BACTERIAL MORPHOLOGY AS SHOWN BY THE ELECTRON MICROSCOPE

I. Structural Differentiation within the Streptococcal Cell[1]

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Asked what he saw in an electron micrograph of streptococci at 18,000 diameters magnification, a bacteriologist replied, "A problem." It is scarcely to be expected that insight into the nature and meaning of fine structures revealed by the electron microscope will necessarily increase in proportion to the increase in resolving power. There are, moreover, the limitations imposed by the present necessity of having the object under study in a vacuum, and by the extreme thinness of objects which the electron beam of present speed can traverse, and the further question of what changes in, for instance protoplasm, may be induced by the electron beam itself.

With all reservations made, however, it is clearly apparent, both from the published work, (bibliography in preceding paper (Marton, 1940b)), and from experience with the instrument just described (Marton, 1940a, Zworykin, 1940), that the electron microscope is a magnificent new implement for the study of many biological problems.

The present communication presents electron micrographs of β-hemolytic streptococci of Lancefield's Group A in their mucoid, smooth, and rough phases. These micrographs have been made as described (Marton 1940b), without fixing or staining. The micrographs reproduced have been selected principally for their

[1] Study of the streptococcus in the University of Pennsylvania is maintained with the aid of a generous grant from the Commonwealth Fund.

bearing upon the fundamental question of structural differentiation within the bacterial cell. Is the streptococcal cell actually what it appears to be when studied with the aid of our current, extremely crude staining methods, a tiny lemon-shaped mass of undifferentiated protoplasm? Or has it an outer cell membrane which is differentiated from an interior protoplasm?

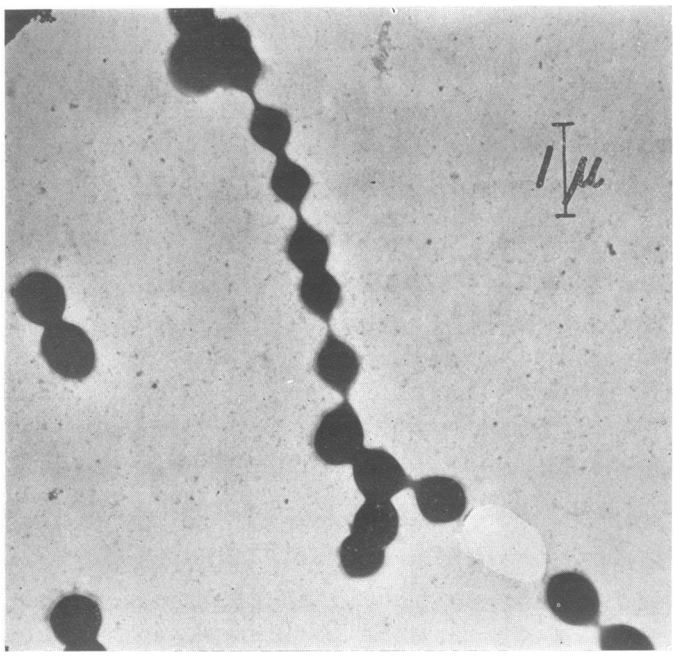

FIG. 1. C203 MUCOID, PREPARED FROM AN 18-HOUR BLOOD AGAR PLATE CULTURE; MAGNIFICATION 12,000 DIAMETERS

Figures 1, 2 and 3 show that the outer surfaces of the streptococcal cells have continuity throughout the chain. Adjacent cells may be separated only by a slight constriction, by a deeper constriction, by a relatively broad band or by a filament which is just resolved even at a magnification of 12,000 diameters.

Figure 4 gives direct evidence of the rigidity of the streptococcal cell. A droplet of bacterial suspension in distilled water was allowed to dry on the collodion mount, which in this case broke and retracted. The margin of this collodion mount is

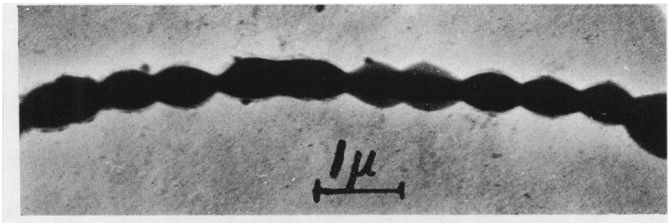

FIG. 2. C203 MUCOID; PREPARED FROM AN 18-HOUR BLOOD AGAR PLATE CULTURE; MAGNIFICATION 11,000 DIAMETERS

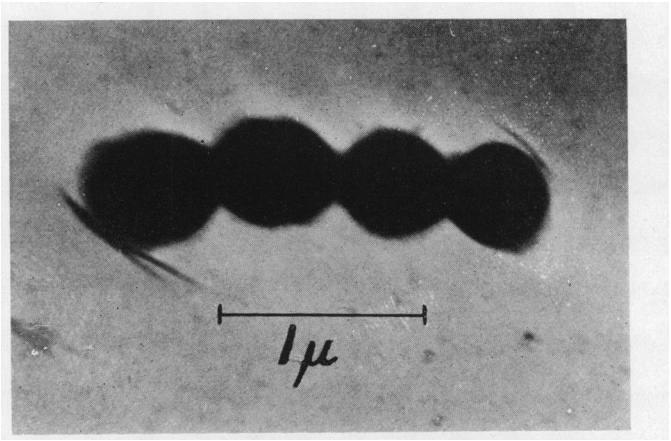

FIG. 3. C203 SMOOTH; PREPARED FROM AN 18-HOUR BLOOD AGAR PLATE CULTURE; ELECTRONIC MAGNIFICATION 12,000 DIAMETERS; TOTAL MAGNIFICATION 26,500 DIAMETERS

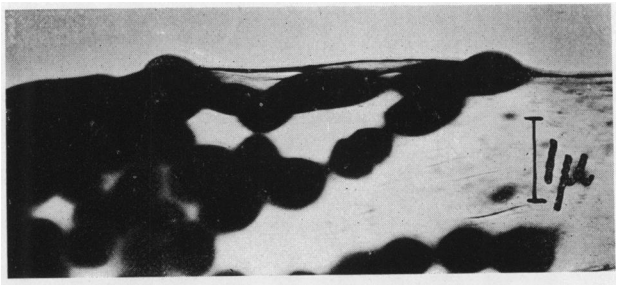

FIG. 4. C203 MUCOID; PREPARED FROM AN 18-HOUR BLOOD AGAR PLATE CULTURE; MAGNIFICATION 11,000 DIAMETERS; FILM BROKEN, SHOWING STREPTOCOCCI IN PROFILE

seen running parallel with the top of the picture; two streptococcal cells appear in profile projecting above the margin of the

collodion. The external structure of the bacteria, at least, is maintained without collapse from desiccation or action by the electron beam.

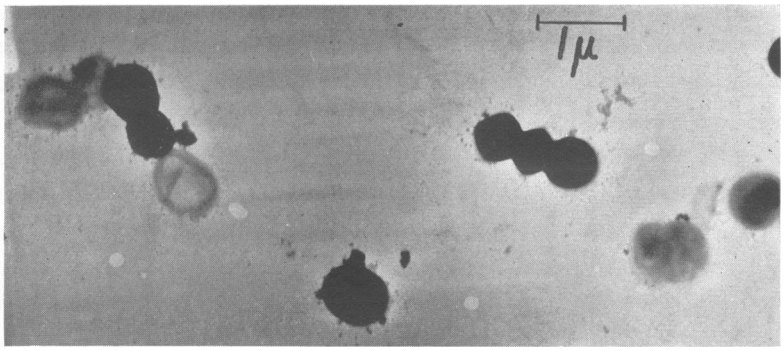

FIG. 5. C203 MUCOID; PREPARED FROM A SONICALLY VIBRATED SUSPENSION OF A NINETEEN-HOUR EXTRACT BROTH CULTURE; MAGNIFICATION 12,500 DIAMETERS

FIG. 6. C203 ROUGH; PREPARED FROM AN 18-HOUR BLOOD AGAR PLATE CULTURE; MAGNIFICATION 12,700 DIAMETERS

Figure 5 shows cytolyzed and intact streptococci in the same chain. The streptococci in this preparation had been subjected

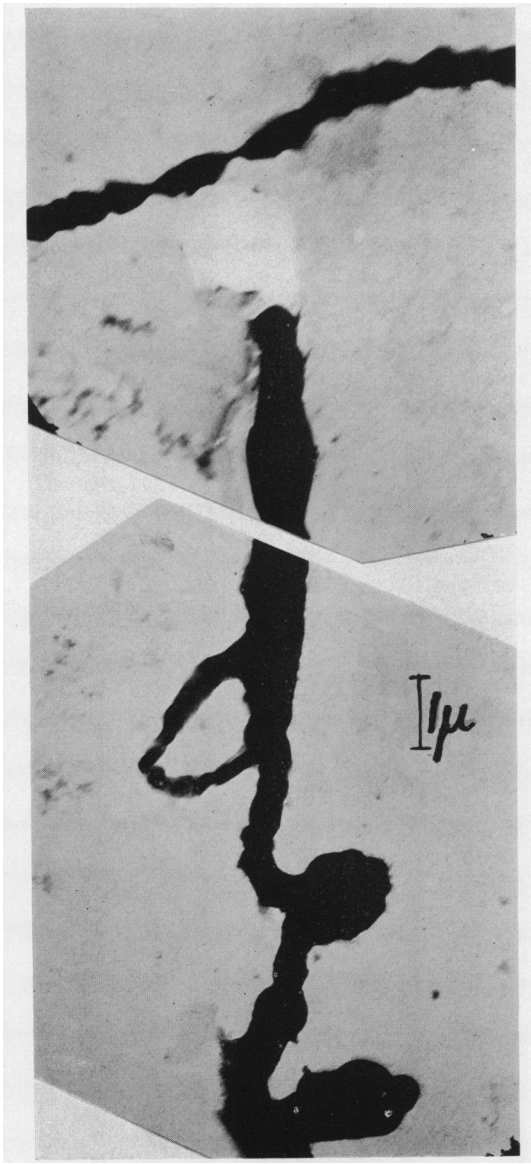

FIG. 7. C203 ROUGH; PREPARED FROM AN 18-HOUR BLOOD AGAR PLATE CULTURE; MAGNIFICATION 9,000 DIAMETERS

to a short period of sonic vibration[2] with the purpose of cytolyzing some cells and leaving others intact. One chain may be seen to contain two "ghost" cells, two intact cells and then one "ghost" cell. The noteworthy fact is that the "ghost" cells do not differ significantly in outline from the intact cells but only in their "opacity" to the electron beam, due doubtless to the escape of protoplasm from the interior of the cell. In the light of these appearances we may perhaps venture an interpretation of the two central cells seen in figure 2, i.e., the cells showing gray cell outlines with black axial portions; these would seem most probably to be instances of plasmolysis or shrinkage of the central protoplasm from the cell wall.

Taken together the phenomena shown would seem very definitely to indicate that streptococcal cells do possess rigid outer membranes whose continuity accounts for the grouping in chains, and which are differentiated from the inner protoplasm.

Figures 6 and 7 show some of the bizarre cells and filaments which appear in cultures of rough variants.

The electron micrographs here reproduced were all taken by Dr. L. Marton at the RCA Manufacturing Company. They are reproduced by permission of Dr. Marton and of Dr. V. K. Zworykin, Associate Director of Research, RCA Manufacturing Company.

The cultures of C203 Mucoid and C203 Smooth were originally received from Dr. Rebecca C. Lancefield and the culture of C203 Rough from Dr. Martin H. Dawson.

REFERENCES

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MARTON, L. 1940b The electron microscope. A new tool for bacteriological research. J. Bact., **41**, 397.

ZWORYKIN, V. K. 1940 An electron microscope for the research laboratory. Science, **92**, 51–53.

[2] The period of vibration was 11 minutes. For purposes of disintegrating streptococci for the preparation of their soluble components a vibration period of 1 hour is used (Chambers, L. A. and Flosdorf, E. W., Proc. Soc. Exptl. Biol. Med., 1936, **34**, 631; Mudd, S. and Lackman, D. B., J. Immunol., 1940, in press.)