JOURNAL OF BACTERIOLOGY, Apr., 1965

Vol. 89, No. 4
Printed in U.S.A.

Plasmolysis in *Bacillus megaterium*

CLAES WEIBULL

Central Bacteriological Laboratory of Stockholm City, Stockholm, Sweden, and Rocky Mountain Laboratory, U.S. Public Health Service, Hamilton, Montana

Received for publication 10 December 1964

ABSTRACT

WEIBULL, CLAES (Central Bacteriological Laboratory of Stockholm City, Stockholm, Sweden). Plasmolysis in *Bacillus megaterium*. J. Bacteriol. **89**:1151-1154. 1965.—Sucrose solutions stronger than 1 M caused plasmolysis in *Bacillus megaterium* strain M, whereas concentrated NaCl and KNO_3 solutions were ineffective. In plasmolyzed cells, mesosome bodies were found in pockets between the cytoplasmic membrane and the cell wall. After plasmolysis, the cytoplasmic membrane appeared as a triple-layered structure, a "unit membrane." Plasmolysis did not markedly influence the viability of the cells.

A detailed electron microscopic study of plasmolysis in a gram-negative organism, *Escherichia coli*, was carried out by Cota-Robles (1963). The present report deals with the same phenomenon as manifested in a gram-positive organism, *Bacillus megaterium*. Electron microscopic observations of plasmolyzed cells of *B. subtilis* have been reported by van Iterson (1961), Ryter and Jacob (1963), and Ryter and Landman (1964). Reviews on bacterial plasmolysis in general have been given by Knaysi (1951), Mitchell and Moyle (1956), and Robinow (1960).

MATERIALS AND METHODS

Organism and growth conditions. Bacillus megaterium strain M (Baumann-Grace and Tomcsik, 1957) was grown at 30 C in 200-ml Erlenmeyer flasks, each containing 50 ml of nutrient broth. The cultures were incubated overnight on a rotary shaker (100 rev/min). The organisms were thus in the stationary-growth phase when harvested.

Production of plasmolysis. Bacteria harvested by centrifugation were suspended in the plasmolyzing solution, which usually was 2 M sucrose.

Microscopy. For light microscopy, bacterial suspensions were spread on agar. The suspending medium and the agar gel contained the same amount of plasmolyzing agent per milliliter. The organisms thus immobilized were studied with a Leitz phase-contrast microscope equipped with an oil-immersion lens.

For studies with the electron microscope, the bacteria were prefixed for 1 hr with formaldehyde (final concentration, 4%), centrifuged, fixed with OsO_4 by the method of Ryter and Kellenberger (1958), and embedded in Epon 812 (Luft, 1961). Thin sections of the embedded material were cut with a LKB ultrotome or Porter-Blum microtome equipped with glass knives, and examined in a Siemens Elmiskop I working at 80 kv and with an instrumental magnification of ×20,000; 20- to 50-μ objective apertures were used.

Counting procedures. Viable counts were determined after spreading suitably diluted samples on agar containing the growth medium. Total counts were made with a Bürcker counting chamber.

RESULTS

In 2 M sucrose, plasmolysis occurred instantaneously, or nearly so, and remained unchanged for at least 24 hr. Sucrose solutions of a concentration less than 1 M were not effective, nor were saturated KNO_3 or NaCl solutions. Phase-contrast microscopy revealed that the protoplasm of the plasmolyzed cells decreased in size by about 50% upon plasmolysis (Fig. 1). Usually, the protoplasts of one cell chain assumed the appearance of cylindrical, rounded, or dumbbell-shaped bodies separated from each other by less-dense regions. When plasmolyzed cells were fixed with formaldehyde and transferred to a medium of low osmotic pressure, the protoplasts retained this appearance but regained much of their original size. Figures 2 and 3 show sections of unplasmolyzed cells. Usually, the mesosomes of these cells were located rather centrally in the protoplasm (Fig. 2). Sometimes, however, there were indications of a connection between a mesosome and the cytoplasmic surface (Fig. 3). A cytoplasmic membrane clearly differentiated from the rest of the protoplasm was not observed. As can be seen from Fig. 4 and 5, plasmolysis markedly changed the cell structure. The cell wall became thinner and more distinctly stratified. The cytoplasmic membrane appeared as a triple-layered structure (two electron-dense layers surrounding a less dense one) at several points widely separated from the cell wall. This membrane, however, always adhered closely to the rest of the cytoplasm. As a rule, spherical bodies appeared in the pockets formed between the wall and the cyto-

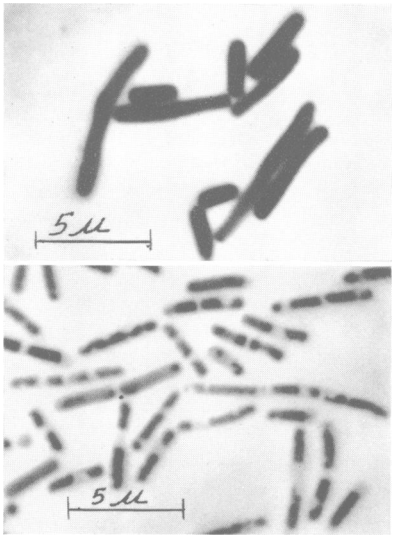

FIG. 1. *Cells of Bacillus megaterium strain M before (top) and after (bottom) plasmolysis in 2 M sucrose. Phase-contrast microscopy.*

plasmic membrane. Bodies of the same appearance were seen by Fitz-James (1964) in rapidly growing cells of *B. megaterium* KM, which had been washed in phosphate or sucrose-phosphate buffers (concentration of sucrose, 0.3 M). It thus seems evident that plasmolysis in 2 M sucrose of stationary-phase cells of *B. megaterium* M caused the expulsion of the centrally located mesosomes into pockets between the cytoplasmic membrane and cell wall. According to Ryter and Jacob (1963) and Ryter and Landman (1964), similar processes take place in cells of *B. subtilis* subjected to solutions of high osmotic pressures. The presence of lysozyme accelerated these processes.

The viability of the *B. megaterium* cells was not significantly affected by the plasmolysis process. Thus, in one experiment viable counts indicated the presence of 4.27 ($\pm$0.50) $\times$ 10^8 viable organisms per milliliter of the bacterial culture before plasmolysis, and 3.60 ($\pm$0.72) $\times$ 10^8 after (P = 0.05). The total number of cells per milliliter was 5.02 ($\pm$0.35) $\times$ 10^8.

DISCUSSION

The present investigation confirms the main results of previous studies in the same field, namely, that most bacteria can be plasmolyzed but that media of a very high osmotic pressure are required to effect plasmolysis in gram-positive organisms. The marked resistance of gram-positive bacteria to plasmolytic agents can be explained by a firm adherence of the cytoplasmic membrane to the highly rigid cell wall (Mitchell

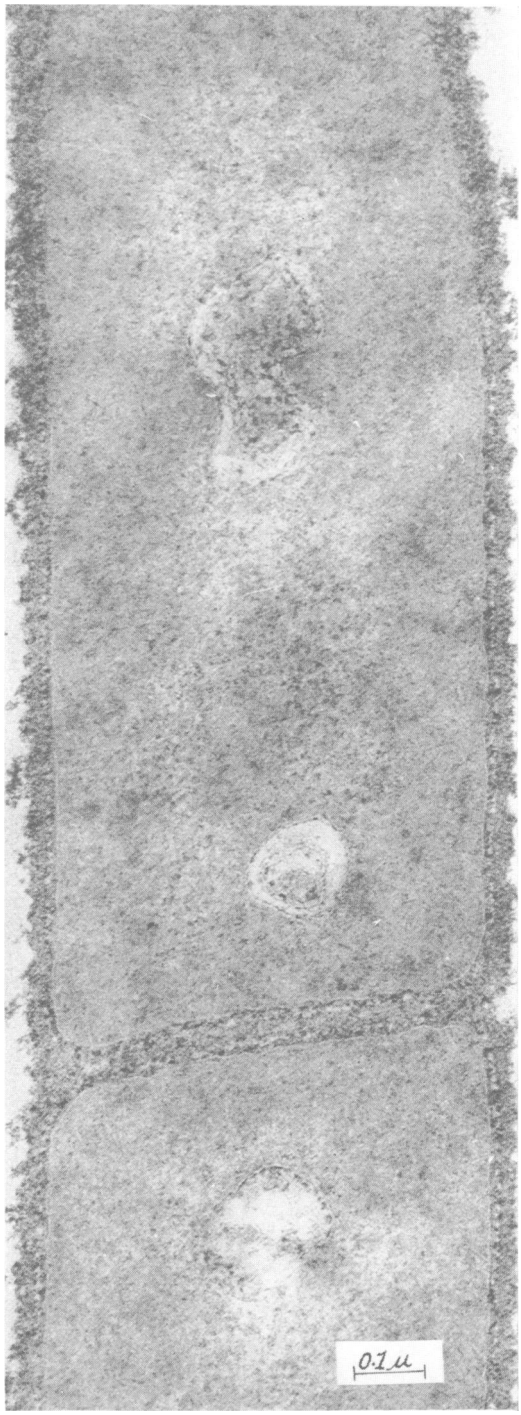

FIG. 2. *Thin section of unplasmolyzed Bacillus megaterium cell. Note mesosomes located approximately centrally in the cytoplasm.*

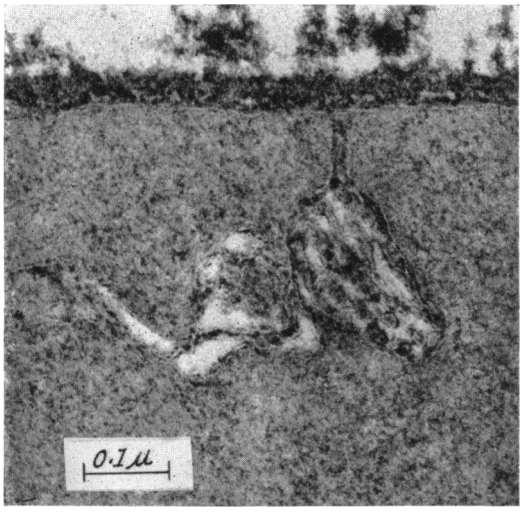

FIG. 3. *Section as in Fig. 2. Note mesosome evidently connected with the surface of the cytoplasm.*

and Moyle, 1956). It is of interest in this connection that cells of *B. megaterium*, strain M, could not be plasmolyzed with strong salt solutions, whereas such solutions were effective in the case of other *Bacillus* spp. (Knaysi, 1951). Robinow (1960) reported plasmolysis of *B. megaterium* cells in 25% NaCl solutions, but probably he did not use the M strain. The shrinkage of cells of this strain accompanying plasmolysis was appreciably greater than that noted in other *B. megaterium* strains (Mitchell and Moyle, 1956; Robinow, 1960).

As evidenced by the studies of Cota-Robles (1963) and by the present investigation, plasmolysis causes a retraction of the cytoplasmic membrane from the cell wall in *E. coli* (gram-negative) as well as in *B. megaterium* (gram-positive). Perhaps invaginations at the sides of the rod-shaped cells were found more frequently in the latter organism. A much more striking difference is, however, that mesosome bodies were not found between the cell wall and the cytoplasmic membrane in plasmolyzed *E. coli* cells, whereas this was the rule in *B. megaterium* cells that had undergone plasmolysis. This suggests that mesosomes of gram-positive and gram-negative bacteria are not strictly comparable organelles, a view supported by the work of Vanderwinkel and Murray (1962) on mesosome-containing cells of *B. subtilis*, *Spirillium serpens*, and *E. coli*.

Another difference between plasmolysis in *B. megaterium* and *E. coli* is that plasmolysis in the latter organism is of a more transient nature, as is also the case in the gram-negative organism *Proteus vulgaris* (Taubeneck, 1955).

Plasmolysis in *B. megaterium* strikingly demonstrates the flexibility of the cytoplasmic membrane as opposed to the high degree of rigidity of the cell wall.

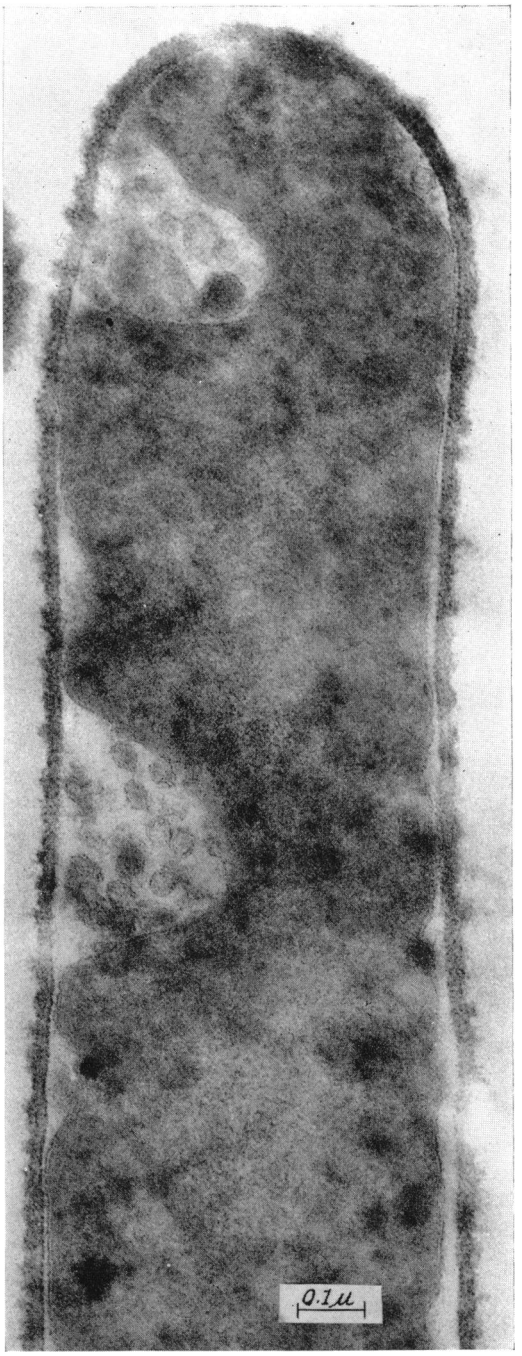

FIG. 4. *Thin section of plasmolyzed Bacillus megaterium cell. Note mesosome-containing pockets between cell wall and cytoplasmic membrane.*

The cytoplasmic membrane of this organism

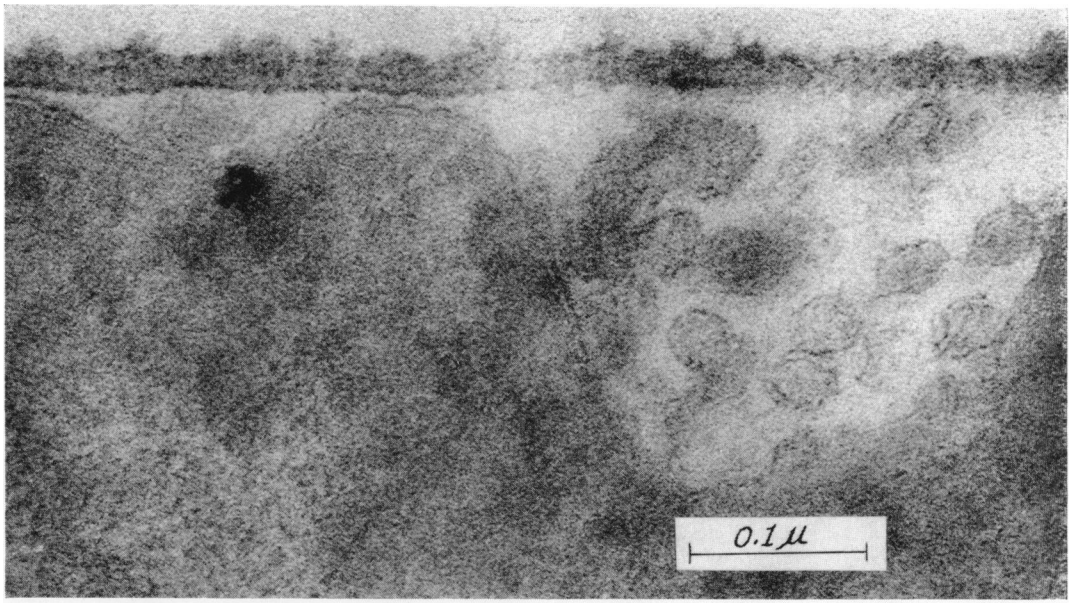

FIG. 5. *Detail of Fig. 4 at higher magnification. Note the straight cell wall, the curved, triple-layered cytoplasmic membrane, and the approximately spherical mesosome bodies. Triple-layered membranes can be seen at the periphery of these bodies.*

appears as a triple-layered structure ("unit membrane") only in plasmolyzed cells. However, this structural change, like other morphological alterations accompanying plasmolysis, does not affect the viability of the *B. megaterium* cells. Similarly, Taubeneck (1955) found that *P. vulgaris* cells that had undergone plasmolysis were able to divide.

ACKNOWLEDGMENTS

I wish to express my sincere gratitude for facilities put at my disposal at the Rocky Mountain Laboratory. I also wish to thank T. Mohri and T. Weibull for skillful assistance.

This investigation is part of a program on the fine structure of the bacterial cell financially supported by the Swedish Natural Science Research Council.

LITERATURE CITED

BAUMANN-GRACE, J. B., AND J. TOMCSIK. 1957. The surface structure and serological typing of *Bacillus megaterium*. J. Gen. Microbiol. **17**:227-237.

COTA-ROBLES, E. H. 1963. Electron microscopy of plasmolysis in *Escherichia coli*. J. Bacteriol. **85**:549-555.

FITZ-JAMES, P. 1964. Fate of the mesosomes of *Bacillus megaterium* during protoplasting. J. Bacteriol. **87**:1483-1491.

KNAYSI, G. 1951. Elements of bacterial cytology, 2nd ed. Comstock Publishing Co., Inc., New York.

LUFT, J. H. 1961. Improvements in epoxy resin embedding methods. J. Biophys. Biochem. Cytol. **9**:409-414.

MITCHELL, P., AND J. MOYLE. 1956. Osmotic function and structure in bacteria. Symp. Soc. Gen. Microbiol. **6**:150-180.

ROBINOW, C. F. 1960. Outline of the visible organization of bacteria, p. 45-108. *In* J. Brachet and A. E. Mirsky [ed.], The cell, vol. 4. Academic Press, Inc., New York.

RYTER, A., AND F. JACOB. 1963. Étude au microscope électronique des relations entre mésosomes et noyaux chez *Bacillus subtilis*. Compt. Rend. **257**:3060-3063.

RYTER, A., AND E. KELLENBERGER. 1958. L'inclusion au polyester pour l'ultramicrotomie. J. Ultrastruct. Res. **2**:200-214.

RYTER, A., AND O. E. LANDMAN. 1964. Electron microscope study of the relationship between mesosome loss and the stable L state (or protoplast state) in *Bacillus subtilis*. J. Bacteriol. **88**:457-467.

TAUBENECK, U. 1955. Über Plasmolyseversuche mit penicillininduzierten Umbildungsformen von *Proteus vulgaris*. Zentr. Bakteriol. Parasitenk. Abt. I. Orig. **163**:477-483.

VAN ITERSON, W. 1961. Some features of a remarkable organelle in *Bacillus subtilis*. J. Biophys. Biochem. Cytol. **9**:183-192.

VANDERWINKEL, E., AND R. G. E. MURRAY. 1962. Organelles intracytoplasmiques bactériens et site d'activité oxydo-réductrice. J. Ultrastruct. Res. **7**:185-199.