk. Abt. I*, 53 (1910) 38.
[26] A. PRENANT, *Revue gén. d. sci.*, 26 (1915) 41.
[27] PH. LASSEUR ET P. VERNIER, *Bull. soc. sci. Nancy*, Juillet 1923, Mai 1924.
[28] S. MUDD, K. POLEVITSKI AND TH. F. ANDERSON, *Arch. Path.*, 34 (1942) 199.
[29] G. KNAYSI, *Elements of Bacterial Cytology*, Ithaca, N.Y., 1944.
[30] W. VAN ITERSON, *Biochim. Biophys. Acta*, 1 (1947) 527.
[31] F. COHN, *Beitr. Biol. Pflanz.*, 1 (1872).
[32] W. H. DALLINGER AND J. DRYSDALE, *The Monthly M.J.*, 1875.
[33] E. WARMING, *Medd. naturhist. För. Kjöbenhavn*, 1875.
[34] R. KOCH, *Beitr. Biol. Pflanz.*, 2 (1876-1877).
[35] F. LOEFFLER, *Centr. Bakt. Parasitenk.*, 6 (1889) 209.
[36] AL. MESSEA, Refer. in *Centr. Bakt. Parasitenk.*, 9 (1891) 107.
[37] F. NEUMANN, *Centr. Bakt. Parasitenk. Abt. I*, 96 (1925) 250.
[38] F. NEUMANN, *Centr. Bakt. Parasitenk. Abt. I*, 109 (1928) 143.
[39] NEUMÜLLER, *Centr. Bakt. Parasitenk. Abt. I*, 102 (1927) 90.
[40] R. P. LOVELAND, *J. Biol. Phot. Assoc.*, 1 (1933) 128.
[41] H. WEI, *China Med. J. Suppl., I*, (1936) 135.
[42] T. Y. KINGMA BOLTJES, *J. Path. Bact.*, 60 (1948) 275.
[43] N. H. SWELLENGREBEL, *Ann. inst. Pasteur*, 21 (1907) 448.
[44] N. CHOLODNY, *Arch. Mikrobiol.*, 1 (1930) 620.
[45] A. T. HENRICI, *J. Bact.*, 25 (1933) 277.
[46] C. E. ZOBELL AND E. C. ALLEN, *J. Bact.*, 29 (1935) 239.
[47] J. HILLIER, G. KNAYSI AND R. F. BAKER, *J. Bact.*, 56 (1948) 569.

[48] M. JONES, *Centr. Bakt. Parasitenk. Abt. II*, 14 (1905) 459.
[49] W. OMELIANSKI, *J. Microbiol. (Russian)*, 1 (1914) 24.
[50] A. T. HENRICI AND D. E. JOHNSON, *J. Bact.*, 30 (1935) 61.
[51] J. ØRSKOV, *Acta Path. Microbiol. Scand.*, 24 (1947) 181.
[52] F. KAUFMANN, *Schweiz. Z. allgem. Path. u. Bakt.*, 11 (1948) 378.
[53] E. LEIFSON, *J. Bact.*, 21 (1931) 357.
[54] C. WEIBULL, *Biochim. Biophys. Acta*, 2 (1948) 351.
[55] W. T. ASTBURY AND C. WEIBULL, *Nature*, 163 (1949) 280.
[56] E. FRACHE, *Les jouets et le mouvement des bactéries*. Thèse, Nancy, 1926.
[57] A. HINTERBERGER, *Centr. Bakt., Parasitenk. Abt. I*, 30 (1901) 417.
[58] C. E. ZOBELL, *J. Bact.*, 46 (1943) 39.
[59] H. HEUKELEKIAN AND A. HELLER, *J. Bact.*, 40 (1940) 547.

Received July 1st, 1949