THE STRUCTURE OF BACTERIAL CELLULOSE

by

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INTRODUCTION

Electron microscopic studies[1] have shown that the cellulose from several different plants occurs as fibres of a diameter of ca 250 Å. How these fibres are built up and why they have so constant a diameter is still unknown and the investigation of such questions is made especially difficult by the fact that the cellulose is produced within the cells of these plants. They could be studied in thin section but the necessary techniques are not yet sufficiently developed to make this possible. It therefore has seemed more profitable to evade these difficulties by choosing for study an organism which builds cellulose extra-cellularly.

As long ago as 1886 A. J. BROWN[2] described a bacterium which produced a solid membrane when growing on a carbohydrate-rich medium. He found that these membranes were soluble in ammoniacal copper hydroxide and gave reducing sugars when hydrolysed with sulphuric acid. Since he knew that cotton gave these reactions he called the organism *Bacterium xylinum* (following PLINY who used the word *xylium* for cotton). Later investigations by VAN WISSELINGH[3] and HIBBERT[4] demonstrated that the material of these membranes is indeed cellulose. HIBBERT found that membrane is produced only in a culture medium containing hexose sugars, their anhydrides or substances which the organism can readily convert into hexoses. MARK AND VON SUSICH[5] confirmed the identity of bacterial cellulose with cotton cellulose by producing well-oriented x-ray diagrams from stretched bacterial membranes that were the same as those from cotton. The original electron micrographs of FRANZ AND SCHIEBOLD[6] showed that these bacterial membranes consist of a thick network of fibres and ribbons. They concluded that the ribbons had a thickness of ca 100 Å, a breadth of ca 5000 Å and that they were composed of smaller fibres of ca 200 Å diameter. The fact that the cellulose fibrils in the walls of plant cells have nearly the same size now raises the question of whether these are indeed primary structural units of cellulose itself. The present paper describes results obtained in attempts to examine this question using electron micrographs of bacterial cellulose.

EXPERIMENTAL

Our previous work has shown (FREY-WYSSLING AND MÜHLETHALER[7]; MÜHLETHALER[8]) that liquid media are best for making electron microscopic preparations of

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bacterial cellulose. We have accordingly grown *Bacterium xylinum* for this purpose in WATERMAN's sucrosebeer solution. This is prepared by adding 40 grams of sucrose to a liter of beer. A portion of such a medium was inoculated by dropping into it a small piece of membrane from an old culture. After a short time slimy fibres extended from this inoculum towards the surface. When they reached it they spread as a fine water-clear skin which quickly thickened and after a few days was too tough to be torn or cut without altering its natural texture. To make preparations for electron microscopy the thin, early film was lifted from the culture medium with a glass microscope slide, washed several times in water and dried, still on the slide. Pseudoreplicas like those used for studying the growth of bacteriophage on an agar surface (EDWARDS AND WYCKOFF[9]) have been made of these. This has been done by pouring collodion diluted with amyl acetate over the surface and draining until dry. The resulting film was floated off onto a water surface, cut into pieces, mounted on grids in the usual fashion and shadowed with either chromium or palladium. It should be emphasized that though these preparations are made as if they were replicas they do not replicate the cellulose film which remains embedded in the collodion and is itself observed in the microscope. In some instances a different kind of preparation was made for microscopy by removing a bit of the bacterial colony with a platinum needle, suspending it in water, drying a droplet of this suspension on a collodion covered screen and shadowing.

RESULTS

The young rapidly growing bacteria have characteristic shapes as seen in the electron microscope. They are 2–4 μ long and are flattened on one end (Fig. 1). Midportions of the bacteria have been coated with an amorphous homogeneous substance which increased with bacterial age and eventually embedded the organisms (Fig. 2). Cellulose fibres have begun to form after this has happened. They appeared first as short-fibred thickenings distributed here and there in the amorphous material (Fig. 3). Long fibres have rapidly grown from these thickenings, seemingly at the expense of the capsular substance. As Fig. 3 indicates this development of cellulose has not taken place within a bacterium or from its surface, but rather at some distance from the organism. This suggests that cellulose results from a sort of enzymatically induced polymerization of the embedding slime that can accordingly be thought of as a kind of cellulose-precursor.

As can be seen from Fig. 4 the cellulose fibres have had from the very first a diameter of ca 250 Å. The individual strands were separated from one another by the amorphous substance which decreased as the cellulose developed and had nearly vanished from older colonies (Figs 5 and 6). The separate fibrils have intertwined to form tangled masses, and in this way built tough membranes which could be torn apart only with difficulty. Where the slimy substance has been lacking, several individual fibres have often been seen lying side by side (Fig. 7) to give the bands already described by FRANZ AND SCHIEBOLD. These bands have commonly been twisted about one another into rope-like structures, but it could not be determined with certainty whether this expressed the original arrangement or was caused by the drying. The piece of membrane shown in Fig. 8 shows this phenomenon especially well. Transverse band structures resembling those observed by FRANZ AND SCHIEBOLD are also discernible here, but it is probable that they are the consequence of mechanical damage.

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Fig. 1. Individual bacteria from a young colony of *Acetobacterium xylinum*. Slime has begun to precipitate upon the bacteria. Magnification — 20000 ×

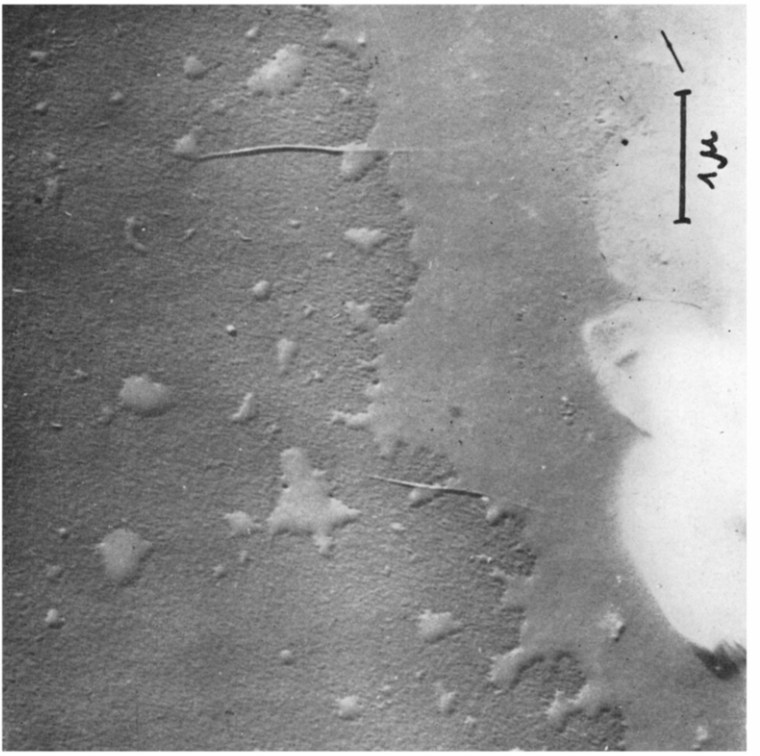

Fig. 2. At a later stage of growth the cellulose is recrystallized in slime which is still devoid of structure. This photograph shows slime about the edge of a micro-colony. Magnification 18000 ×

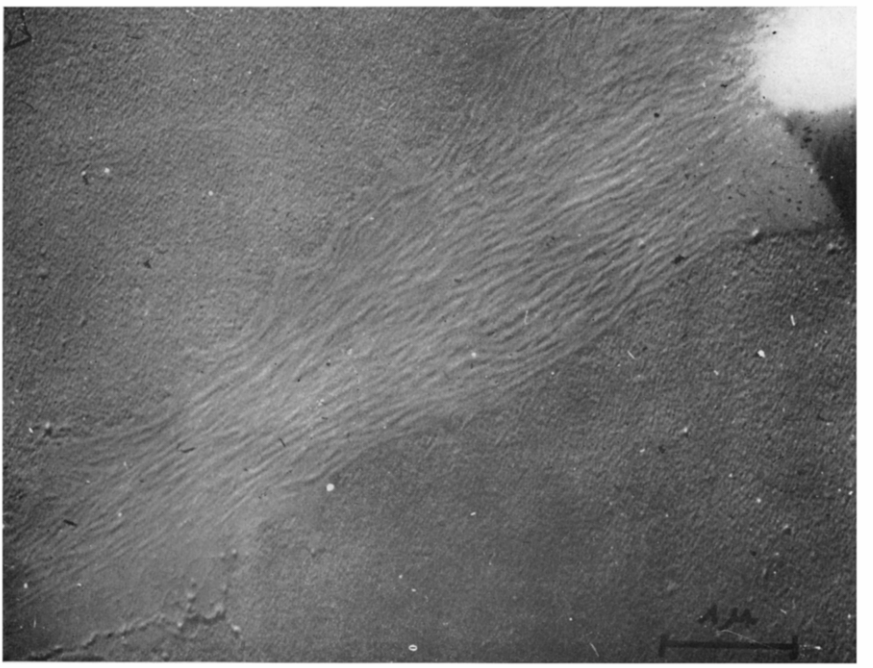

Fig. 3. Strands of cellulose can here be seen forming within the slime. Magnification 18000 ×

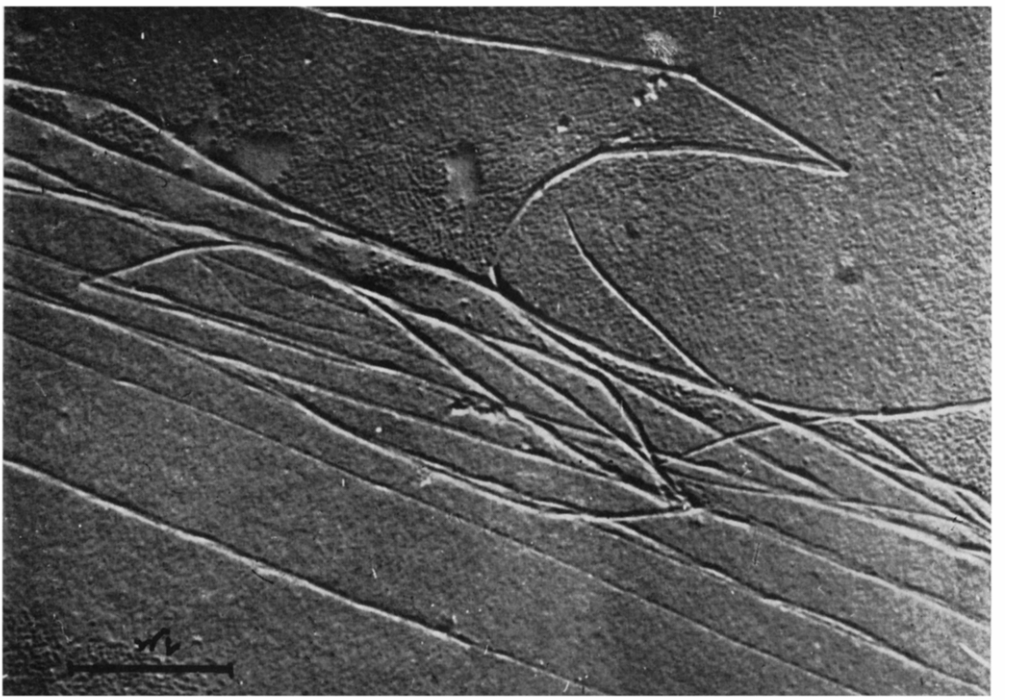

Fig. 4. Several formed cellulose fibrils still embedded in a thin layer of amorphous slime. Magnification = 22000 ×

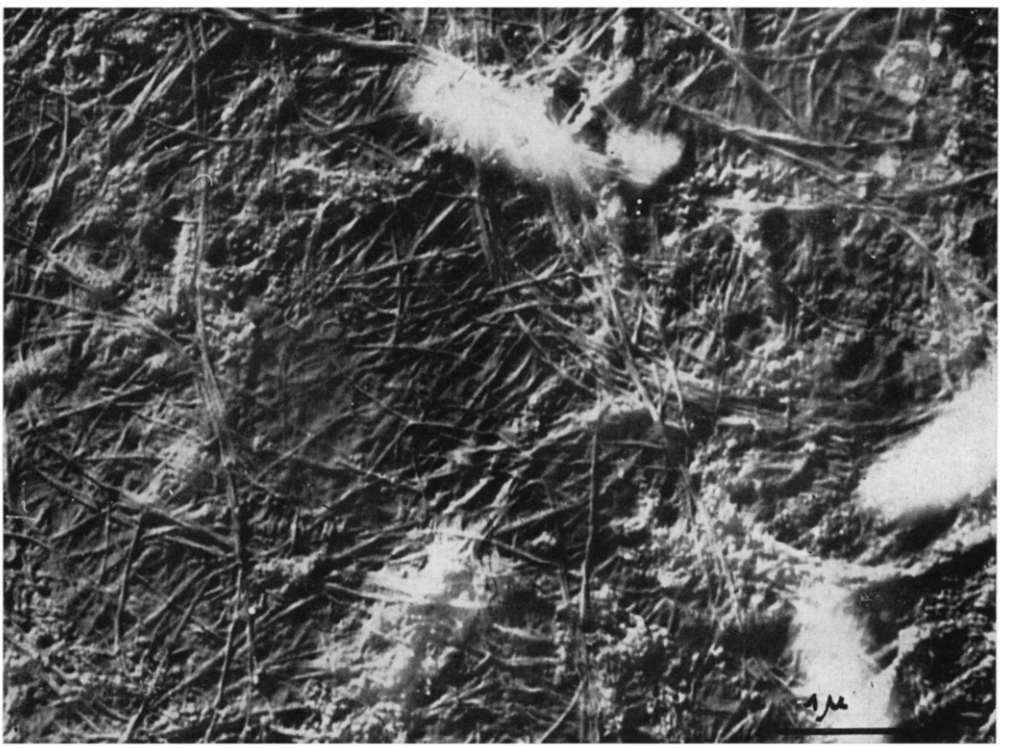

Fig. 5. A dense network of cellulose fibrils. The amorphous stuff has for the most part disappeared. Magnification = 18000 ×

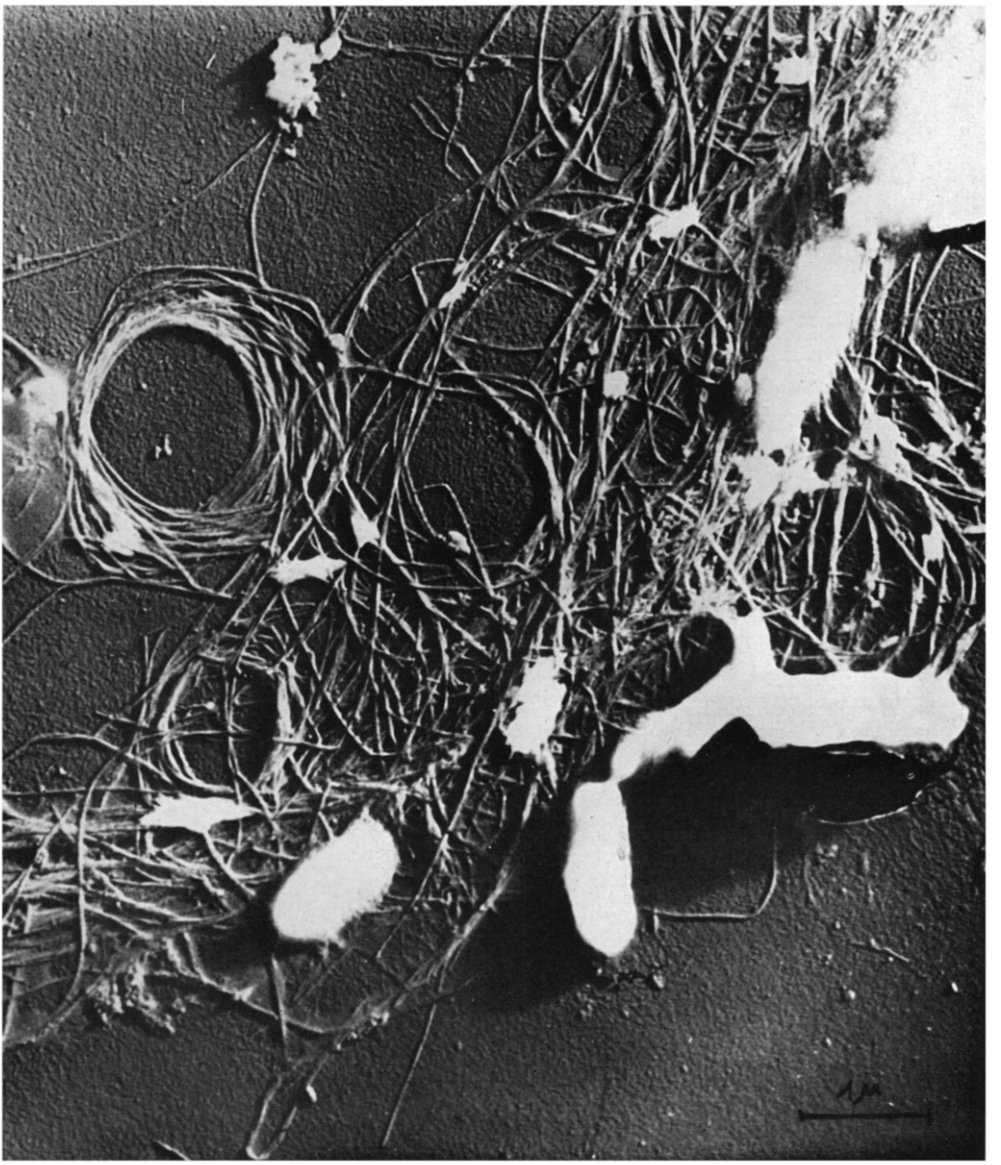

Fig. 6. Tangled cellulose fibrils in an old culture. Magnification 22000 ×.

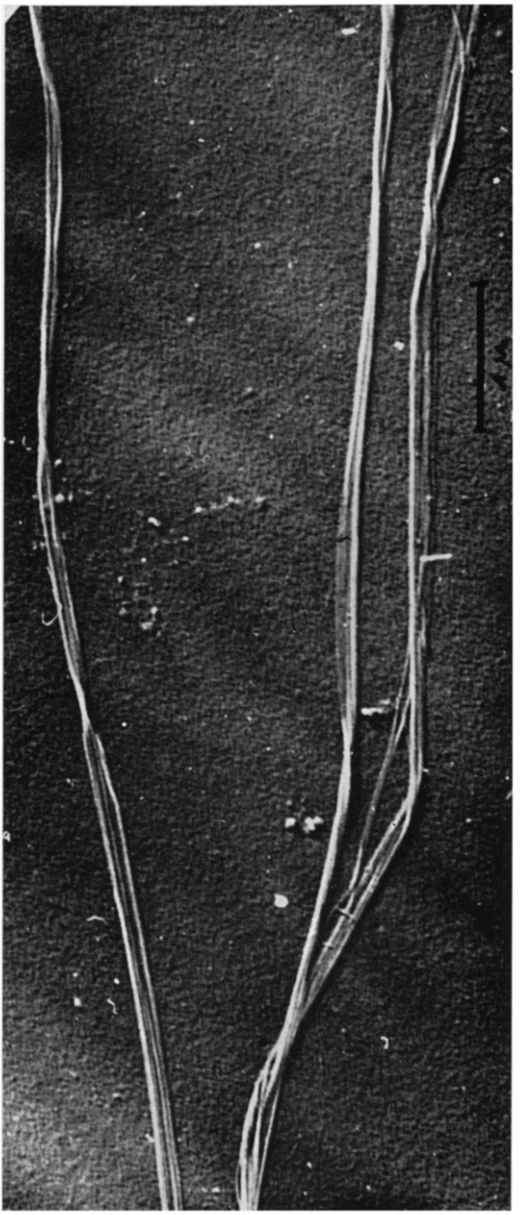

Fig. 7. Long bands formed by the parallel association of several cellulose fibrils. Magnification = 20000 ×

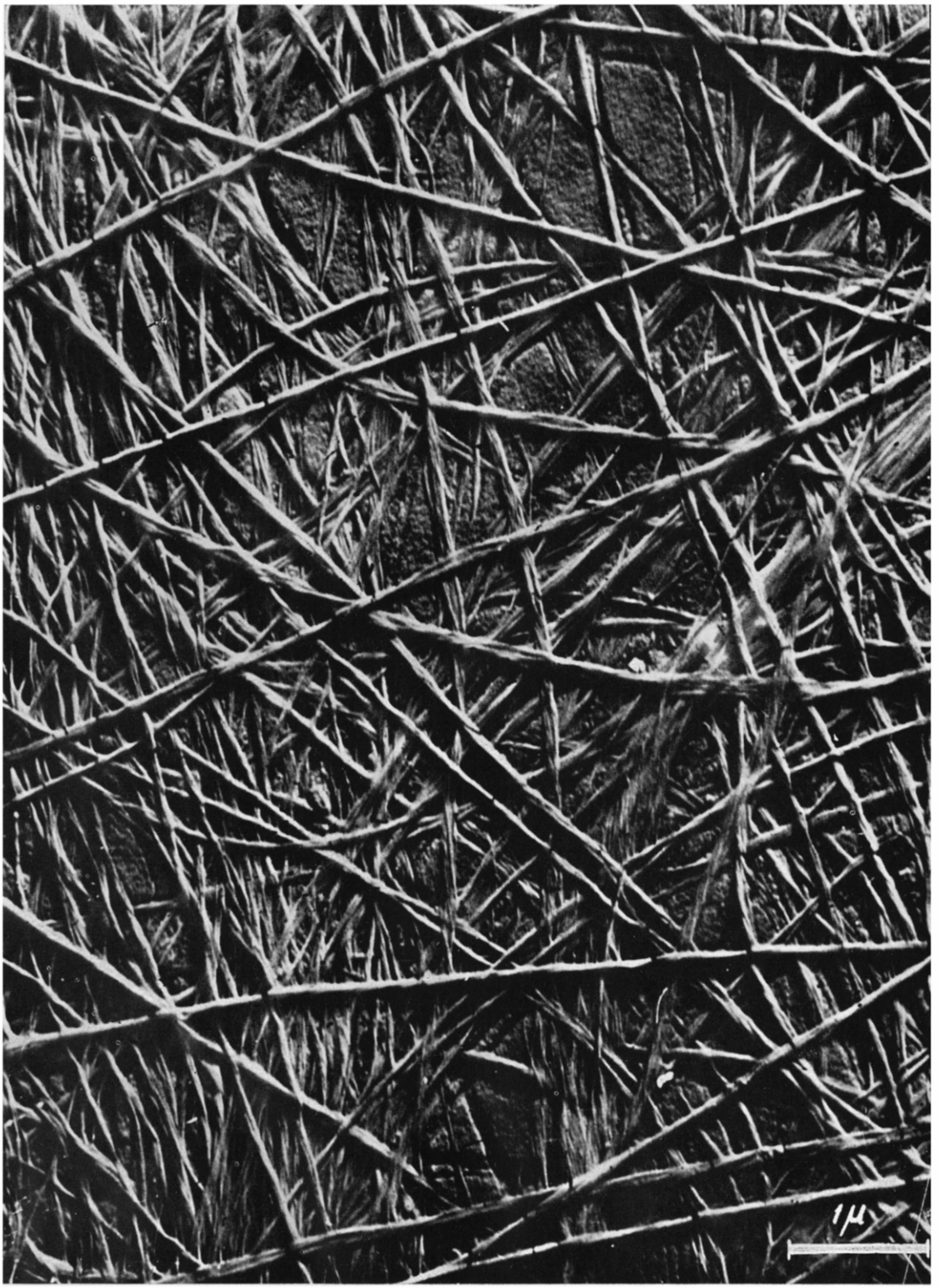

Fig. 8. A network of cellulose containing many twisted bands of fibres. Magnification 22000

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SUMMARY

Electron microscopic observations show that the cellulose built by *Acetobacterium xylinum* occurs in the form of fibres having about the same diameter (*ca* 250 Å) as that of the fibrils of cellulose from the cell walls of numerous plants. In *Bacterium xylinum* these strands are synthesized outside the cells and can therefore be examined especially well under the electron microscope.

The bacteria first secrete a structurally homogeneous slimy substance within which, after a short time, the cellulose fibres can be seen forming. This secreted substance appears to be a precursor of cellulose whose polymerization proceeds outside the organisms presumably with the help of an enzyme. In older cultures the cellulose strands have become so intertwined as to build a rigid framework.

RÉSUMÉ

Les observations faites au microscope électronique montrent que la cellulose synthétisée par *Bacterium xylinum* se présente sous forme de fibres ayant à peu près le même diamètre (environ 250 Å) que celui des fibrilles de cellulose constituant les parois cellulaires de nombreux végétaux. Chez *Bacterium xylinum*, ces fibres sont synthétisées à l'extérieur des cellules et peuvent par conséquent être examinées particulièrement facilement au microscope électronique.

Les bactéries secrètent d'abord une substance mucilagineuse homogène, au sein de laquelle on peut voir se former après un temps court, les fibres de cellulose. La substance ainsi secrétée est un précurseur de la cellulose dont la polymérisation se fait à l'extérieur des organismes, et probablement sous l'action d'une enzyme. Dans les cultures âgées, les fibres de cellulose sont tellement entrelacées qu'elles constituent un entrelac rigide.

ZUSAMMENFASSUNG

Beobachtungen mit Hilfe des Elektronenmikroskops lassen erkennen, dass die durch *Bacterium xylinum* synthetisierte Cellulose aus Fasern besteht, die ungefähr den gleichen Durchmesser haben (*ca.* 250 Å) wie die Fasern der Zellwände vieler Pflanzen. Bei *Bacterium xylinum* werden diese Fasern ausserhalb der Zelle synthetisiert und können deshalb besonders leicht im Elektronenmikroskop beobachtet werden.

Die Bakterien scheiden zuerst eine schleimige homogene Substanz aus und nach kurzer Zeit kann man sehen, wie sich innerhalb dieser Substanz die Cellulosefasern bilden. Diese Substanz ist ein Vorläufer der Cellulose deren Polymerisation sich ausserhalb der Organismen, wahrscheinlich unter dem Einfluss eines Enzyms vollzieht. In älteren Kulturen sind die Cellulosefasern so verzweigt, dass sie ein steifes Netzwerk bilden.

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