SOME ELECTRON-MICROSCOPICAL OBSERVATIONS ON BACTERIAL CYTOLOGY

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I. INTRODUCTION

During the earlier years of the war, in the Physical Institute of the Technische Hoogeschool at Delft an electron microscope has been designed by Mr. J. B. LE POOLE who also supervised its construction[1]. In this period only preliminary investigations regarding the usefulness of the instrument could be made, and in the last year of the war it had to be dismounted in order to safeguard it from undesirable attention from the side of the occupying authorities. After the liberation of the country the instrument was reinstalled, and it then became possible to compare its merits with those of the various designs developed in other countries. It is here not the place to enter into details of this comparison, it may suffice to state that the Delft electron microscope proved to have some characteristic features of its own. The great interest aroused by the development of electron-microscopical studies in other parts of the world led immediately to a great demand for similar investigations from the side of industry and other institutions of applied science. In consequence hereof a heavy claim was laid upon the instrument and its operators, and this to such a degree that no operation time was left for a more systematic investigation of problems of purely scientific interest.

Notwithstanding this the author who as a biologist had joined the staff of the electron-microscopical institute in 1943 has now and then had the opportunity to make more or less incidental observations in the field of bacterial cytology. Now her activity in the Delft Institute has temporarily come to an end, it seems worth while to report here briefly on some of the micrographs obtained, as well as on some conclusions which may be derived therefrom.

II. METHODS

The bactreia used in the preparations w re grown either on agar slants or on plates. After one night at 30–35° C in the incubator the cultures were kept for 24–72 hours at room temperature, since I had got the impression that this rest period favourably influenced the demonstration of internal structures in the cells. With a platinum loop a small part of the culture was removed and suspended in some distilled water so that the liquid acquired a slight turbidity. From this suspension a tiny droplet was brought on the film on top of the object holder of the instrument.

In the Delft microscope conical metal object holders are used in the center of which a small hole is bored to allow for the passage of the electron beam. These holders are covered with a very thin film of "Geiseltal-lack" or collodion which is obtained by bringing a drop of a solution on a water

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surface in a Petri-dish. When the film has attained sufficient strength, it is lifted from the water by bringing the object holder in touch with the film and applying a rotatory movement.

After the drop of bacterial suspension had dried up in the air, the object holder was placed either directly into the microscope or into a vacuum chamber in which the "shadow-casting" procedure according to WILLIAMS and WYCKOFF[2] was carried out. In a highly evacuated chamber ($5 \cdot 10^{-5}$ mm Hg) a small piece of gold wire is electrically heated so that gold atoms are evaporated. In the chamber the object holders have been placed in an oblique position to the gold wire. In consequence hereof the gold atoms settle all over the preparation sparing, however, those parts which are protected by protruding structures. Here no gold can settle, whereas the contrast of the other parts of the preparation is raised by a gold layer. Printed in negative, micrographs of specimens prepared in this way have in the first place the advantage of disclosing fine structures which otherwise would have been lost owing to lack of contrast. Moreover, the shadows bring about a threedimensional effect.

A too high intensity of the electron beam tends, however, to coagulate the gold particles which leads to undesirable structural effects. Further it has to be taken into account that the shadowcasting procedure also brings to light superficial irregularities in the molecular structure of the "Geiseltallack"-film, whilst casual large molecules possibly present in the evaporated water may also show up.

III. DISCUSSION OF SOME CYTOLOGICAL CHARACTERISTICS AS DERIVED FROM THE MICROGRAPHS PRESENTED

As has already been remarked in the Introduction no attempt will be made to give here a harmonious survey of the present state of our knowledge regarding bacterial cytology. I shall confine myself to a brief discussion of the more important data which can be derived from the micrographs, in the hope that this discussion may contribute in a modest way to the answering of some open questions in the cytological field.

It seems of advantage to consider successively the main cytological characteristics.

a. Cell shape

First of all it should be observed that one may rightly question whether electron micrographs of bacteria which have been prepared in the primitive way as has been applied in this study are apt to furnish useful information regarding the cell shape. The author is aware that at present more adequate preparation methods (freeze drying, replica technique) are available, and for this reason only a few remarks will be made.

Although one can fully agree with PIJPER's view[3] that a study of bacteria in the living state offers many advantages over a mere reconstruction based on fixed preparations, the pictures obtained of *Vibrio metchnikovii* (Fig. 9) and *Spirillum serpens* (Fig. 10) offer a very satisfactory confirmation of the current conceptions regarding the shape of the bacteria as based on light-microscopical studies.

Another question is whether the micrographs can throw any light on the thesis recently advanced by PIJPER[4] that, although the word bacterium means rod, in reality there are no "rod-shaped" bacteria, the cell shape being in all cases that of a spirillum, *i.e.*, of a coil.

If we check this point of view on the picture of *Pseudomonas fluorescens* (Fig. 5) which according to orthodox bacterial classification should, indeed, present a rod-shape, we cannot escape the conclusion that this picture strongly supports PIJPER's opinion. In this micrograph all cells show a marked curvature. It remains to be seen, however, whether this holds good for all bacteria until now supposed to be rod-shaped. More in particular there may be some reason to distinguish in this respect between the bacteria with polar and those with peritrichous flagella. Although realizing that the available data are fully inadequate to allow of a definite answer to this question I will not omit to refer to Fig. 2, in which a perfectly straight rod-form of *Bacillus subtilis* is presented.

References p. 548. *Text continued p. 541*

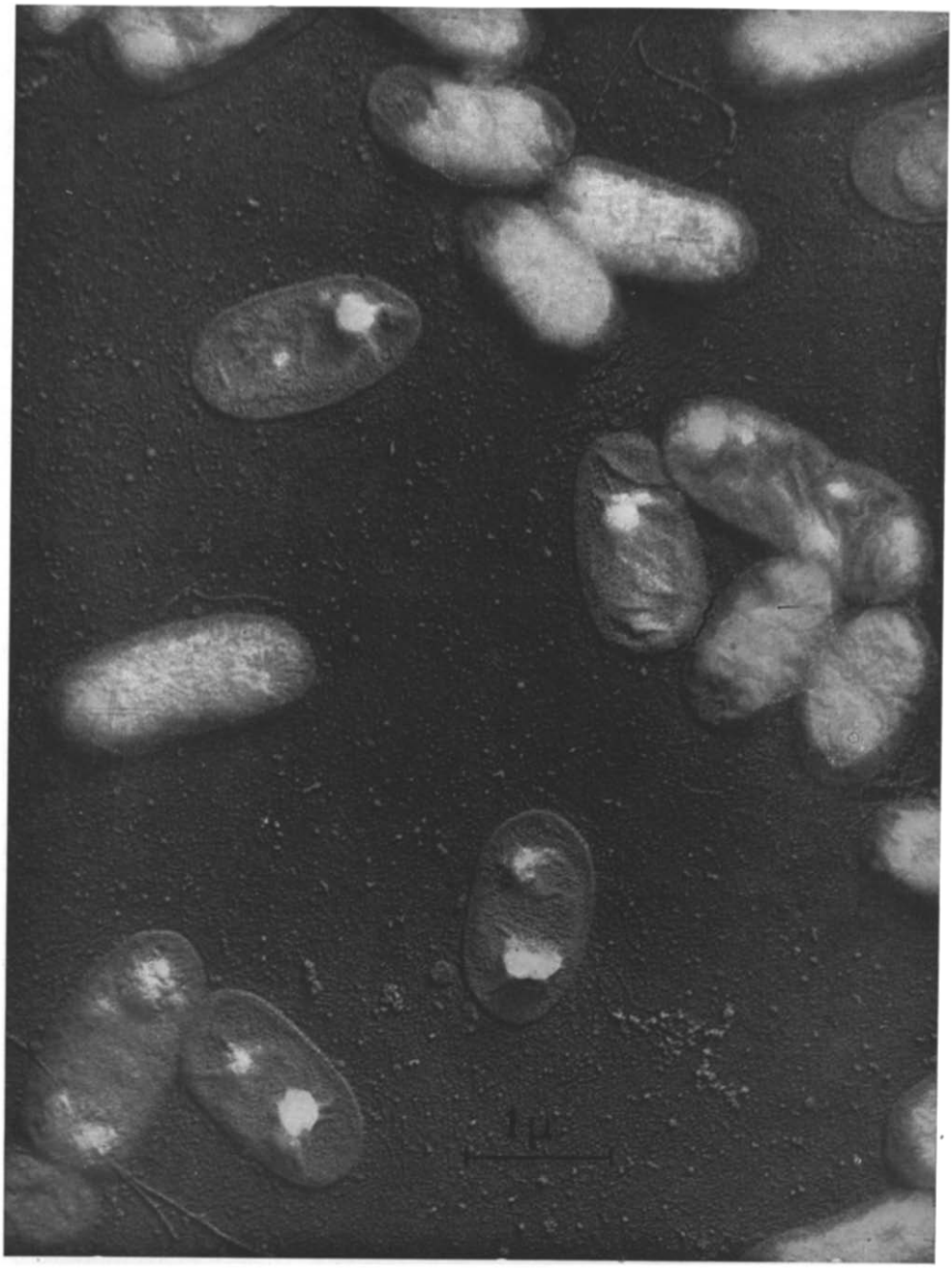

Fig. 1. *Serratia marcescens* (*Bacterium prodigiosum*) 20 000 ×; 90 kv; shadow-cast. The diversity in the appearance of the individual cells is striking. In some cells the protoplasm has only slightly receded from the distinct, rigid cell wall, whereas other cells appear nearly empty but for a rest of very dense, strongly contracted and more or less elevated protoplasm. Flagella were scanty in all preparations examined, and if present usually broken off.

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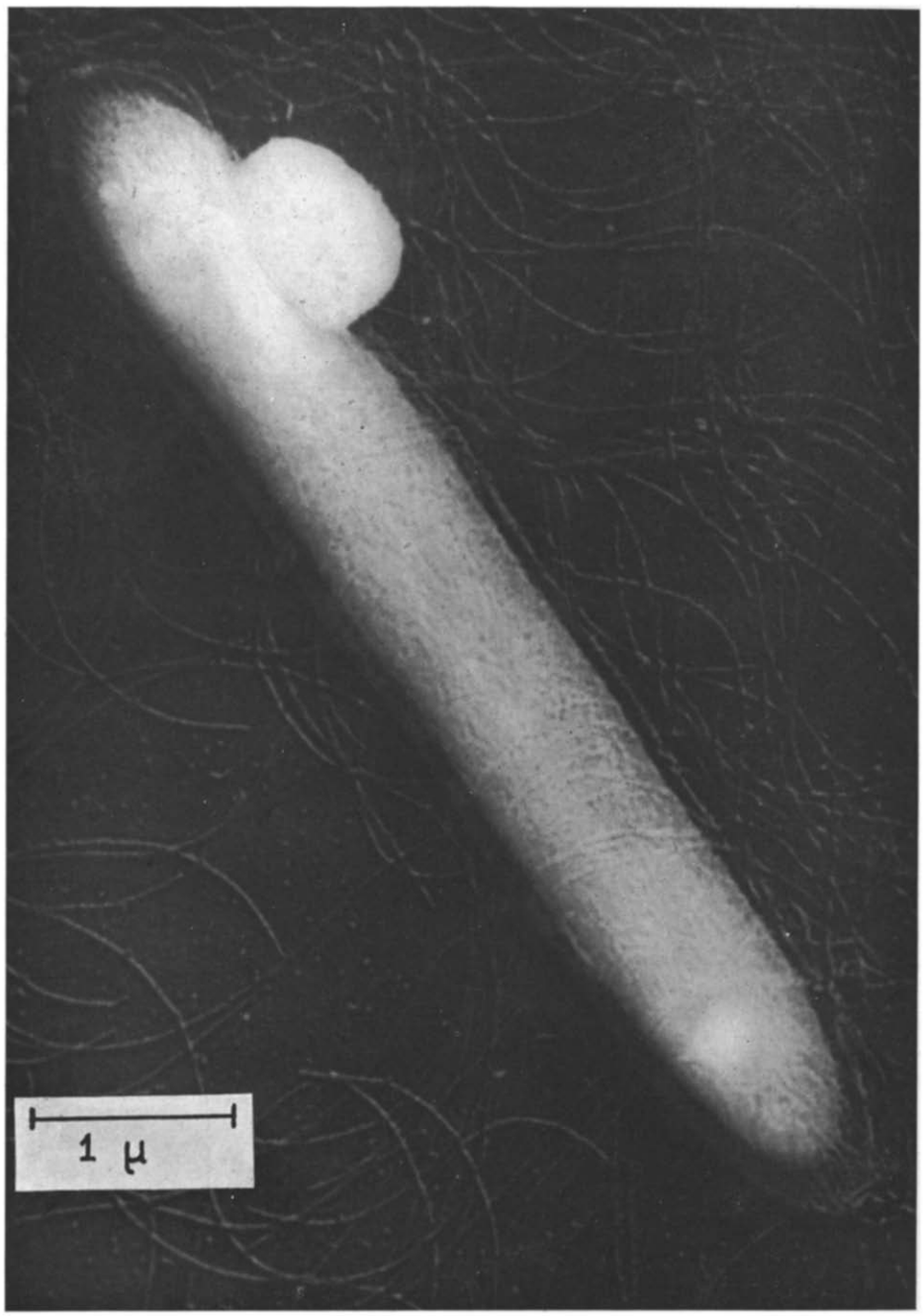

Fig. 2. *Bacillus subtilis* 27000 ×; 90 kv; shadow-cast. Printed in negative. Typical example of a vegetative rod. The oval body at the side of the rod is apparently a spore originating from a second bacterium, and which only incidentally has stuck to the cell. A spherical body in both extremities of the cell is discernible. A large number of entangled flagella covers the preparation.

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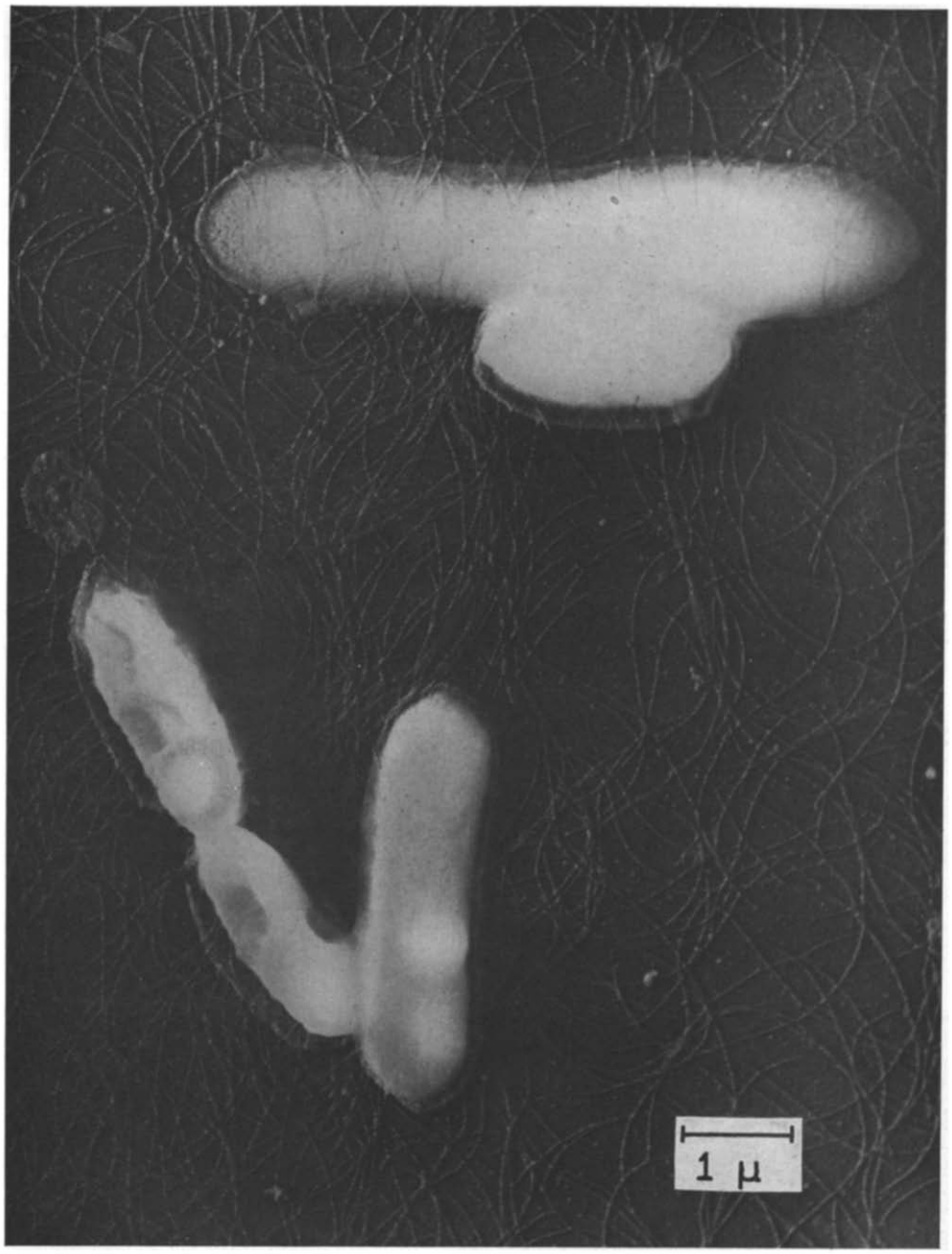

Fig. 3. *Bacillus subtillis*. 15000 ×; 90 kv; shadow-cast. Printed in negative.

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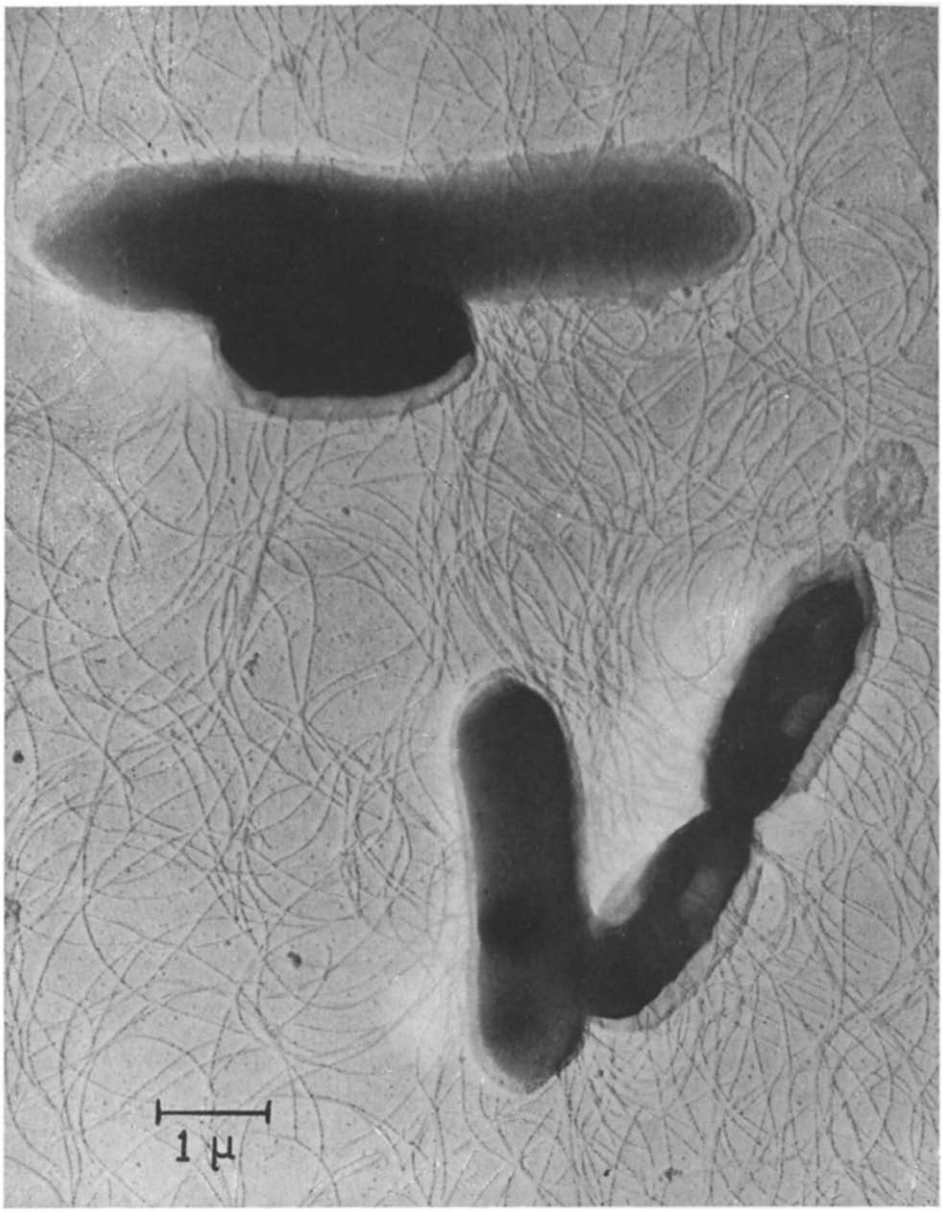

Fig. 4. The same as Fig. 3, printed in positive. The micrographs show amongst others two smaller cells, shortly after cell-division. In Fig. 4 in each of these cells towards one end a round body is clearly discernible. In the adjoining longer cell a similar body is situated near the center. One of the first mentioned cells shows a rest of empty cell wall material at its top. In the other half of the picture a large bacterium in state of spore formation is to be found, a small cell being incidentally attached. In these pictures too the numerous flagella scattered all over the field, most of which may be broken off, are highly characteristic.

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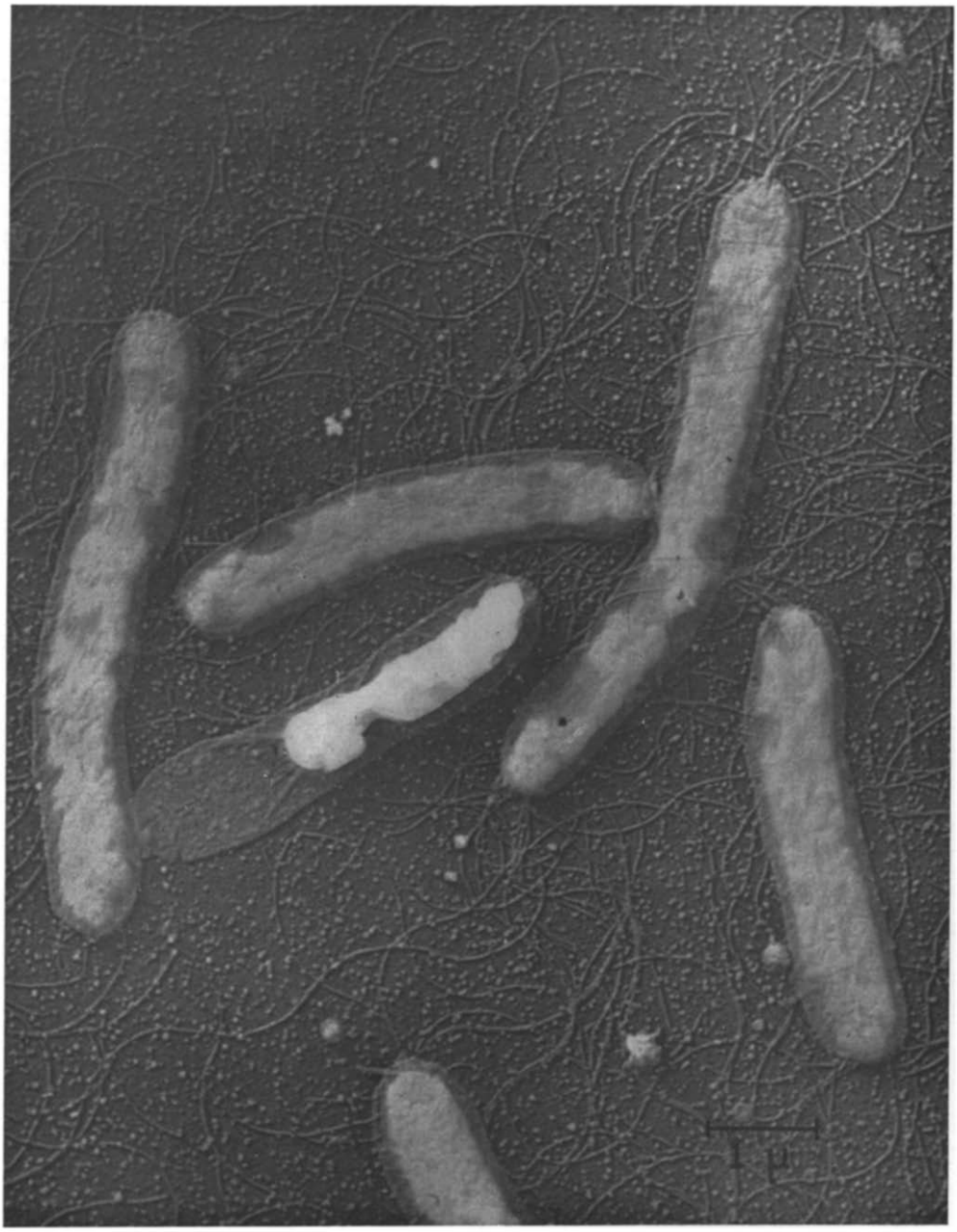

Fig. 5. *Pseudomonas fluorescens.* 15000×; 90 kv; shadow-cast. The picture suggests two types of cells, possibly primary and secondary cells in the sense of RUSKA and PIEKARSKI (Cf. Chapter 4d). In the secondary cells the protoplasm has strongly contracted from the cell wall, and is much denser than in the more dispersed state characteristic of the other cells. The secondary cells — as is also the case in other unpublished micrographs — often show a large empty bladder at one extremity (Cf. Fig. 6). There are many broken off and displaced flagella. However, at least at the one end of the largest cell a clear cut flagellar bundle can be seen. The small granules scattered all over the film are a constant feature of preparations of this strain and may constitute some excretion product.

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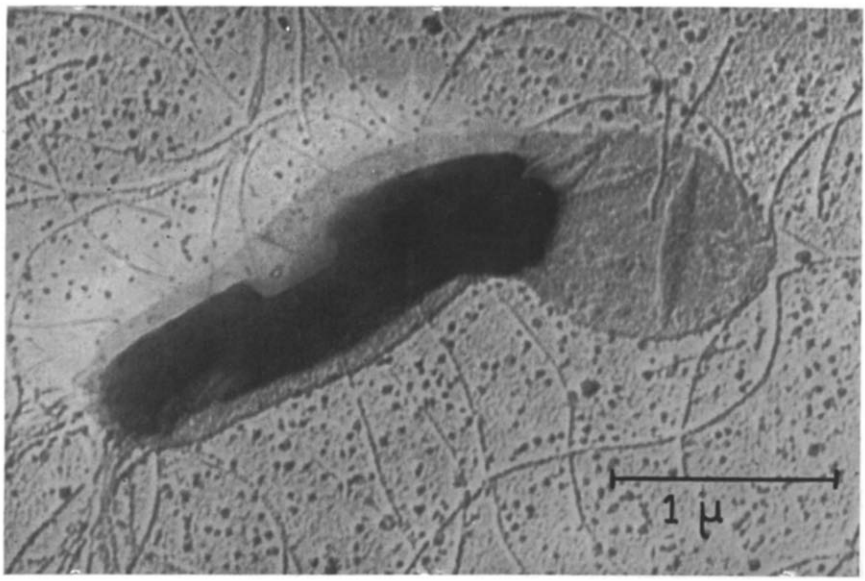

Fig. 6. *Pseudomonas fluorescens.* 30000 ×; 90 kv; shadow-cast. Printed in positive. A typical secondary cell with large cell wall bladder on which runs a transverse fold. Special attention may be drawn to the two dark round bodies in the part of the protoplasm near to the bladder.

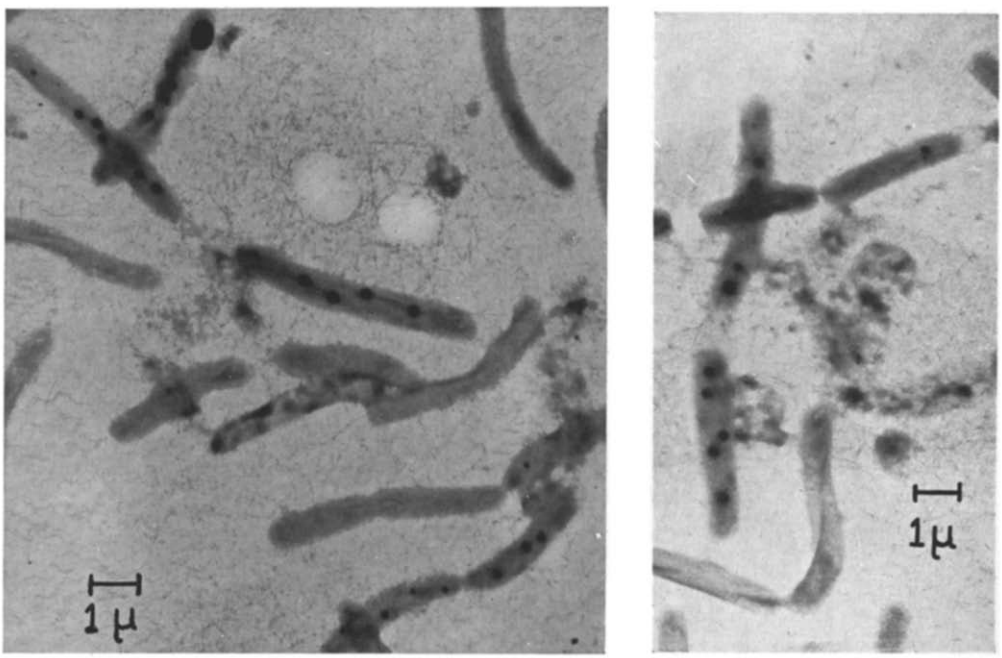

Fig. 7 and 8. *Pseudomonas fluorescens.* 6400 ×; 100 kv; not shadow-cast. Characteristic for this picture is the occurrence in part of the cells of pairs of discrete granules mutually connected by fine threads. In consequence of the coarse grain of the photographic film — at that time the only available — the evaluation of the finer structural details has to be restricted.

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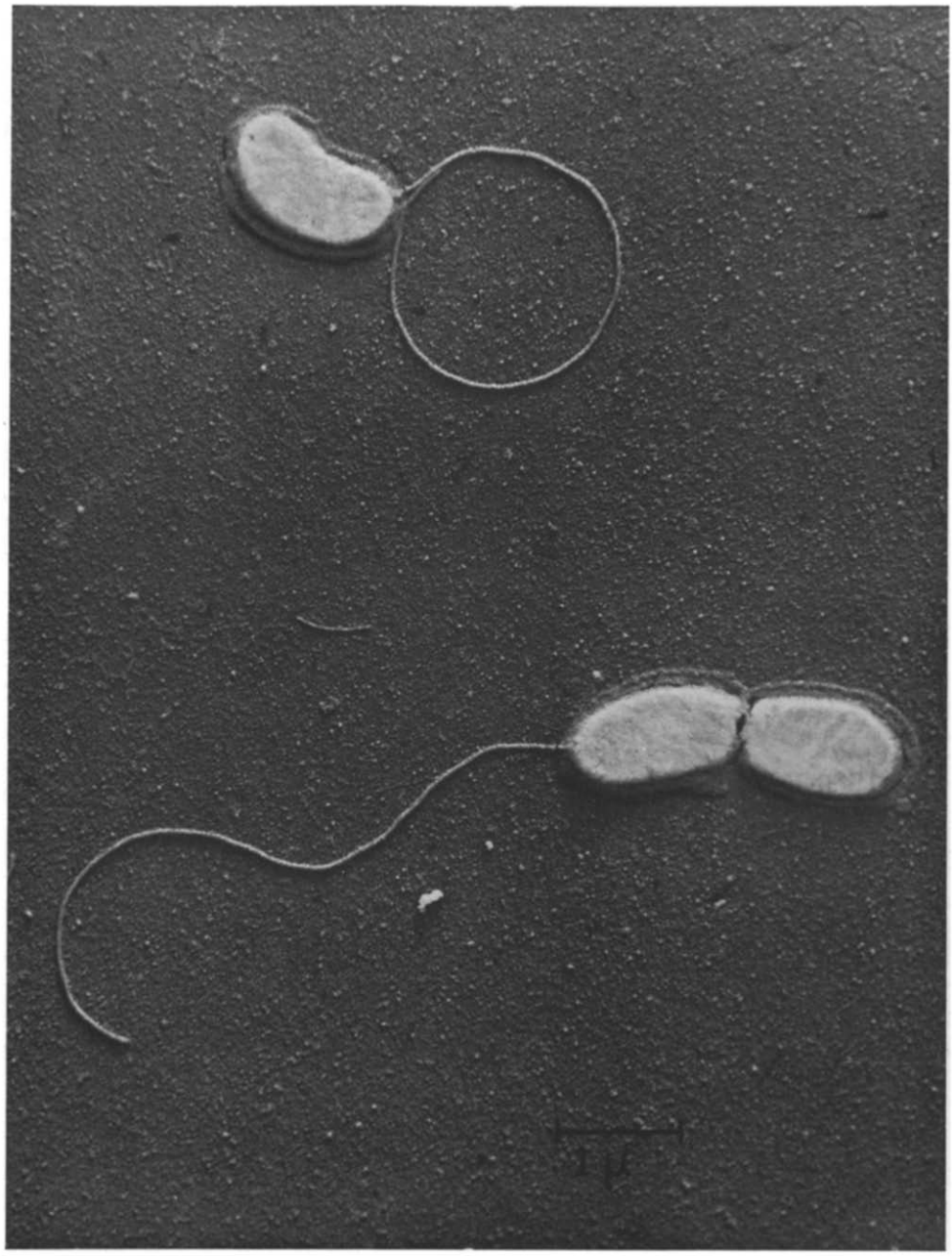

Fig. 9. *Vibrio metchnikovii*. 17000 ×; 90 kv; shadow-cast. Beautiful example of an undoubtedly monotrichous bacterium. The single flagellum has a much more robust character than the tender threads in the preceding pictures. Attention should be drawn to the way in which the circularly bent flagellum is attached to the cell.

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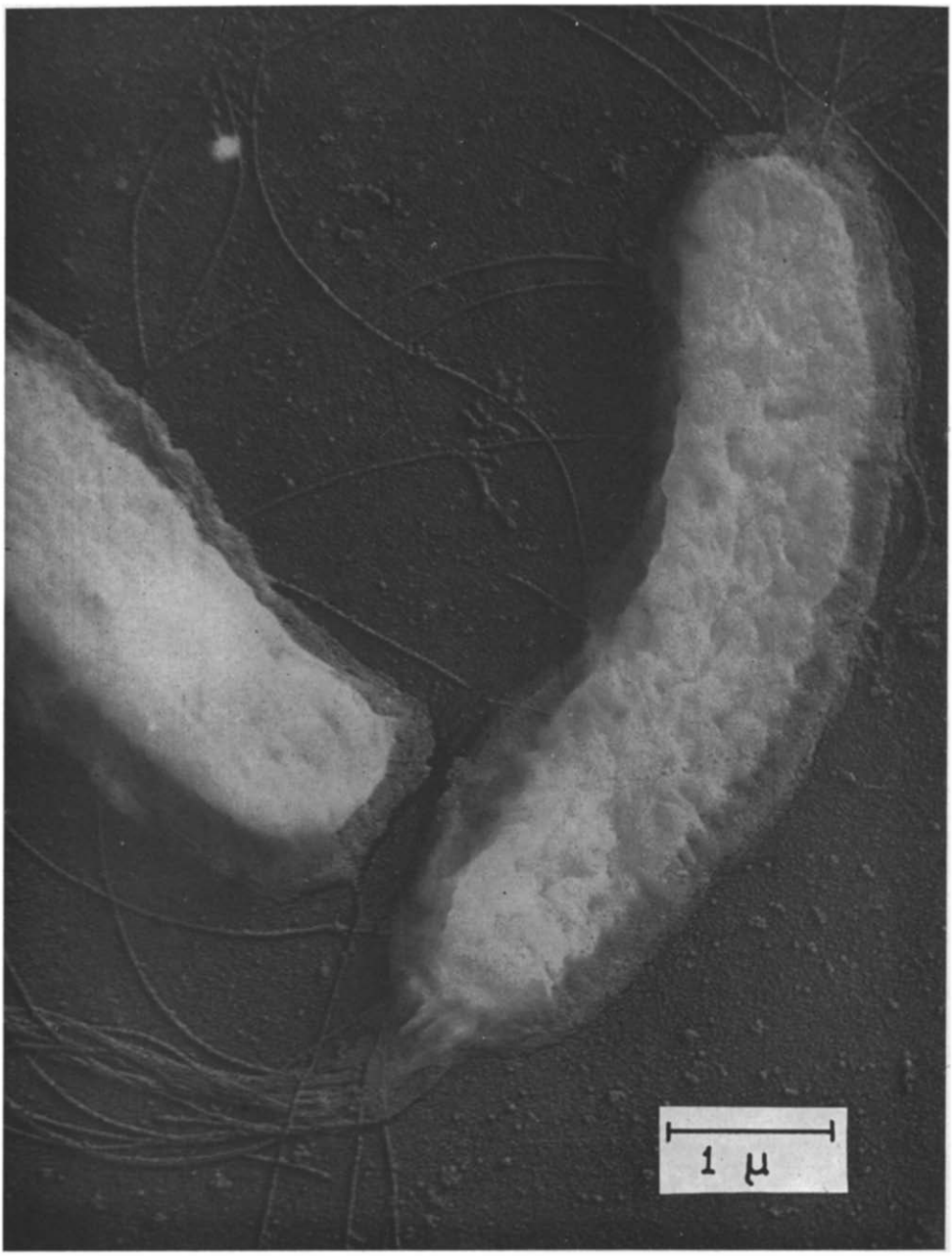

Fig. 10. *Spirillum serpens*. 22 500 ×; 90 kv; shadow-cast. A convincing demonstration of the lophotrichous mode of flagella insertion. Characteristic is the difference in development of the flagellar bundles at both cell ends. In the original print the left cell faintly shows a spherical body.

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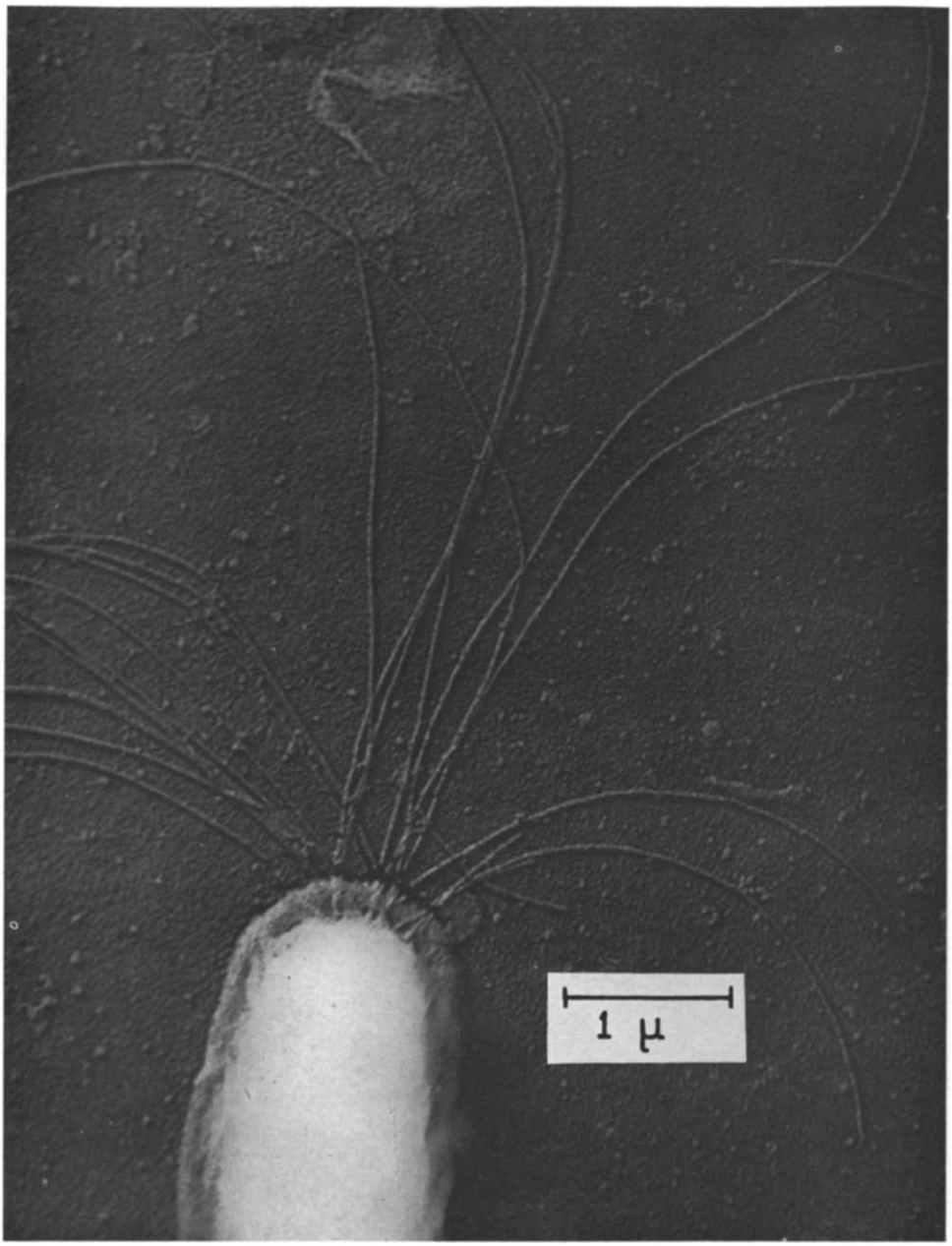

Fig. 11. *Spirillum serpens.* 22 500 ×; 90 kv; shadow cast. Picture of the extremity of a cell with the minor bundle of flagella, suggesting that the latter pierce the cell wall, and are connected with the prot plasm (Note the conical basal parts of the flagella).

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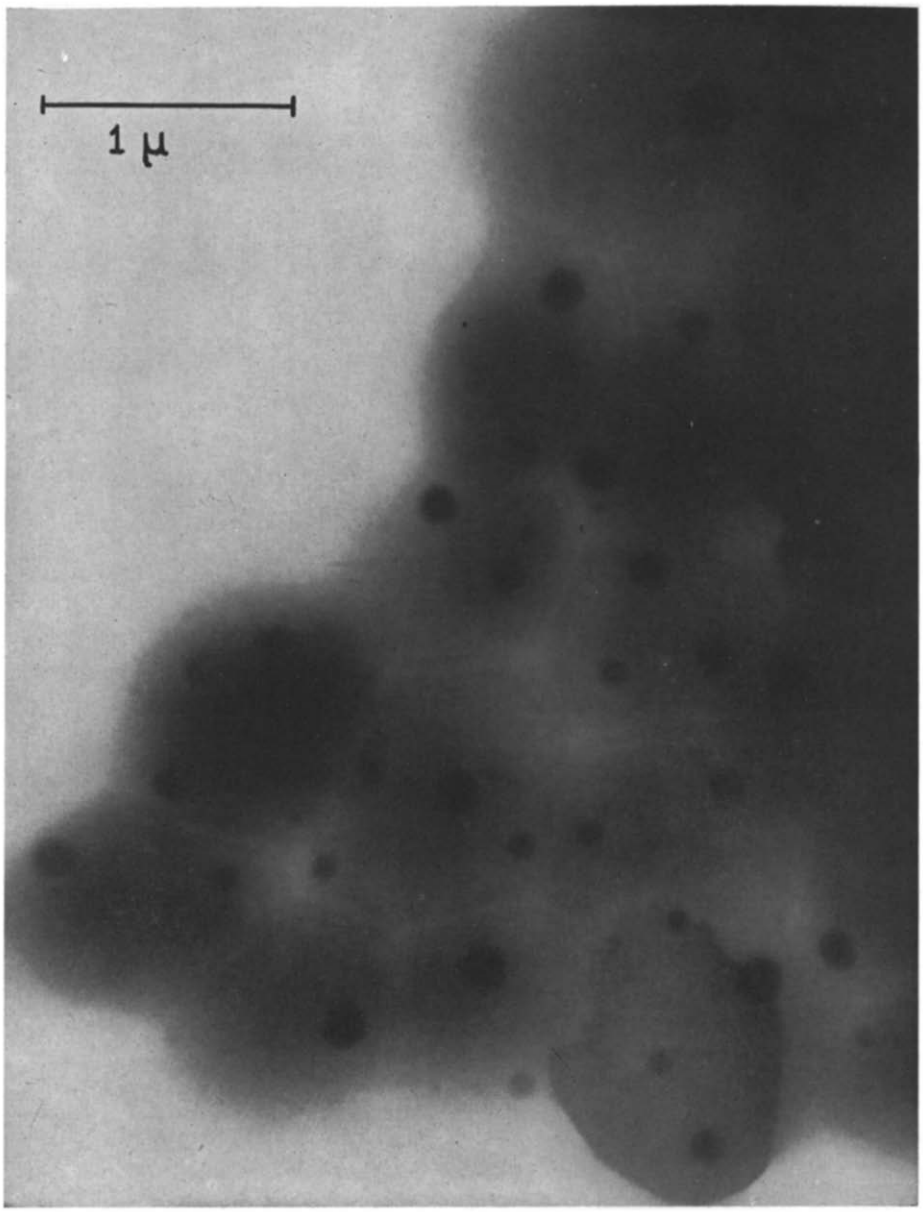

Fig. 12. *Sarcina flava.* 33000 ×; 110 kv; shadow-cast. A remarkable feature of this micrograph is the occurrence of several dark granules of variable size in the cells. These granules became only clearly perceptible when the voltage of the electron emission was raised to 110 kv or higher. The cell wall can be seen as a double clear line.

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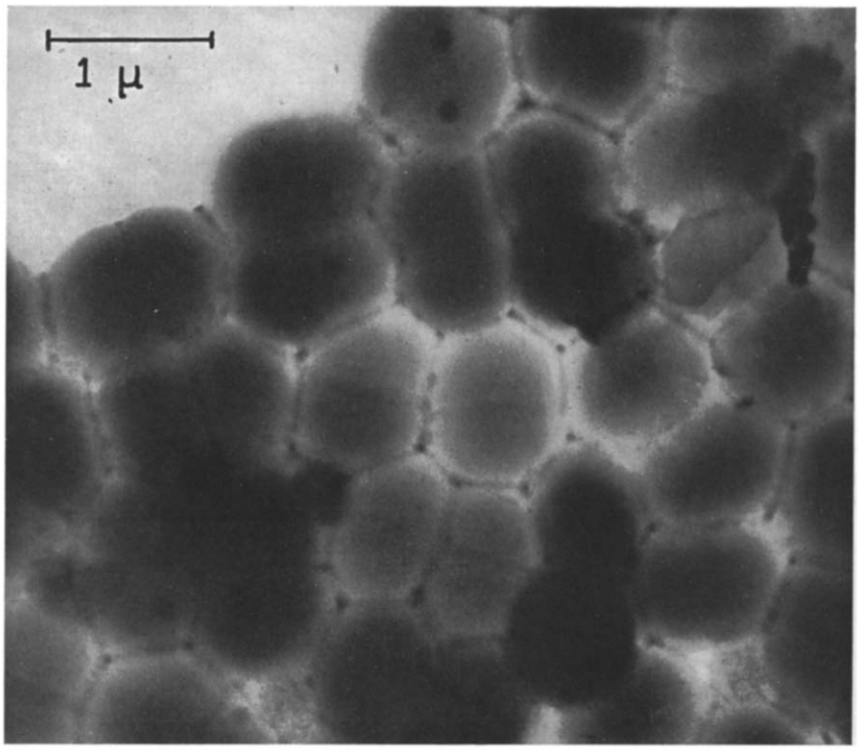

Fig. 13. *Micrococcus varians*. 21 000 ×; 110 kv; not shadow-cast. Even with the high voltage used only a few cells show the dark granules so characteristic of *Sarcina flava*. The most striking feature is the mainly hexagonal cell shape, obviously resulting from the mutual pressure of the various cells. The cell walls themselves are not so distinct as in the preceding figure, but the dark lines which separate the cells are suggestive of the presence of a "middle lamella" and triangular intercellular spaces. Attention is drawn to the first indication of cell division as evidenced by the appearance of a broad lamella dividing the cell.

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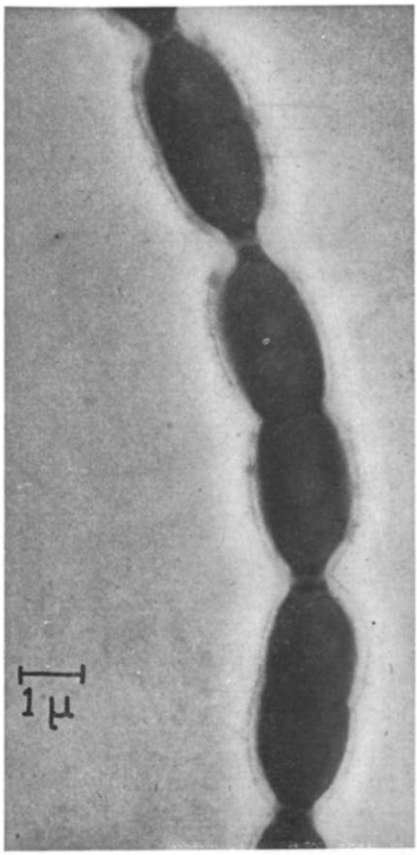

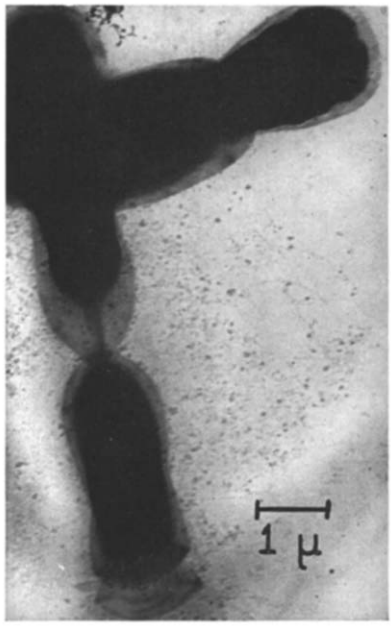

← Fig. 14. *Bacillus mycoides.* 9000 × ; 70 kv; not shadow-cast. This picture is illustrative of the first phases of cell wall formation. The first symptom of cell division is again the appearance of a dark lamella in the center of the cell, accompanied by a notch in cell wall and protoplasm. At both sides more transparent parts of the protoplasm reminiscent of vacuoles show up.

Fig. 15. *Bacillus mycoides.* 10000 × ; 110 kv; not shadow-cast. This picture is only reproduced in order to show the presence of a plasmodermid connecting two dividing cells.

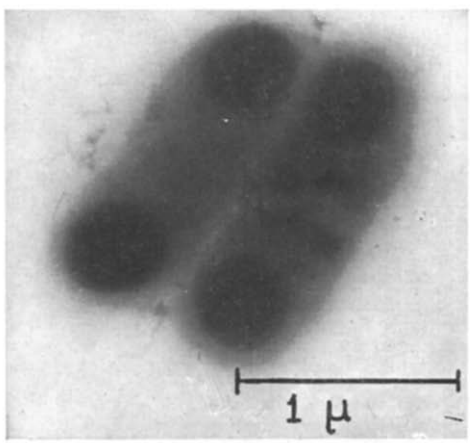

Fig. 16. *Corynebacterium xerose.* 30000 × ; 90 kv; not shadow-cast. A demonstration of polar bodies so typical for the bacteria of the genus *Corynebacterium*, well-known from light-microscopical observation after staining of the cells.

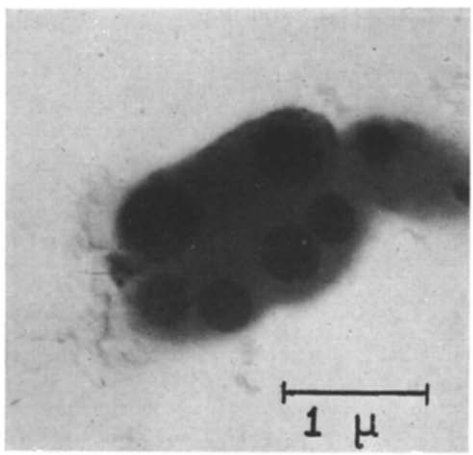

Fig. 17. *Corynebacterium xerose.* 19000 × ; 90 kv; not shadow-cast. This picture gives evidence that cell division is accompanied by a multiplication of the polar bodies.

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It is, however, possible that this shape — as is suggested by PIJPER[3] — has to be attributed to a "rigor mortis" responsible for the stretching of an in the living state slightly curved rod.

A few words may be devoted to the empty cell wall bladders which are a more or less constant feature of the "secondary" cells of *Pseudomonas fluorescens* (Fig. 5 and 6). It cannot be decided how these bladders originate, a contraction of the protoplasm during the preparation may well be responsible. Anyhow, it is noteworthy that the occurrence of unilateral balloon-like swollen empty spaces within the cell wall has so far only been encountered in the mentioned species.

The figures 12 and 13 which are dealing with coccoid cells are characteristic for the hexagonal shape of the cells in the conglomerates. It is obvious that this particular shape results from the mutual pressure which the individual cells exert on each other. Whether this state is already present during the growth of the bacteria in the colony, or whether it is produced only in the drying on the "Geiseltallack"-membrane must be left undecided.

b. Cell wall

All figures yield a confirmation of the result of earlier investigations (Cf. for instance 5, 6, 7, 8, 9, 10) that the cells of all representatives of the *Eubacteriae* are characterized by the presence of a distinct cell wall. This is particularly manifest in the picture of *Serratia marcescens* (Fig. 1) and in that of *Pseudomonas fluorescens* (Fig. 6).

It may be remarked by the way that unpublished investigations on the cytology of a representative of the Myxobacteria, *viz.*, *Cytophaga myxococcoides*, and a corresponding investigation of the Spirochete *Leptospira biflexa*[11] have learned that in these cases an equivalent formation fails.

The micrograph dealing with *Sarcina flava* (Fig. 12) is remarkable for the dark lines which separate the individual cells. It is obvious that these lines mark the spots or surfaces of adhesion of the walls of two adjoining cells. Although strongly suggestive of a distinct middle lamella as well-known from the lightmicroscopical studies of cell walls in higher plants, such a conclusion could only be made with considerable reserve. It seems possible that the mutual flattening of the cells leads to a more or less perpendicular position of the walls, so that the electron beam has to pass here through a thick layer of the wall material. The same holds for the picture of *Micrococcus varians* (Fig. 13), where the same dark lines are conspicuous. Here, however, the triangular dark spots which suggest intercellular spaces are an additional feature. Their opaqueness to the electron beams proves that these spaces are not empty, possibly they have been filled up with some slimy excretion product. Once accepting this there is no reason to reject the same explanation for the dark lines themselves, implying a difference in chemical constitution between the material of the lines and the cell wall proper.

c. Cell wall formation

It seems worth while to dwell upon some data which can be derived from the pictures as regards the formation of a new cell wall in the process of cell division. In Fig. 13 of *Micrococcus varians* attention should be drawn to the presence of faint dark lines dividing several of the hexagonal cells into two pentagonal parts. Moreover here and there I could observe these lines to continue in adjacent cells. It seems not doubtful

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that we are dealing here with the beginning of a membrane formation induced by a protoplasmic differentiation or concentration. On the basis of this picture no conclusions are possible as to the process which leads from this beginning to the formation of two separate cells, yet it is tempting to suggest a possible connection between this initial lamella and the "middle lamella" previously discussed.

Another picture of interest for cell wall formation is offered by Fig. 14 representing *Bacillus mycoides*. The first symptom of cell division is again the appearance of a dark lamella in the middle of the cell (the top cell of the chain). This lamella seems usually to extend also in the transparent zone of the cell wall. A notch in the cell wall and in the protoplasm marks the beginning of the constriction ultimately leading to the formation of the two daughter cells. As shown by the central pair of cells the constriction progresses which may be due to a contraction of the protoplasmatic lamella. In a final stage the constriction is so accentuated that both sides of the cell wall contact each other, and it is noteworthy that the shortened protoplasmic lamella is still present in the form of two separated broad bands which may well have been responsible for the interknitting of the originally separated cell walls. In a later stage the cytoplasm of the daughter cell may sometimes recede leaving, however, a plasmodesmid as evidenced in Fig. 15. The latter figure corresponds rather well with the still more marked plasmodesmid shown in JOHNSON's picture of *Bacillus cereus*[12].

d. The cytoplasm

Our pictures of *Serratia marcescens* (Fig. 1) and of *Pseudomonas fluorescens* (Fig. 5, 6 and 7) tend to support the division which PIEKARSKI and RUSKA[5, 6, 13, 14] have advocated between primary and secondary bacterial cells. In the first type of cells the cytoplasm is homogeneously distributed inside the cell wall cavity, whilst in the secondary type the protoplasm has contracted, and here and there — often at one or both of the extremities of the cell — has receded from the cell wall. Whether this protoplasmatic contraction has already proceeded in the living cell, or only marks a difference in behaviour of both types of cell towards the drying process during the preparation may be considered to be of secondary importance. It should be remarked that the German authors cited base their differentiation also on observations apart from the electron microscopical data. As further support for the reality of the difference in the two cell types I wish to stress that whenever the electron microscope shows the presence of dark spherical bodies, suggesting nuclei, this always pertains to cells of the secondary type. This is the more remarkable because, if such bodies were also present in primary cells, one should expect that they would be more visible in the much more transparent protoplasm of the cells of this type.

e. The nucleus problem

It is not at all the intention to discuss here the delicate problem of the occurrence or non-occurrence of discrete nuclei in bacterial cells. For this I refer to the review of LEWIS[15] and the books of KNAYSI[16] and of DUBOS[17]. It is at once clear that electron-microscopical observations on unstained cells can only contribute in a restricted way to the solution of the nucleus problem, in so far that if discrete nuclei of a type comparable to the nuclei of the cells of higher plants are present, there will be a chance — but not more than a chance — that they will show up in the micrographs made. If, however,

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the cells in these micrographs appear to be devoid of characteristic bodies it would be incorrect to conclude that discrete nuclei are absent.

On the other hand if the micrographs show the presence of bodies which reasonably correspond to our conception of a bacterial nucleus one should still always realize that all proof that one is, indeed, dealing with a structure worthy of the name of nucleus is completely lacking.

All this implies that the only contribution which we can make on the basis of more or less incidentally made micrographs is to answer the question whether we find in the cells bodies which *might* represent nuclei. It is true that the chances for the correctness of such an identification will be enhanced, if indications are found that cell division is accompanied by a multiplication of these bodies, and also if there is correspondence between the observed structures and those revealed by application of specific staining methods such as FEULGEN's in light-microscopical work.

However, the following inspection of micrographs is mainly confined to an enumeration of those cases in which nuclear-like bodies are discernible. It is only for shortness' sake that we shall designate these structures as "nuclei".

In the picture of *Serraria marcescens* (Fig. 1) in the primary cells all structure is lacking, whilst in the secondary cells any possibly present "nucleus" will be hidden in the shrunken protoplasm. In the picture of *Bacillus subtilis* (Fig. 2) at each end of the evidently grown-out cell discrete bodies are present which might well represent a nucleus after division. Several additional pictures (not reproduced) of the same bacterium show the same characteristic in so far that immature cells have only one "nucleus", apparently mature ones generally two "nuclei". Figures 4 and 5 show that the "nucleus" may under certain conditions also occur near the center of the cell. In the original print reproduced in Fig. 5 one small "nucleus" may be discerned in the spore-forming cell, quite near to the spore. A similar situation was found in other spore-forming cells. These observations are in agreement with STILLE[18], but are in contrast to the earlier light-microscopical observations of BADIAN[19] and KLIENEBERGER–NOBEL[20] who report the presence of three nuclear bodies besides the spore.

In the picture of a secondary cell of *Pseudomonas fluorescens* (Fig. 6) two "nuclei" may be signalized, occurring in the dense contracted protoplasm. In the survey of the pictures attention has already been drawn to the occurrence in Fig. 7 and 8 of several "nuclei", probably in a state of division, mutually connected by fine threads. These structures taken as a whole are strongly reminiscent of the dumbbell-shaped figures revealed amongst others by ROBINOW[21] in several types of bacteria. The variable size of these "nuclei", and the fact that they sometimes occur in odd numbers in the cell should be noted.

Although in the pictures of *Spirillum serpens* reproduced in Fig. 10 and 11 nuclei are not clearly discernible, it may be remarked that in other (not reproduced) pictures often two large "nuclei" situated at both ends of the cell have been observed.

Since, however, *Spirillum serpens* is known to produce under certain conditions volutin globules, an interpretation of the structures observed is especially delicate in this case.

The picture of *Sarcina flava* (Fig. 12) is characterized by the occurrence of an irregular number of dark granules of variable size in the cells. We should hesitate to designate these granules as "nuclei", were it not that KNAYSI and MUDD[22] who present an almost identical picture of *Staphylococcus flavo-cyaneus* — also made with high voltage — give arguments in favour of the nuclear nature of the structures in question.

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The picture of *Corynebacterium xerose* (Fig. 16) gives a clear demonstration of the polar bodies characteristic for the bacteria of this genus, and well-known from light-microscopical observations of stained preparations. The large dimensions of these bodies as compared to the diameter of the cell are noteworthy. Fig. 17 gives evidence that cell division is accompanied by a multiplication of the polar bodies, which fact could be explained as being in favour of their "nuclear" nature.

An important objection to be expected from the side of those readers acquainted with the classical conception of the single nucleus in higher plant cells, will be the revelation of the sometimes large and inconstant number of the bodies described in some of the present bacteria (in particular in Fig 12 of *Sarcina flava* and Fig 7 and 8 of *Pseudomonas*). In the absence of additional data in favour of a nuclear nature it may seem rather speculative to dwell upon a possible explanation. Still the author is tempted to emphasize the bacteria's active state of cell multiplication, which might involve in these primitive organisms a rapid nuclear division going ahead of the cell division.

As to the repeatedly made observation of two nuclei situated each in one end of rod-shaped cells (*Bacillus subtilis* amongst others in Fig 3; *Spirillum serpens*, and perhaps in *Corynebacterium xerose*) it might be remarked by the way that in general this is in rather satisfactory agreement with the descriptions given for cells from young cultures by authors using the FEULGEN technique (STILLE[18], PIEKARSKI[13]).

But the author is fully aware that the incidental data reported in the foregoing discussion at best contribute only in a very modest way to an elucidation of the nuclear problem; moreover the observations made may act as a stimulant for future more systematic studies.

f. Flagellation

The problem of bacterial flagellation is nowadays again in the lime light owing to the fundamentally important and challenging studies of PIJPER[3, 4, 23]. These studies have the great merit that they are based on observation of bacteria in living, and even active state. In as far as these studies deal with the mode of motility of the bacteria electron-microscopical investigations of dead and desiccated cells are of no avail. However, such observations may serve to check the correctness of PIJPER's revolutionary conclusion that bacterial flagella are not organs of locomotion, but only "products of motility". It seems appropriate to give here a short quotation taken from one of PIJPER's publi-cations:

"Flagella" are not motor organs but rather fortuitous appendages. The gyrating and undulating movement of the bacterial body originates in the protoplasm which lines the inner surface of the cell wall. It forces the cell wall into the shape of a moving spiral. As a result, the outer covering of the cell wall, which consists chiefly of polysaccharide material, is during fast movement, with the added effect of friction, mechanically twisted into a tail . . . At other times this twisted material untwists into a varying number of wavy threads, which have so far gone under the name of "flagella".

In the following review of the pictures in which flagella occur I shall chiefly confine myself to a discussion of the question whether these pictures offer arguments pro or contra PIJPER's views.

In the Fig. 2 and 3 of *Bacillus subtilis* the abundance of very distinct wavy threads is the most striking fact. Further it is clear that these threads are very fragile. Several of them are obviously broken off, so that there is not the slightest guarantee that they

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all belong to the pictured cell. Nevertheless it is good to state that the examination of the preparation which preceded the taking of the micrograph had shown that the whole field was covered with scattered threads. It seems, therefore, highly probable that one cell is responsible for a very large number of these threads. The presence of so many loose threads makes it impossible to give an opinion regarding the way in which they are inserted into the cell. Another feature is the uniformity of diameter both over the whole length of one thread and of various threads when mutually compared. The characterisation of bacterial flagella as "hollow tubes" as was advocated by POLEVITZKY[24] on the ground of his electron-microscopical observations does not find any support in the pictures obtained in this study.

Taking all together it seems likely that the threads in question must be identified with the elementary flagella which have been described by several light-microscopists as characteristic for peritrichous bacteria. On the other hand — taking into account the diameter of the threads — it must be deemed probable that the restricted number of flagella which usually are made visible by the ordinary flagella stains can only be explained by accepting a twisting or a sticking together of the threads revealed by the electron microscope.

As for PIJPER's theory that bacterial flagella are "polysaccharide twirls" originating from the mucous covering of the cell wall in consequence of the motility of the cell we wish only to remark that the more or less perfect standardization of the threads as apparent from the micrographs is not in favour of the theory.

Fig. 5 of *Pseudomonas fluorescens* which pertains to a lophotrichous bacterium in the classical sense is on the whole not very instructive. At the one end of the largest cell one gets the impression that a flagellar bundle is present, and could be connected with the cell interior.

A more clear-cut example of lophotrichy is, however, found in Fig. 10 and 11, dealing with *Spirillum serpens*. The first picture shows conclusively that this bacterium is equipped with a polar flagellar bundle at each end of the cell. One of these bundles is much more developed than the other, suggesting a polarity of the cell. In this picture it is difficult to arrive at a sharp conclusion as to the way in which the flagella are attached to the cell. For the main flagellar bundle it is impossible to exclude a direct connection with the cell wall, although there is some indication that protoplasm has also extended into the basal part of the bundle.

The situation, however, appears quite different in Fig. 11. Here the flagella of a minor bundle are unmistakably connected with the protoplasm of the cell, and at least for a few of these flagella it is almost certain that they have their origin in small rhizoid protoplasmic extensions. It must be added to this description that it cannot be decided at which spots the flagella pierce the cell wall: it seems possible that in the region where the protoplasm had receded the flagella in question are lying on the surface of the cell wall, but it is neither excluded that the flagella pierce the wall at a greater distance from the protoplasmatic contact, and only show up because of the great transparency of the wall.

Finally Fig. 9 dealing with *Vibrio metchnikovii* gives a striking illustration of a monotrichous bacterium. The picture is not in need of much comment, but here too a protoplasmatic connection of the single flagellum is strongly suggested whilst one gets the impression that in the cell with the circularly bent flagellum the latter originates from a basal granule (blepharoplast). In this respect it should be mentioned that

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KNAYSI[16] claims to have encountered the same situation in the electron micrograph of *Vibrio cholerae* made by MUDD and ANDERSON[8]. Moreover an analogous structure can be observed in the micrograph of *Vibrio schuylkiliensis* by MUDD, POLEVITZKY, and ANDERSON[25].

In the light of the foregoing it does no longer seem possible to subscribe to PIJPER's recent statement:

"Even the electron microscope has not brought evidence that "flagella" pierce the cell wall, witness the comprehensive review of MUDD and ANDERSON".

But even if one should not be inclined to consider the foregoing micrographs as definite proof of a protoplasmatic origin of bacterial flagella it must be difficult in view of these micrographs to accept the general validity of PIJPER's thesis that flagella are merely "products of motility" originating from mucous polysaccharides.

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SUMMARY

Some micrographs from bacteria taken with the Delft electron microscope are reproduced. The text is principally confined to a discussion of some cytological characteristics as shown by these micrographs.

a. Although it is questionable whether micrographs of bacteria prepared in a primitive way are apt to furnish useful information regarding the *cell shape*, the electron micrographs obtained were in satisfactory agreement with the current conceptions as based on light microscopical observations (Fig. 9 and 10). *Pseudomonas fluorescens* (Fig. 5) was found to be slightly bent like a spirillum, which tends to confirm PIJPER's recently advanced theory, however. Fig. 2 of *Bacillus subtilis* shows a perfectly straight rod. *Pseudomonas fluorescens* (Fig. 5 and 6) often shows characteristic cell wall bladders. Coccoid cells often assume hexagonal shape (Fig. 12 and 13).

b. A distinct *cell wall* was clearly perceptible in all investigated Eubacteria. Special attention has been paid to the appearance in conglomerates of coccoid cells of structures reminiscent of "middle lamellae" and "triangular spaces" in tissues of higher plants.

c. With regard to *cell wall formation* there is reason to conclude that the initial phenomenon is the appearance of a dark "lamella" in the central part of the cytoplasm (Fig. 13). In bacteria usually designated as rods, this phenomenon is accompanied by a notch in protoplasm and original cell wall, as a first indication of the progressing cell constriction, ultimately leading to cell division (Fig. 14).

d. Micrographs of some strains of bacteria (*Serratia marcescens* and *Pseudomonas fluorescens* show characteristic differences in *cytoplasm* which support a subdivision into primary and secondary cell types as proposed by PIEKARSKI and RUSKA.

e. The frequent occurrence of distinct globular bodies in many bacterial cells which becom eclearly visible only at 75–110 kv has tempted us to make a few remarks regarding the *nucleus problem.* Noteworthy is the fact in case of occurrence of primary and secondary cell types these bodies were exclusively found in the very opaque protoplasma concentration of the latter type. In Fig. 7 and 8 of *Pseudomonas fluorescens* the bodies seem mutually connected by threads, suggesting a recently accomplished division.

f. The problem of flagellation is mainly considered in the light of PIJPER's recent studies. *Bacillus subtilis* micrographs (Fig. 2 and 3) show a striking number of perfectly uniform threads, a great number of which is evidently broken off from the cells. Several micrographs (especially Fig. 11 of *Spirillum serpens* and Fig. 9 of *Vibrio metchnikovii*) are strongly suggestive of a protoplasmatic origin of the flagella.

Taking together all micrographic evidence it is hard to accept PIJPER's thesis that flagella are mere "products of motility", originating from a twisting of mucous polysaccharides.

RÉSUMÉ

Quelques micrographies de bactéries obtenues avec le microscope électronique de Delft sont présentées. Le texte a été limité à une discussion des caractères cytologiques ainsi qu'ils sont démontrés par ces photographies.

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a. On peut se demander si des micrographies de bactéries qui ont subi un traitement assez primitif peuvent donner des renseignements utiles sur la *forme bactérienne*. Pourtant les micrographies électroniques ne sont pas en contradiction avec les idées générales concernant la forme des bactéries, déduites des observations microscopiques ordinaires (comp. Fig. 9 et 10). Conformément aux vues exprimées par PIJPER, *Pseudomonas fluorescens* (Fig. 5) paraît légèrement courbé, tandis que *Bacillus subtilis*, par contre, présente la forme d'un bâton parfaitement droit. Plusieurs cellules de *Pseudomonas fluorescens* (Fig. 5 et 6) sont caracterisées par une grande vésicule de la membrane cellulaire. Dans les préparations les cellules cocciformes montrent souvent une forme hexagonale (Fig. 12 et 13).

b. Chez toutes les Eubactéries étudiées on trouve une *paroi* bien distincte. La présence dans les conglomérats de cellules cocciformes d'éléments de structure qui rappellent les "lamelles moyennes" et les "espaces intercellulaires" des tissus de cormophytes a été signalée.

c. En ce qui concerne la *formation des parois* on a constaté comme phénomène initial l'apparition de "lamelles" dans la partie centrale du cytoplasme (Fig. 13). Dans les bactéries auxquelles en général une forme bâtonnaire est assignée, ces lamelles sont accompagnées d'une légère entaille dans le protoplasme et dans la paroi indiquant le commencement du rétrécissement menant à la division des cellules (Fig. 14).

d. Les micrographies de cellules individuelles de certaines bactéries (*Serratia marcescens* et *Pseudomonas fluorescens*) montrent des différences dans l'apparence du *cytoplasme*, qui semblent soutenir la distinction faite par PIEKARSKI et RUSKA entre types cellulaires "primaires" et "secondaires".

e. La présence fréquente dans les cellules de plusieurs bactéries de corps rond — qui deviennent nettement perceptibles quand on élève la tension d'émission jusqu'à 75–110 kv — nous a conduit à faire quelques remarques concernant le problème du *noyau bactérien*. Il est digne de remarque que dans les bactéries présentant de types cellulaires "primaires" et "secondaires" des corps se trouvent exclusivement dans les concentrations protoplasmiques très opaques du type "secondaire". Dans les Fig. 7 et 8 de *Pseudomonas fluorescens*, ces corps se présentent en paires, liées par des fils, ce qui suggère une division récemment accomplie.

f. Surtout en vue des études récentes de PIJPER, nous avons aussi donné quelque attention au *problème de la flagellation*. Les micrographies de *Bacillus subtilis* (Fig. 2 et 3) montrent un nombre considérable de fils uniformes, bien souvent détachés des cellules. Plusieurs micrographies (surtout Fig. 11, de *Spirillum serpens* et Fig. 9 de *Vibrio metchnikovii*) semblent indiquer une origine protoplasmique de ces flagelles. La totalité des observations n'est pas en faveur de la thèse de PIJPER, que les flagelles ne sont que des "produits de la motilité" des bactéries.

ZUSAMMENFASSUNG

Einige Mikrographien von Bakterien, die mit dem Delfter Elektronenmikroskop aufgenommen wurden, werden abgebildet. Der Text beschränkt sich prinzipiell auf eine Diskussion einiger zytologischer Charakteristika, die diese Mikrographien zeigen.

a. Obwohl es fraglich ist, ob Mikrographien von Bakterien, die auf eine primitive Art bereitet wurden, geeignet sind, um wertvolle Auskünfte über die *Zellform* zu ergeben, stimmten die Elektronenmikrographien, die erhalten wurden, befriedigend mit den gangbaren Auffassungen, die auf lichtmikroskopischen Beobachtungen beruhen, überein (Abb. 9 und 10). *Pseudomonas fluorescens* (Abb. 5) war, wie gefunden wurde, leicht gebogen wie ein Spirillum, was PIJPER's vor kurzem aufgestellte Theorie bestätigen könnte; dagegen zeigt Abb. 2 von *Bacillus subtilis* einen vollkomen geraden Stab. *Pseudomonas fluorescens* (Abb. 5 und 6) zeigt oft charakteristische Zellwandblasen. Kokkenartige Zellen nehmen oft eine sechseckige Form an (Abb. 12 und 13).

b. Eine deutliche *Zellwand* war bei allen untersuchten Eubacteria gut zu erkennen. Besondere Aufmerksamkeit wurde der Tatsache gewidmet, dass in Konglomeraten kokkenartiger Zellen Strukturen erscheinen, die an "Mittellamellen" und "Dreiecksräume" in Geweben höherer Pflanzen erinnern.

c. Hinsichtlich der *Zellwandbildung* ist Grund zu der Folgerung vorhanden, dass das Beginnphänomen das Auftreten einer dunklen "Lamelle" im Mittelteil des Zytoplasmas ist (Abb. 13). Bei Bakterien, die gewöhnlich als Stabbakterien angedeutet werden, wird dieses Phänomen von einer Einkerbung im Protoplasma und in der ursprünglichen Zellwand begleitet, als einem ersten Anzeichen der fortschreitenden Zelleinschnürung, die schliesslich zur Zellteilung führt (Abb. 14).

d. Mikrographien von einigen Stämmen der Bakterien *Serratia marcescens* und *Pseudomonas fluorescens* zeigen charakteristische Unterschiede im *Zytoplasma*, die eine Unterteilung in primäre und sekundäre Zelltypen, wie sie von PIEKARSKI und RUSKA vorgeschlagen wurde, unterstützen.

e. Das häufige Auftreten deutlicher, kugelförmiger Körper in vielen Bakterienzellen, die nur bei 75–110 kV deutlich sichtbar werden, hat uns in Versuchung gebracht, einige wenige Bemerkungen über das *Kernproblem* zu machen. Bemerkenswert ist die Tatsache, dass im Falle des Auftretens pri-

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märer und sekundärer Zelltypen diese Körper ausschliesslich in der sehr undurchsichtigen Protoplasmakonzentration des letzteren Typs gefunden wurden. In Abb. 7 und 8 von *Pseudomonas fluorescens* scheinen die Körper miteinander durch Fäden verbunden zu sein, was eine kürzlich vollzogene Teilung suggeriert.

f. Das Problem der Flagellation wird hauptsächlich im Lichte von PIJPER's jüngsten Studien betrachtet. *Bacillus subtilis*-Mikrographien (Abb. 2 und 3) zeigen eine schlagende Anzahl vollkommen gleichförmiger Fäden, von denen eine grosse Anzahl offensichtlich von den Zellen abgebrochen ist. Mehrere Mikrographien (besonders Abb. 11 von *Spirillum serpens* und Abb. 9 von *Vibrio metchnikovii*) suggerieren stark einen Protoplasma-Ursprung der Flagella.

Wenn man das gesamte mikrographische Beweismaterial zusammen betrachtet, ist es schwierig PIJPERS These zu akzeptieren, dass die Flagella blosse "Beweglichkeitsprodukte" sind, die von einer Drehstreckung schleimiger Polysaccharide herrühren.

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