A MACROMOLECULAR MONO-LAYER IN THE CELL WALL OF *SPIRILLUM* SPEC.*

by

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A big, rod-like bacterium, isolated some time ago by Mr G. W. WIERINGA**, interested this investigator by showing a type of motility not found with other motile bacteria. Though isolated from an enrichment culture for *Beggiatoa*, it is not to be classed as a sulfur bacterium but most probably as a *Spirillum*. The way it generally moves may be described as floundering rather than as swimming. Although it revolves rapidly about its axis the simultaneous displacement in axial direction is insignificant and rarely exceeds the length of the cell. Maximum motility is shown when it is romping with its fellow bacteria, the more the merrier. Occasionally, however, in some preparations the various bacteria are found to travel straight for a 100 μ or so, but then, without any perceptible cause, they switch to full speed astern, and so on.

The flagella. The phase-contrast microscope makes visible what looks like one big flagellum on each end of the rod-like body. In a resting specimen both of these "flagella" have the form of a tightly coiled spring. In a motile specimen the distal "flagellum" is a narrower and much longer spiral. It should not be said to be drawn out and to trail behind the bacterium, since this would be an explanation rather than a description of the observed facts, and in this paper the problem of the mechanism of bacterial motility is better left out of consideration. The apical "flagellum" winds round the bacterium in wide curves. Each time locomotion stops the flagella make a swift movement that looks like uncoiling. The next moment locomotion has started again in reversed direction and simultaneously the flagella have taken on the shapes proper to their interchanged positions.

Electron micrographs of these bacteria show that in reality each of these appendages is a bundle of flagella (Fig. 1). The fact that the latter, though individually only *ca.* 125 A wide, are visible in the phase-contrast microscope, and sometimes even in the bright-field of the ordinary microscope, is easily explained by the twined condition as shown in this and many other micrographs (Fig. 2). The basal part of each flagellum is a small hook, similar to that found in other bacteria[1] (Fig. 5).

The cell wall. Close inspection of Fig. 3—a magnification of Fig. 1—revealed that the cell wall of this species must contain either fibrils in two or three intercrossing layers, or else globules or nearly globular particles arranged in a hexagonal pattern. Similar micrographs have been obtained from *Spirillum serpens*.

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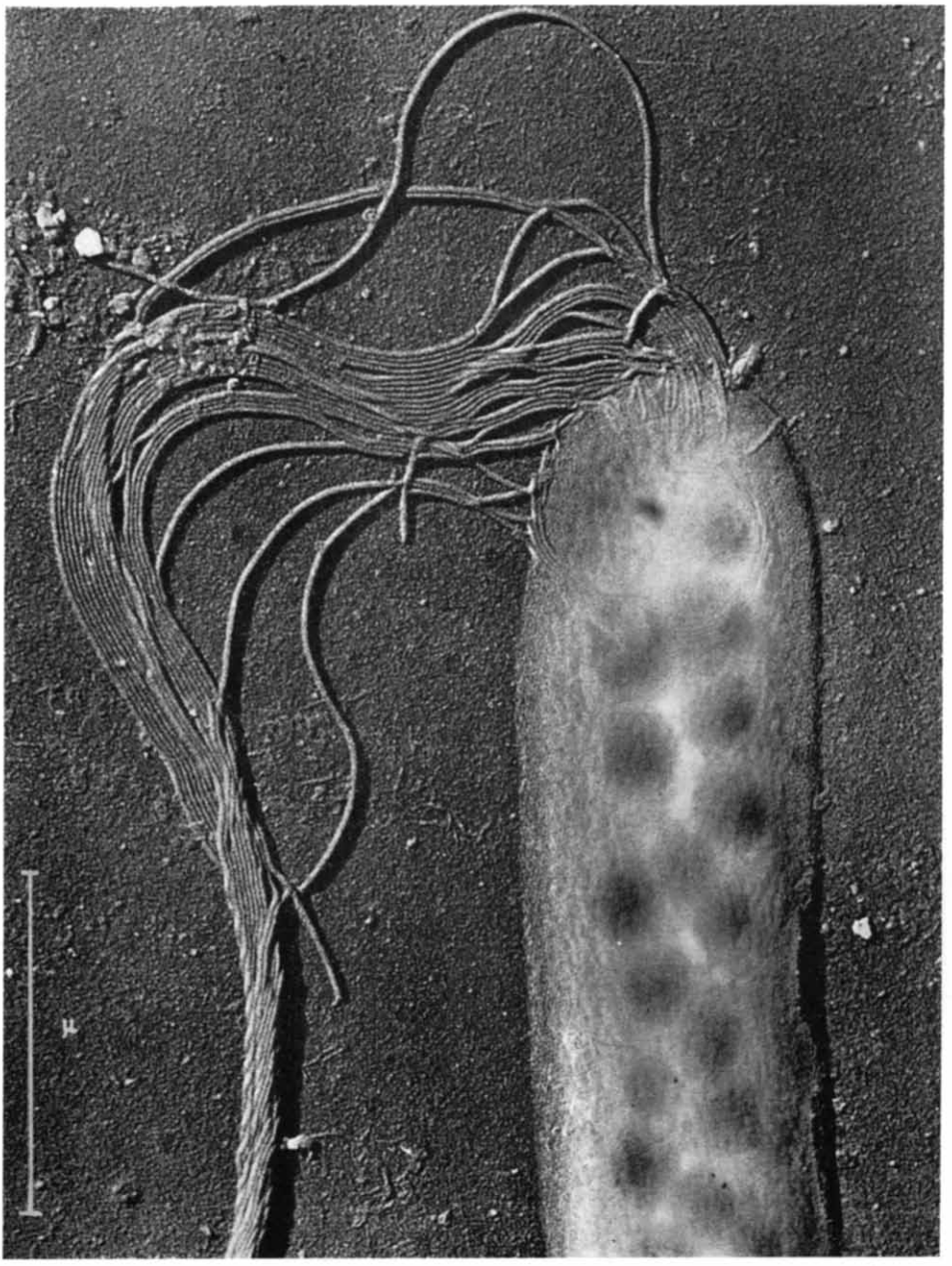

Fig. 1. *Spirillum* spec. isolated by G. W. WIERINGA. Flagella twined to form a thick bundle. Large dark areas in the bacterium may be vacuoles. Structure of the cell wall just visible (*cf.* Fig. 3). 45,000 ×
(Fig. 1–5. Micrographs taken with the modified Philips E.M. of the T.P.D., Delft. Fig. 4 with the short focal-length objective lens designed by J. B. LE POOLE.)

Fig. 2. Tuft of twined flagella. 35,000 ×

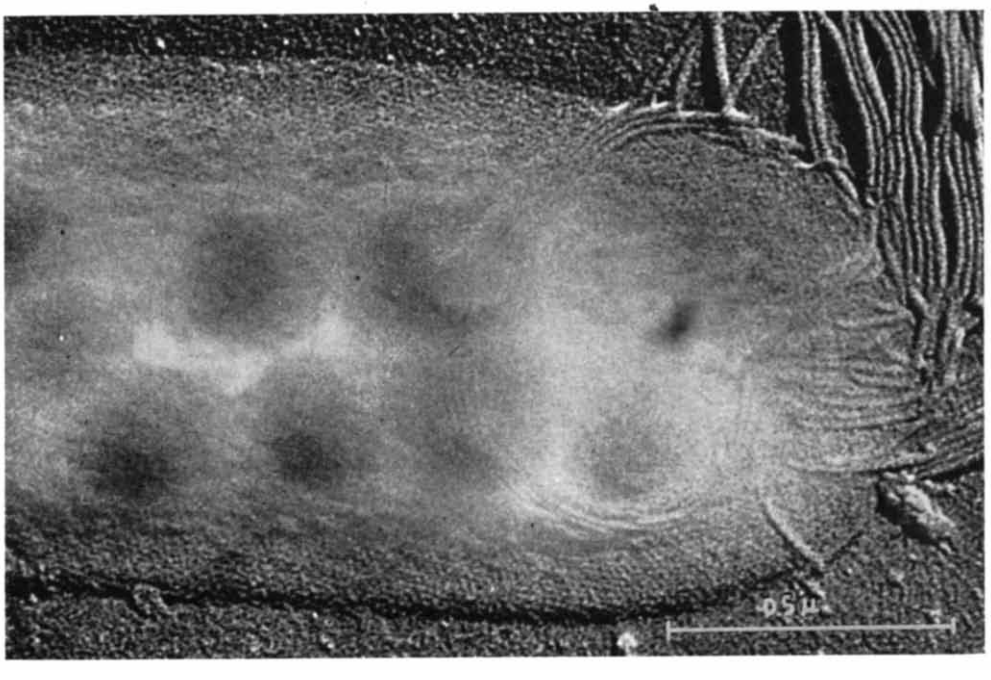

Fig. 3. Detail of Fig. 1, enlarged to show hexagonal pattern on outside of cell wall. 80,000 ×

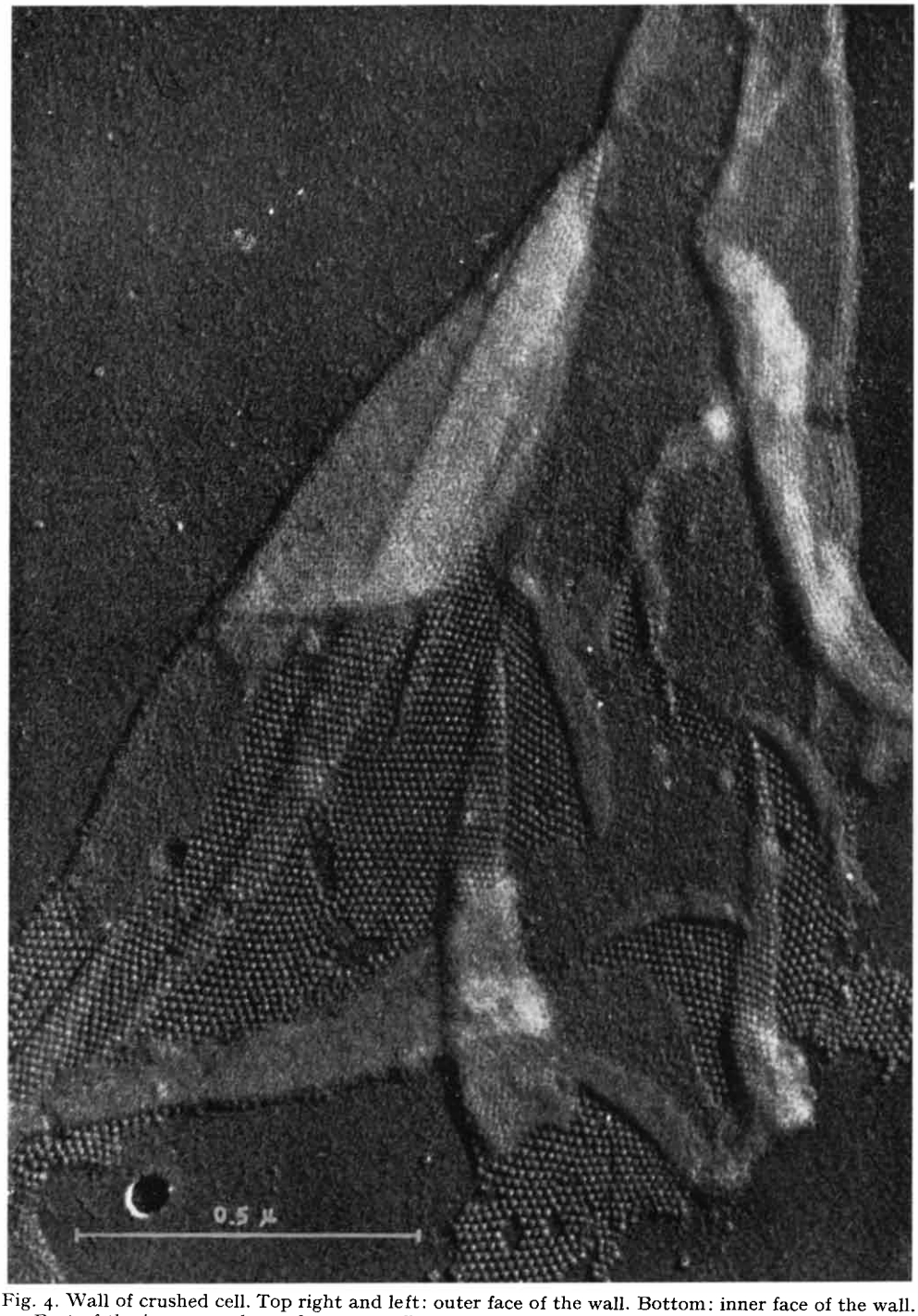

Fig. 4. Wall of crushed cell. Top right and left: outer face of the wall. Bottom: inner face of the wall. Part of the inner membrane has come off, exposing inner face of outer membrane. 100,000 ×

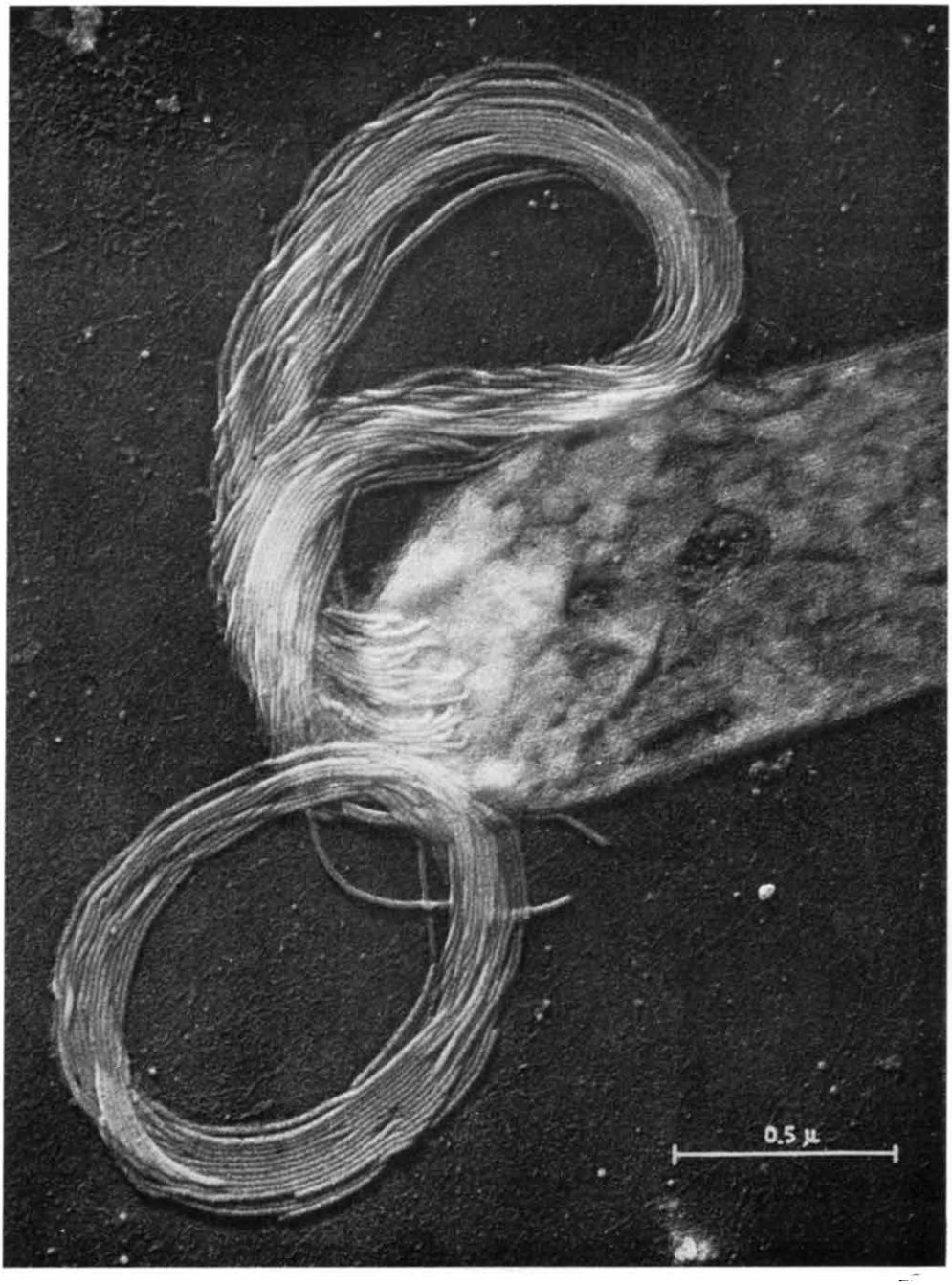

Fig. 5. Bacterium, lysed by unknown cause, shows insertion of flagella. Basal end of each is a small hook. Pattern on outside of cell wall faintly visible. 60,000 ×

In order to become better informed on this unexpected structure, isolated cell walls of the bacterium, as well as of *Spirillum serpens*, have been examined. These were prepared by crushing the cells in a "Mickle Microshaker" and washing the walls on the centrifuge (*cf.* SALTON AND HORNE[2]).

The pattern on the outside of the isolated cell walls is clearly identical with that observed with intact cells. When viewed from the inside, however, the wall looks structureless except in some areas where an apparent inner membrane has come off, so that the inner face of an outer membrane is exposed (Fig. 4). The latter contains or consists of spherical particles arranged in a single layer that resembles the crystals of macromolecular proteins, such as have been studied by WYCKOFF[3]. The dimensions (diameter 120–140 A) fall well within the macromolecular range.

Though the pattern of the inner face of the outer membrane is hexagonal as is that of the outer face, the appearances of the two are different and this presents a problem that remains to be solved. One possible explanation would be that what has been called the outer membrane is composed of two layers instead of one, in which case the cell wall would consist of three layers in total, the layer of globules being situated between the other two. However, no trace of a separate outside sheet, which would cover the globules, has been found. Another explanation would be that the apparent globules are cup-shaped particles instead, with the concave side directed outwards and the rims fused. This would lend the outer face the appearance of a honey-comb.

No data can be given so far regarding the chemical nature of the globules. Any reference to the findings of those authors who studied the composition of the bacterial cell wall seems useless since, on the one hand, no species of the genus *Spirillum* is found among the bacteria investigated and, on the other hand, there is no indication that the structure described here is also a feature of the species that have been included in chemical studies. SALTON AND HORNE[2] did not find any submicroscopical structure in the cell walls of *Escherichia coli* nor in those of *Salmonella pullorum* and *Streptococcus faecalis*. This is corroborated by the results of the present work as far as *E. coli* is concerned.

Neither did we succeed in obtaining evidence for the presence of a similar structure in the wall of the vegetative cells of *Bacillus subtilis*, whereas KNAYSI[4] has reported that the cell wall of a *Bacillus*—but especially that of the spores—contains particles of the same order of magnitude. The latter would not be spherical, though, and a regular pattern like that described in the present paper was not observed by this author.

In the present study only the cell wall isolated by centrifugation has been studied, whereas the protoplasm was discarded. By ultra-centrifugation of the supernatant, however, the macromolecular components of the protoplasm can be investigated, and this has been performed by SCHACHMAN, PARDEE AND STANIER[5]. In the "extract" of all the species investigated "three principal components were found with sedimentation constants of approximately 40, 29 and 5 S". Electron micrographs of the purified 40 S particle fraction show spherical particles with a diameter of *ca.* 140 A. Now, applying the same procedure to our *Spirillum*, one would expect to find a certain percentage of the cell wall globules in the particle fractions. Therefore, the question arises whether part of the macromolecules studied by means of this technique belong to a layer of the cell wall, especially in the case of bacteria related to *Spirillum*.

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SUMMARY

The flagella and the cell wall have been studied in a bacterium apparently belonging to the genus *Spirillum*.

Owing to the twined condition of each bundle of flagella the bundles are easily visible in the phase-contrast microscope. The cell wall is composed of at least two membranes. The outer one contains or consists of a single layer of what may be spherical macromolecules with a diameter of 120–140 A. These are arranged in a hexagonal pattern.

RÉSUMÉ

L'auteur a étudié les flagelles et la paroi cellulaire d'une bactérie faisant partie, apparemment, du genre *Spirillum*.

Les faisceaux de flagelles sont bien visibles au microscope de contraste de phases à cause de l'entrelacement des flagelles appartenant à un même faisceau. La paroi cellulaire se compose de deux membranes au moins. La membrane externe contient ou consiste en une seule couche de ce qui pourrait être des macromolécules sphériques de 120–140 A de diamètre. Ces dernières sont disposées en un dessin hexagonal.

ZUSAMMENFASSUNG

Es wurden die Geisseln und die Zellwände eines Bakteriums untersucht, das wahrscheinlich zur Art *Spirillum* gehört.

Da die Geisselbündel stark aufgedreht sind, sind sie im Phasenkontrastmikroskop leicht sichtbar. Die Zellwand ist aus mindestens zwei Membranen zusammengesetzt. Die äussere enthält oder besteht aus einer einfachen Schicht, die möglicher Weise von sphärischen Makromolekülen mit einem Durchmesser von 120–140 A gebildet wird. Diese sind zu einem hexagonalen Muster angeordnet.

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