BACTERIAL MORPHOLOGY AS SHOWN BY THE ELECTRON MICROSCOPE

III. Cell-Wall and Protoplasm in a Strain of Fusobacterium

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The differentiation of a solid bacterial cell-wall from an inner protoplasm has been demonstrated in favorable cases by plasmolysis, by microdissection, and by differential staining; notable success with mordant and differential staining has recently been attained by Knaysi (1938, 1941); the literature is reviewed by Lewis (1941). Examination of a variety of bacterial species with the aid of the electron microscope has demonstrated this important structural differentiation with particular vividness. The general conclusion is warranted, we believe, that bacteria are cells, with solid cell-wall clearly distinct from inner fluid or potentially fluid protoplasm. The inner protoplasm is frequently observed to be shrunken from the cell-wall by plasmolysis or drying, and readily escapes from the cell-wall following injury. The capacity of undergoing reversible gelation is possessed by protoplasm in general. We know of no convincing evidence, however, as to whether normal bacterial protoplasm is a sol or a gel or whether it may undergo reversible change from one state to the other.

Bacteria of the genera thus far considered in this series, *Streptococcus*, (Mudd and Lackman, 1941), and *Bacillus*, (Mudd, Polevitzky, Anderson, and Chambers, 1941), have been relatively opaque to the electron beam. In the present study, pictures of a strain of fusiform bacillus are presented in which both differentiation of cell-wall from protoplasm and differences of density within the protoplasm itself are particularly clearly shown.

The strain of fusiform bacillus, isolated by Wakeford, the morphology of whose cells is shown herewith, has been described, (Kast, 1928). This strain has been preserved in coagulated serum medium for some thirteen years, and has maintained its characteristic morphology during this time. For the present study transplants were made to cystein-serum broth medium, sealed with vaseline, and incubated for various periods of time. For examination with the electron microscope a small amount of culture was removed with a capillary pipette and suspended in distilled water, centrifugalized, and the sediment resuspended in distilled water; this washing procedure was twice repeated. A droplet of the last suspension in distilled water was placed on the collodion mount and allowed to dry without fixing or staining. All pictures were taken with electrons accelerated by 60 Kilovolts and at an original magnification of 6250 diameters.

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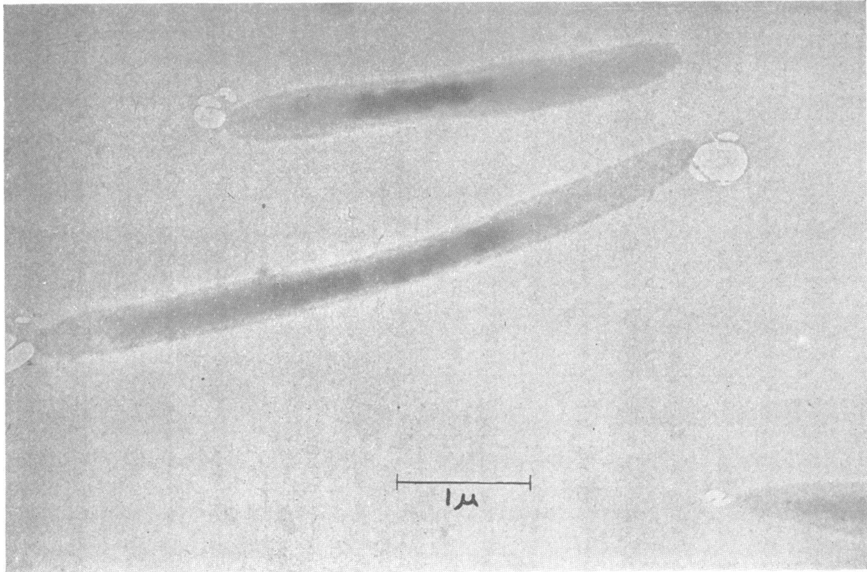

FIG. 1. TWO CELLS FROM YOUNG (2 DAYS OLD) CULTURE
Final magnification as reproduced × 17,500

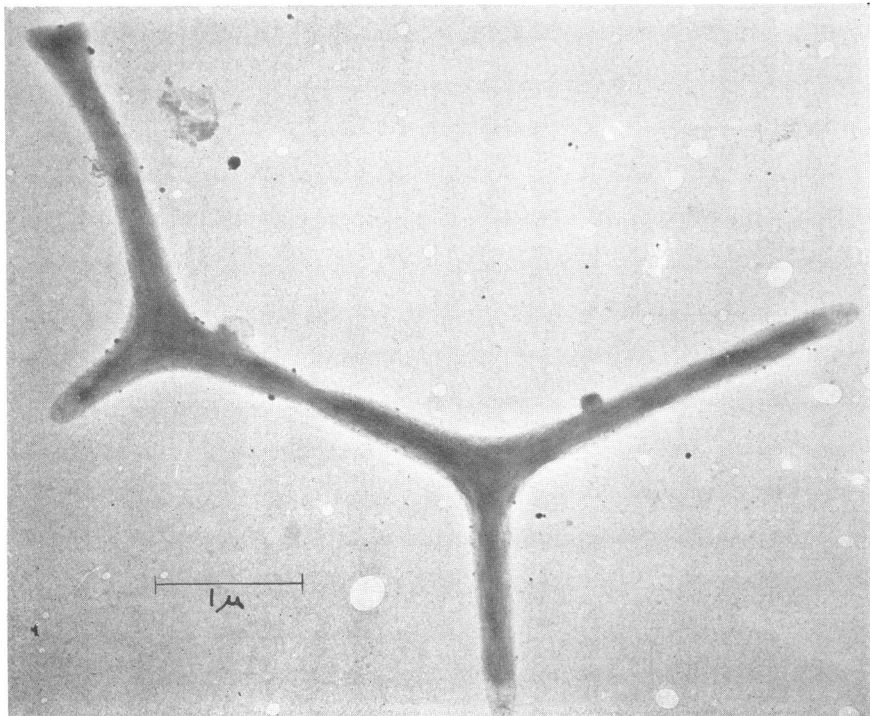

FIG. 2. BRANCHING FORM FROM YOUNG (2 DAYS OLD) CULTURE
Washed bacteria had stood in distilled water overnight. Final magnification × 19,000

In figure 1, two characteristic cells of a young culture are seen. Differences in density are obvious in various parts of the protoplasm. A branching form

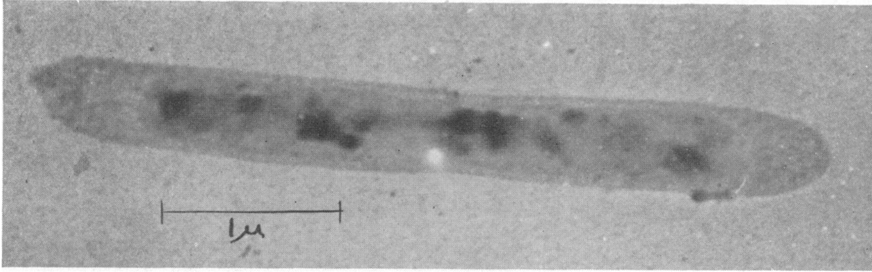

FIG. 3. CELL FROM CULTURE INCUBATED 48 HOURS AND KEPT AT ROOM TEMPERATURE FOR 2 WEEKS × 23,500

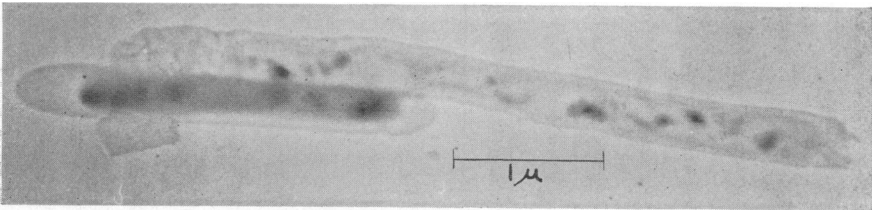

FIG. 4. CELLS FROM CULTURE TREATED AS IN FIGURE 3 × 19,500

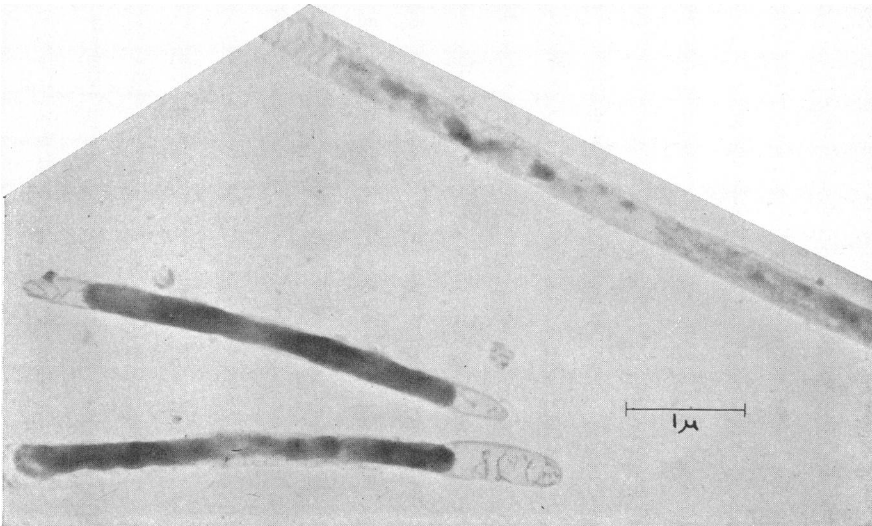

FIG. 5. CELLS FROM CULTURE TREATED AS IN FIGURE 3 × 15,500

is shown in figure 2. A cell from an older culture appears in figure 3. In this cell very dense local areas appear as black granules against a background of relatively "transparent" protoplasm which is retracted from the cell-wall.

Figure 4 is of a preparation from a two-weeks old culture. The end of the cell-wall of one cell has been broken: the jagged line of rupture attests to the solidity of the cell-wall, (as in the previous studies in which fracture by sonic vibration

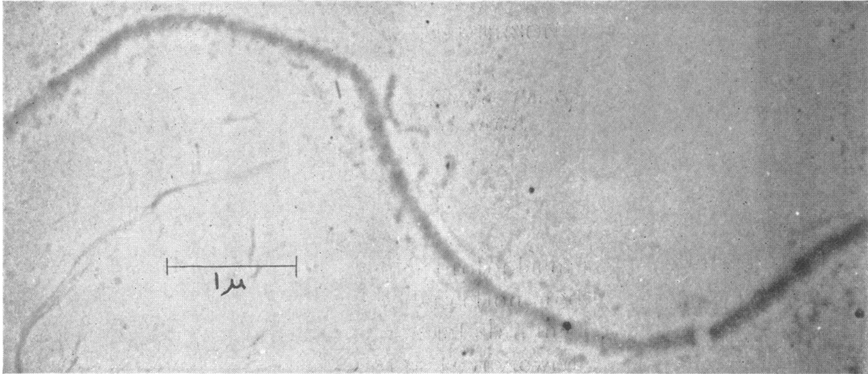

FIG. 6. CURVED FILAMENTOUS BACTERIUM FROM A CULTURE TREATED AS IN FIGURE 3 × 17,000

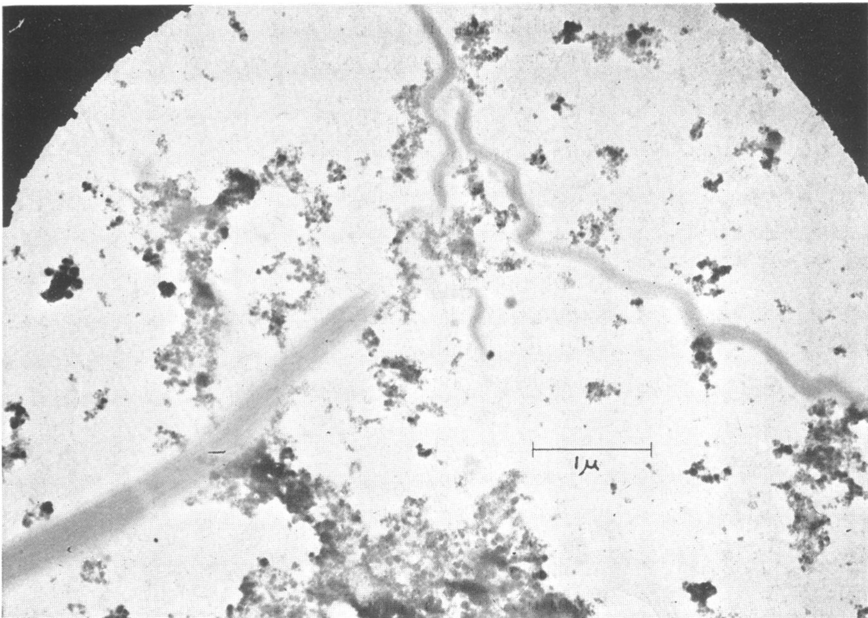

FIG. 7. EXUDATE FROM INFECTED AREA OF A CASE OF VINCENT'S GINGIVITIS OF TWO WEEKS DURATION × 15,500

was employed). The other cell appears to be intact. Its relatively dense protoplasm is retracted from the cell-wall. In figure 5 cells from a two-weeks old culture are again shown. In two of the cells the protoplasm is relatively dense. The tapering ends of the cells are apparently free of protoplasm and the cell-

wall appears to be wrinkled in several places. Part of a larger filamentous cell is also shown, in which dark granules appear in a protoplasm which is relatively transparent to the electron beam. Figure 6 shows a curved, filamentous form found in an old culture of the fusiform bacillus. It is a filamentous bacterial form which might easily be mistaken for a spirochete if studied with the light microscope.

As a matter of interest, a fresh preparation of the exudate from a case of Vincent's gingivitis is shown in figure 7. Fusiform and spiral microorganisms are shown in association.

DISCUSSION

The differentiation of cell-wall from inner protoplasm has been clearly demonstrated in electron micrographs of streptococci (Mudd and Lackman, 1941), of species of the genus *Bacillus* (Mudd, Polevitzky, Anderson, and Chambers, 1941), and in *Thiobacillus thiooxidans* (Umbreit and Anderson, 1942). Cell-wall and inner protoplasm appear as structurally distinct, also, in many of the electron micrographs in the German literature (Piekarski and Ruska, 1939; Jakob and Mahl, 1940), but have not always been correctly interpreted. The differentiation of cell-wall from protoplasm has also been demonstrated by combination of the protoplasm with salts of silver and lead, resulting in greatly increased density of the protoplasm without discernible alteration of the cell-wall (Mudd and Anderson, 1942).

The differences in density of the protoplasm of these unfixed (though dried) and unstained cells of *Fusobacterium* were not unexpected, in view of the uneven staining which has been described as characteristic of fusiform bacilli (Varney, 1927; Hine and Berry, 1937; Bergey's Manual, 1939). Moreover, when the living cells are observed with dark field apparatus[2] they show granular structures essentially similar to those more clearly shown in the electron micrographs. Exact correlation between density as found with the electron microscope and staining behavior has not been attempted in this study, (see, however, Piekarski and Ruska, 1939).

CONCLUSION

Bacteria are cells with solid cell-wall and fluid, or potentially fluid, inner protoplasm distinct from the cell-wall. Electron micrographs of cells of a strain of *Fusobacterium* show striking differences in density within the protoplasm. Correlation of the significance of the differentiations observable with the electron microscope with those observable by microchemical and staining techniques, though hardly more than begun, presents a challenging problem.

REFERENCES

Bergey, D. H. 1939 Manual of Determinative Bacteriology. 5th Ed. Baltimore, p. 586.

Hine, M. K., and Berry, G. P. 1937 Morphological and cultural studies of the genus *Fusiformis*. J. Bact., **34**, 517–533.

[2] Zeiss "Bitumi" binocular attachment, cardioid condenser, quartz objective, carbon arc, magnification approximately × 1000.

JAKOB, A., AND MAHL, H. 1940 Strukturdarstellung bei Bakterien, insbesondere die Kapseldarstellung bei Anaerobiern mit dem elektrostatischen Elektronen-Übermikroskop. Arch. exptl. Zellforsch. Gewebezücht., **29,** 97–104.

KAST, C. C. 1928 Observations on the morphology and motility of fusiform bacilli. J. Lab. Clin. Med., **14,** 112–119.

KNAYSI, G. 1938 Cytology of bacteria. Botan. Rev., **4,** 83–112.

KNAYSI, G. 1941 Observations on the cell division of some yeasts and bacteria. J. Bact., **41,** 141–154.

LEWIS, I. M. 1941 The cytology of bacteria. Bact. Revs. **5,** 181–230.

MUDD, S., AND ANDERSON, T. F. 1942 Differential "staining" for electron microscopy. J. Exptl. Med., in press.

MUDD, S., AND LACKMAN, D. B. 1941 Bacterial morphology as shown by the electron microscope. I. Structural differentiation within the streptococcal cell. J. Bact., **41,** 415–420.

MUDD, S., POLEVITZKY, K., ANDERSON, T. F., AND CHAMBERS, L. A. 1941 Bacterial morphology as shown by the electron microscope. II. The bacterial cell-wall in the genus *Bacillus*. J. Bact., **42,** 251–264.

PIEKARSKI, G., AND RUSKA, H. 1939 Übermikroskopische Untersuchungen an Bakterien unter besonderer Berücksichtigung der sogenannten Nucleoide. Arch. Mikrobiol., **10,** 302–321.

UMBREIT, W. W., AND ANDERSON, T. F. 1942 A study of *Thiobacillus thiooxidans* with the electron microscope. J. Bact., **44,** 317–320.

VARNEY, P. L. 1927 The serological classification of fusiform bacilli. J. Bact., **13,** 275–314.