BACTERIAL MORPHOLOGY AS SHOWN BY THE ELECTRON MICROSCOPE

II. The Bacterial Cell-Wall in the Genus Bacillus

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The fact that most bacteria are not spheres, indicates that they possess rigidity of structure in some degree; otherwise the action of surface forces, relatively powerful at surfaces of such high curvature, would make the bacterial cells spherical. There is a considerable body of evidence to indicate the existence of a surface membrane[3] or cell-wall which is differentiated from an internal protoplasm. This evidence, which includes the differential staining of cell-wall and endoplasm, cytolytic and plasmolytic effects, has been reviewed by Gotschlich (1929), by St. John-Brooks (1930), by Knaysi (1938), and by Henrici (1939). The observations of Emmerich and Saida (1900) and of Eisenberg (1910) on cytolysis of anthrax bacilli, and of Gutstein (1924) on differential staining of cell-wall and endoplasm are particularly relevant to the present study, as are also those of Knaysi (1930) on plasmolysis of *Bacillus subtilis*.

Wámoscher in 1930 succeeded in microdissecting bacteria

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[3] Most recently Knaysi (1938, 1941) has advanced good reasons for referring to the outer limiting structure at the surface of the (non-capsulated) bacterial cell as the "cell-wall," and to the limiting surface of the inner protoplasm as the "cytoplasmic membrane." In this paper we follow Knaysi's terminology in terming "cell-wall" the structure which in the earlier literature has often been called "cell-membrane."

under direct microscopic observation. His principal observations were with a large, aerobic, non-sporing bacterium, "Bacillus Mazun," but were supplemented by experiments with colon, paratyphoid and dysentery bacilli. This important work should be consulted in detail. From it Wámoscher concluded that "the cell membrane" (cell-wall) "has shown itself . . . to be an extremely solid, elastic, extensible structure, enormously resistant to pressure." From the open ends of bacilli which had been cut across by the microdissection needle, Wámoscher described the exudation of tiny globules which dissolved in the suspending medium.

Following observation of the bactericidal action of the magnetostriction oscillator by Williams and Gaines (1930) and Chambers and Gaines (1932), two of us (L. A. C. and S. M., unpublished work) studied cells of *Bacillus megatherium* broken under the action of sonic vibration. Smears of such preparations show gram-negative cell fragments among the gram-positive bacteria. With good dark-ground illumination cell-walls of broken bacteria can be seen, looking like minute, silvery, test-tubes, in contrast with the brilliant outlines of the intact bacteria. These broken cell-walls closely resemble those of bacteria cut by the microdissection-needle as photographed by Wámoscher.

For the present study, broth cultures of *Bacillus subtilis*, *Bacillus megatherium*, and *Bacillus anthracis* were grown for various periods of time, depending on whether a suspension of normal cells or autolyzed cells and spores was desired. The cells were then thrown down in the centrifuge and resuspended in distilled water to remove salts and nutrient, since their presence would be undesirable in the subsequent mounting of specimens for study.

Portions of the resulting suspensions of cells were then placed in the sonic oscillator and subjected to the effects of cavitation for 10 minutes. The cells were then mounted for examination in the electron microscope by placing a minute drop of the desired suspension on a collodion membrane some 15 mμ thick and allowing the drop to evaporate, as described by Marton (1941). The specimen was then placed in the object chamber, which was

evacuated before the mount was placed for observation on the movable stage of the electron microscope.

An electron micrograph illustrating fragmentation of cells of *Bacillus megatherium* is shown in figure 1. The cell-walls in chains of bacilli are continuous from cell to cell, as electron micrographs have shown also to be the case with streptococci (Mudd and Lackman, 1941). The intact cell, which is opaque to the electron beam, is connected with the cell-wall of the adjoining broken cell, much of whose protoplasm has escaped, leaving only

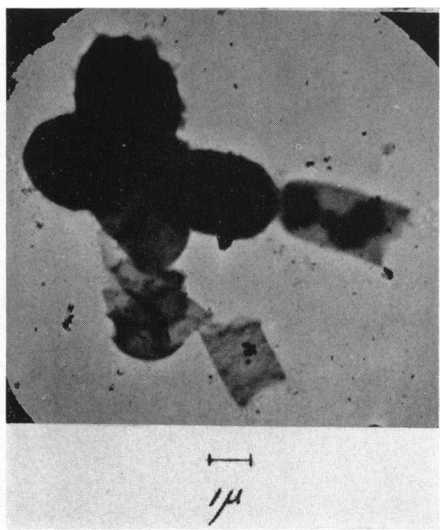

FIG. 1. BACILLUS MEGATHERIUM FRAGMENTED IN SONIC APPARATUS. MAGNIFICATION × 5500

an irregular residue of protoplasm within the cell-wall. Below, a tubular fragment is almost or quite empty of protoplasm. The somewhat jagged lines of fracture emphasize the solid character of the cell-walls. The outline of the uppermost cell in the picture suggests that the preparation may have dried at a moment when protoplasm was escaping through multiple points of injury of the cell-wall. This appearance is quite analogous to the appearance of cells of streptococci which had been exposed to sonic vibration (Mudd and Lackman, 1941, figure 5).

In figure 2 are seen two cells of *Bacillus anthracis* from suspensions which had been subjected to ten minutes of sonic vibration. The lower cell still contains its protoplasm. The upper cell has

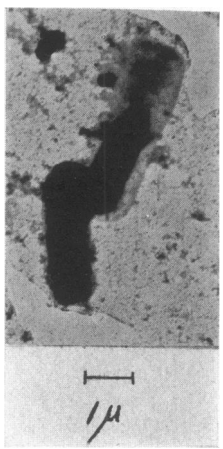

FIG. 2. BACILLUS ANTHRACIS FRAGMENTED BY SONIC VIBRATION. × 6250

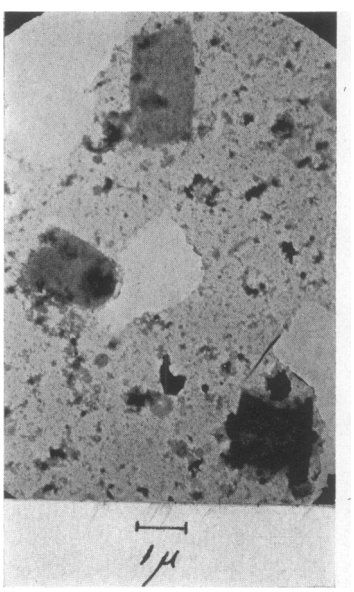

FIG. 3. BACILLUS ANTHRACIS FRAGMENTED BY SONIC VIBRATION. × 6250

apparently been ruptured by the vibration and much of the protoplasm has escaped from its upper end. Some of the protoplasm

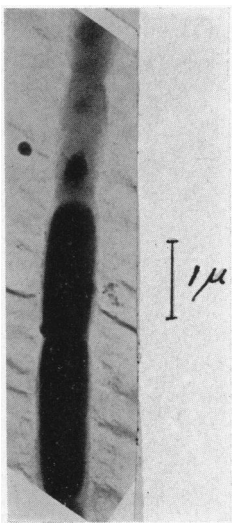

FIG. 4. BACILLUS ANTHRACIS. INTACT CELLS AND "GHOSTS." × 10,000

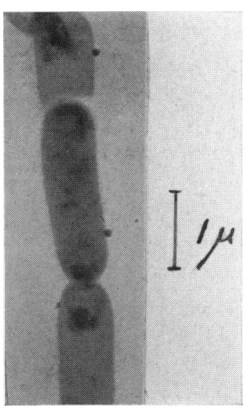

FIG. 5. BACILLUS SUBTILIS. TWO "GHOSTS" AND A BROKEN CELL. × 10,000

still remains in the lower part of the cell, although it has shrunken away from the cell-wall.

In figure 3 are shown several fragments of the cell-wall of cells of *Bacillus anthracis*, broken by sonic vibration. In figures 2 and 3 the cell contents, liberated by sonic disintegration, are

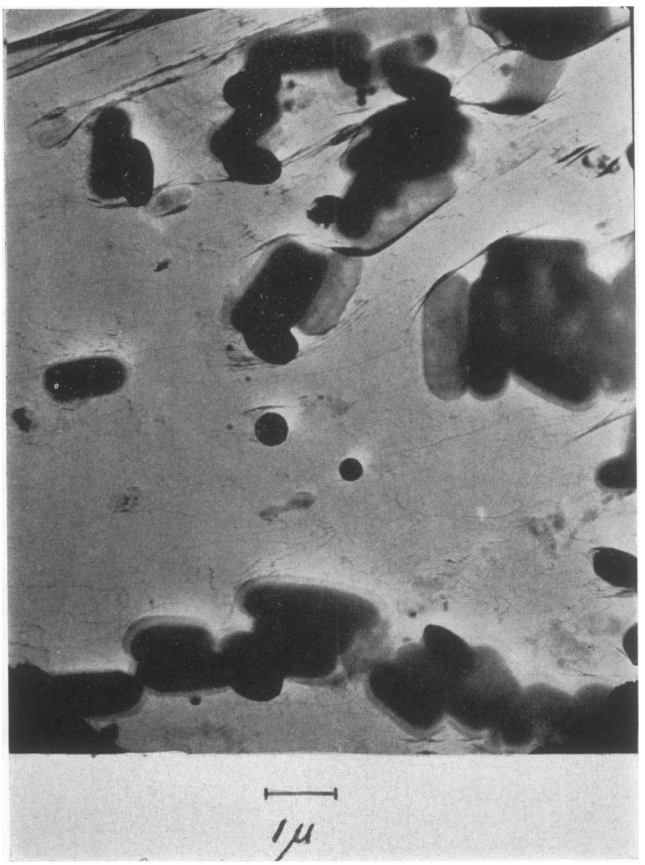

FIG. 6. BACILLUS SUBTILIS. INTACT VEGETATIVE CELLS, CYTOLYSED CELLS AND SPORES. × 9200

strewn over the collodion mount. To what extent these cell contents may have been altered from their original state by escape from the cell and subsequent drying is, of course, at present not known. With further experience, however, the study of such cell

contents, which at least have not been subjected to chemical fixation or staining, may become of value.

Cytolysis is, of course, of common occurrence without the artificial aid of sonic vibration. Intact cells and cytolysed cells of *Bacillus anthracis* are shown in the same chain in figure 4. Figure 5 shows two connected "ghost" cells of *Bacillus subtilis* with a broken cell-wall above them.

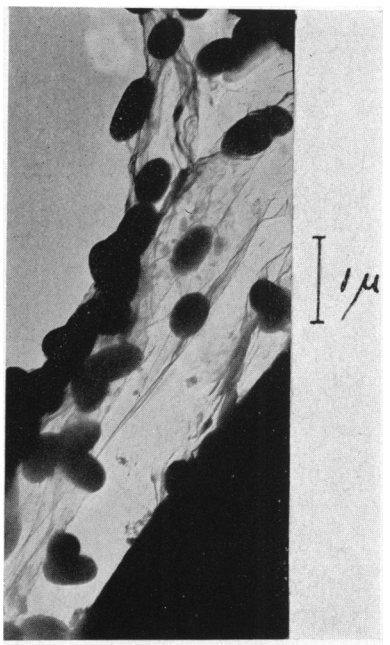

FIG. 7. BACILLUS SUBTILIS. SPORES SEEN IN PROFILE AT EDGE OF MOUNT. × 11,000

Figure 6 shows intact vegetative cells, cytolysed cells and spores of *Bacillus subtilis* in the same field. The spores here shown, resembling those photographed under dark-ground illumination by Barnard (1930), stand out as rigid structures of high density, whereas the vegetative cells consist of an inner, somewhat transparent protoplasm which has separated from an outer cell-wall, possibly in the process of drying. The high relative density of the

spores is further brought out in figure 7, in which the spores are seen in profile where the collodion mount has broken. It is interesting that the spores, having slightly smaller diameters

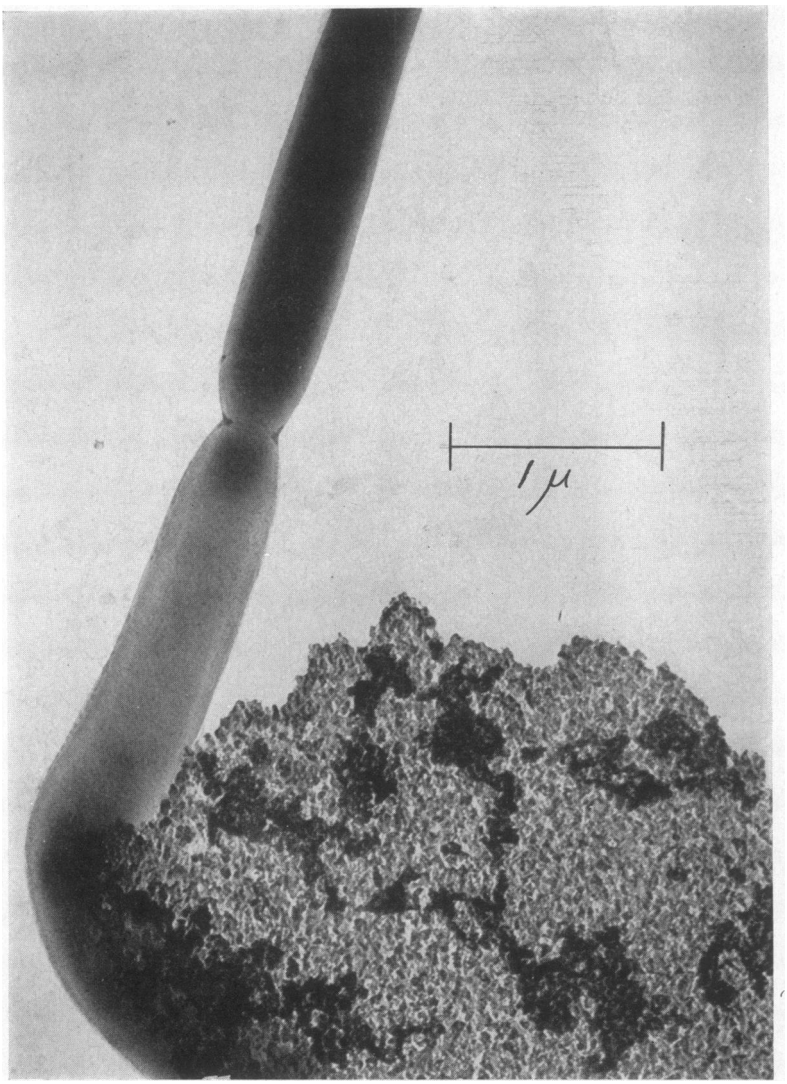

FIG. 8. BACILLUS SUBTILIS; ADJOINING CELLS, ONE OF WHICH IS DEFORMED BY A FOREIGN PARTICLE. × 28,000

than the vegetative cells, should be more opaque. Friedman and Henry (1938) have reported that the total water content is about

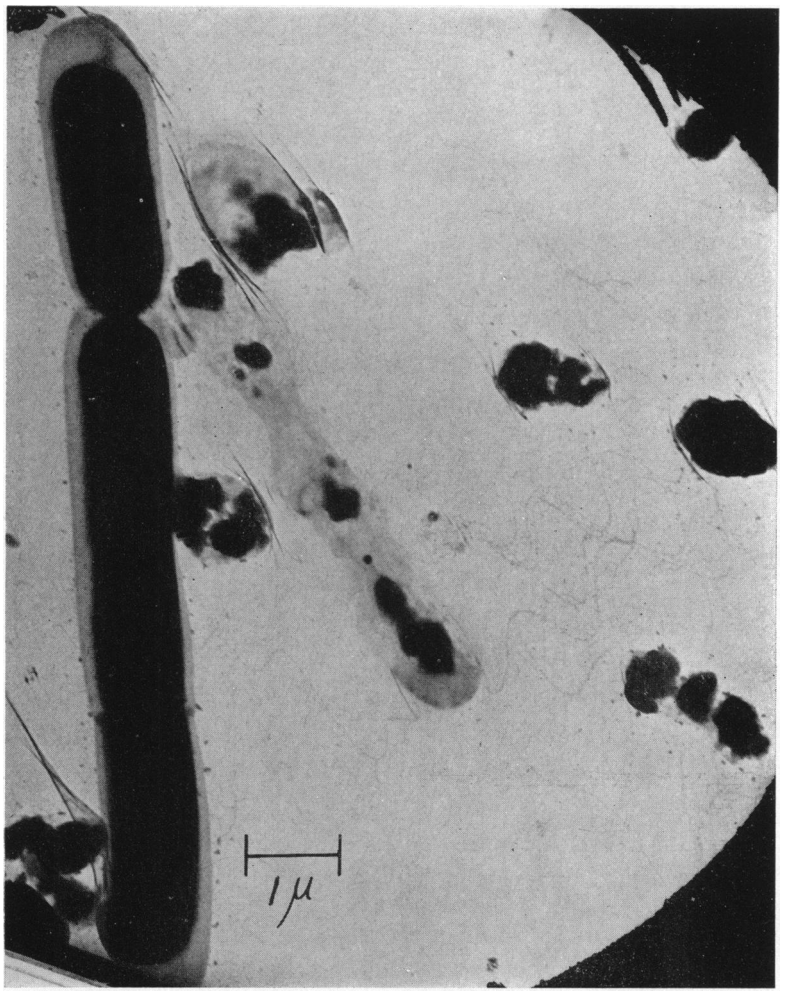

FIG. 9. BACILLUS SUBTILIS. INTACT VEGETATIVE CELLS AND REMAINS OF CYTOLYSED CELLS. NOTE FLAGELLA. × 12,200

the same in spores and in vegetative cells, but that the proportion of bound water is much greater in spores. It may well be that the greater density of the spores is due to the retention of

bound water, whereas the vegetative cells may have lost water by evaporation in the high vacuum of the microscope chamber.

In figure 8 two adjacent cells of *Bacillus subtilis* are seen, one of which is deformed by some particle of foreign material; this cell is semi-transparent, suggesting that some of its protoplasm may have escaped.

FIG. 10. BACILLUS SUBTILIS. OLD CELLS WITH PROTOPLASM SHRUNKEN FROM CELL MEMBRANE. FLAGELLA CONTINUOUS WITH CELL MEMBRANE. × 10,000

Figure 9 is a micrograph of a culture of *B. subtilis*. Two cells are joined with a continuous cell-wall to be seen around each of them with a thin column of protoplasm extending from one cell to the other. Of particular interest is the region in the long lower cell where the cell-wall is slightly indented and apparently thickened at a future point of division. To the right of the two intact cells is to be seen a "ghost" from which most of the protoplasm has escaped and in which the cell-wall has largely dis-

integrated. Note the numerous flagella which appear in this (unstained) preparation. That the flagella are continuous with

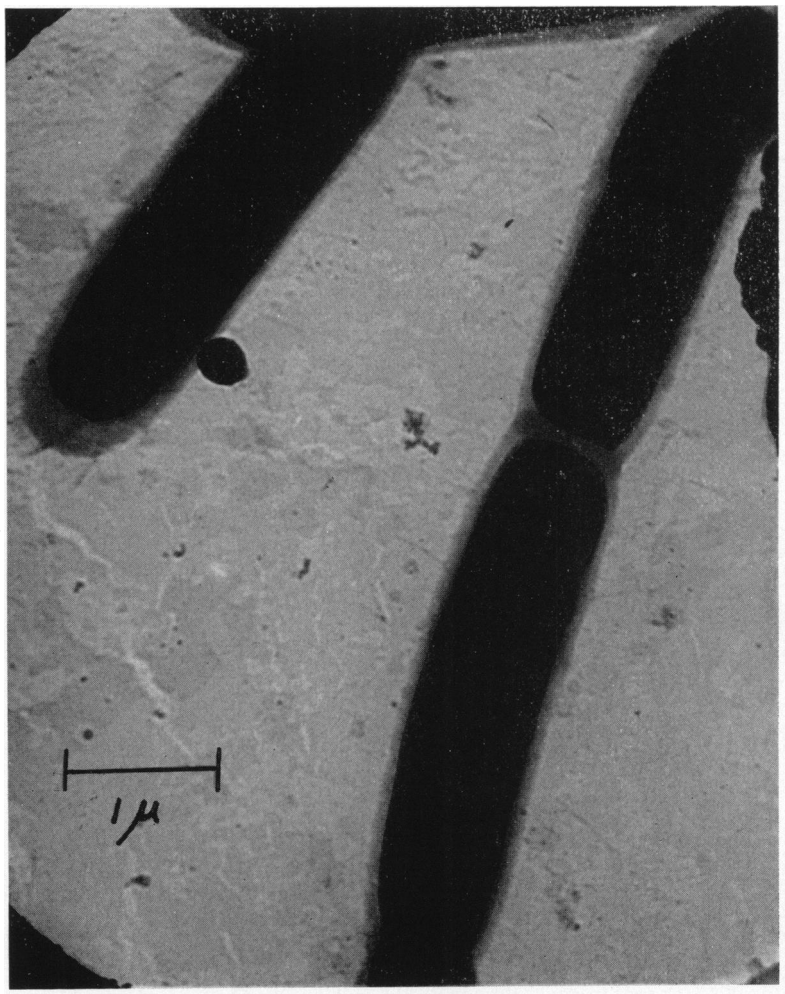

FIG. 11. BACILLUS ANTHRACIS. CELL MEMBRANE THICKENED BETWEEN ADJOINING CELLS IS PARTICULARLY WELL SHOWN. × 20,000

the cell-wall is illustrated in figure 10, which shows cells from an old culture of *Bacillus subtilis* in which the protoplasm has shrunken away from the cell-wall in drying.

The intercellular plate (Knaysi 1938, 1941) at a future point of division of cells of *Bacillus anthracis* is clearly seen in figure 11. The continuity of the outer membrane from cell to cell is particularly obvious in this picture. In figure 12, very delicate strands are to be seen connecting adjacent cells of *Bacillus megatherium.*

In this article and the preceding one (Mudd and Lackman, 1941) we are dealing with the limiting cell-wall of the bacterial

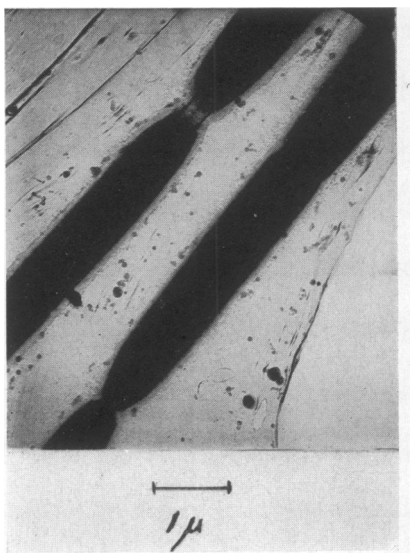

FIG. 12. BACILLUS MEGATHERIUM. × 10,000

cell itself, and not at all with an extracellular capsule, as found, for instance, in virulent pneumococci. An article which has just appeared (Jakob and Mahl, 1940) includes numerous electron micrographs of non-encapsulated anaerobic bacilli, (*Clostridia*). Phenomena of shrinkage of the cell protoplasm from the cell-wall, and of fractured cell-walls empty of inner protoplasm are clearly shown. Nevertheless these appearances are interpreted as the discovery of hitherto unrecognized "capsules" from which the "bacterial body" is supposed, under appropriate conditions, to emerge. This interpretation is, in our belief, erroneous.

It is perhaps worth emphasizing that the very vividness with which structures of minute and unfamiliar dimensions may be revealed in electron micrographs may create the impression that their interpretation is correspondingly unambiguous. This, of course, is not the case. Much that has been learned by other means must serve as a background for interpretation of the new appearances opened to investigation by this instrument.

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SUMMARY

Bacteria of the genus *Bacillus*, (*B. subtilis*, *B. megatherium*, and *B. anthracis*) possess cell-walls which are definite, solid morphological structures. An inner protoplasm may shrink away from this wall, or escape following injury, leaving the cell-wall as a "ghost," which has essentially the shape of the intact cell. The solidity of the wall is sufficient to leave jagged lines of fracture when broken by sonic vibration. The flagella of *B. subtilis* are continuous with the cell-wall. The spores of *B. subtilis* appear to be very dense, rigid bodies.

REFERENCES

BARNARD, J. E. 1930 Morphology of bacteria in the living state, in A system of bacteriology in relation to medicine. Medical Research Council, London, **1**, 115–116.

CHAMBERS, L. A., AND GAINES, NEWTON 1932 Some effects of intense audible sound on living organisms and cells. J. Cellular Comp. Physiol., **1**, 451–473.

EISENBERG, P. 1910 Studien zur Ektoplasmatheorie. III. Weitere Methoden zur Darstellung des Ektoplasmas. Zentr. Bakt. Parasitenk., I, Orig., **53**, 481–485.

EMMERICH, R., AND SAIDA 1900 Über die morphologischen Veränderungen der Milzbrandbacillen bei ihrer Auflösung durch Pyocyanase. Zentr. Bakt. Parasitenk., I, Orig., **27**, 776–787.

FRIEDMAN, C. A., AND HENRY, B. S. 1938 Bound water content of vegetative and spore forms of bacteria. J. Bact., **36**, 99–105.

GOTSCHLICH, E. 1929 Plasma und Membran, in KOLLE, W., KRAUS, R., AND UHLENHUTH, P.: Handbuch der Pathogenen Mikroorganismen, 3rd edition, Berlin, I, 74–75.

GUTSTEIN, M. 1924 Über die färberische Dartstellung des Bakterienektoplasmas. Zentr. Bakt. Parasitenk., I, Orig., **93**, 233–239.

HENRICI, A. T. 1939 The finer structure of bacterial cells, in The Biology of Bacteria, 2nd edition, New York, 117–137.

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

KNAYSI, G. 1930 The cell structure and cell division of *Bacillus subtilis*. J. Bact., **19**, 113–115.

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.

MARTON, L. 1940 A new electron microscope. Physiol. Revs., **58**, 57–60.

MARTON, L. 1941 The electron microscope: A new tool for bacteriological research. J. Bact., **41**, 397–413, and other references contained therein.

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

ST. JOHN-BROOKS, R. 1930 Cell membrane and cell contents, in A system of bacteriology in relation to medicine, Medical Research Council, London, **1**, 106–107.

WÁMOSCHER, L. 1930 Versuche über die Struktur der Bakterienzelle. Z. Hyg. Infektionskrankh., **111**, 422–460.

WILLIAMS, O. B., AND GAINS, NEWTON 1930 The bactericidal effects of high frequency sound waves. J. Infectious Diseases, **47**, 485–489.

ZWORYKIN, V. K. 1940 An electron microscope. Science, **92**, 51.