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NOTES

Mesosomes in *m*-Tyrosine-inhibited *Bacillus thuringiensis*[1]

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Several species of *Bacillus* have been observed to grow at a drastically reduced rate in a chemically defined medium containing the amino acid analogue *m*-tyrosine (J. N. Aronson and G. R. Wermus, J. Bacteriol. **90**:38, 1965). Although the organisms would attain a cell density equivalent to that of control cultures, the processes resulting in the formation of heat-stable spores seemed to be greatly inhibited. This inhibition of sporulation could not be attributed to a block at an early presporulation step from light microscopy alone. An abortive sporulation could have begun, but not culminated in a finished spore. An attempt at answering this question by use of electron microscopy was made with *B. thuringiensis*; this organism also forms another morphologically interesting structure, the crystalline octahedral inclusion body (parasporal crystal), at the same time that the spore is formed. Sporulation of this organism is reduced from the normal value of 5.7×10^7 to 4.0×10^4 cells per milliliter in *m*-tyrosine-inhibited cultures.

The strain of *B. thuringiensis* Berliner used was isolated from a commerical preparation, Parasporin (Grain Processing Corp., Muscatine, Iowa). The organisms were grown in shake flasks in a citrate-salts medium (H. J. Vogel and D. M. Bonner, J. Biol. Chem. **218**:97, 1956) supplemented with 0.15% glucose, 0.01% L-methionine, and 0.01% L-glutamic acid. For preparation of inhibited cultures, cells were grown as above and 10^{-3} M DL-*m*-tyrosine was added. The cells were observed by phase-contrast microscopy, harvested at the proper time with a refrigerated centrifuge, and then fixed by either the glutaraldehyde-osmium (D. D. Sabatini, K. Bensch, and R. Barrnett, J. Cell Biol. **17**:19, 1963) or the Ryter-Kellenberger (A. Ryter and E. Kellenberger, Z. Naturforsch. **13b**:597, 1958) procedures. The material was embedded in an epoxy resin (Epon 812, Shell Chemical Co., New York, N.Y.), sectioned with glass knives on a Porter Blum MT-1 microtome, placed on 200-mesh copper grids, and double-stained with lead citrate and uranyl acetate. The grids were viewed at an accelerating voltage of 60 kv on a Norelco/Philips EM-100B microscope equipped with a 20-μ objective aperture. Thin sections representing five normal cultures in various stages of sporulation and four inhibited cultures were examined.

Normal cells possessed a mature spore and parasporal crystal by 20 hr. Spores and parasporal crystals became evident by light microscopy by 12 to 14 hr. The lack of cytoplasmic membrane invaginations or mesosomes is evident in micrographs of cells during both early and late sporulation The mesosome shown in Fig. 3 was an infrequent finding in uninhibited cells. This contrasts with the extensive distribution of presumed mesosomes in the *m*-tyrosine-inhibited cells (Fig 1 and 2). These cells were taken from cultures 32 to 48 hr old, well after normal culture cells had completed sporulation and autolyzed to release the free spore and parasporal crystal. There was no evidence for the formation of the parasporal crystal in the absence of the spore. These inhibited cells appeared to be actively dividing, as evidenced by the mesosome involved in septum formation (Fig. 2). The physiological state beyond which the inhibited cells did not proceed is that of the chromatin axial filament stage (I. E. Young and P. C. Fitz-James, J. Biophys. Biochem. Cytol. **6**:483, 1959), as shown in Fig. 1. An asporogenic mutant of *B. cereus* has been reported to be blocked genetically at this stage, and also to possess more numerous mesosomes than normal cells (D. G. Lundgren and C. C. Remsen, J. Bacteriol. **91**:2096, 1966).

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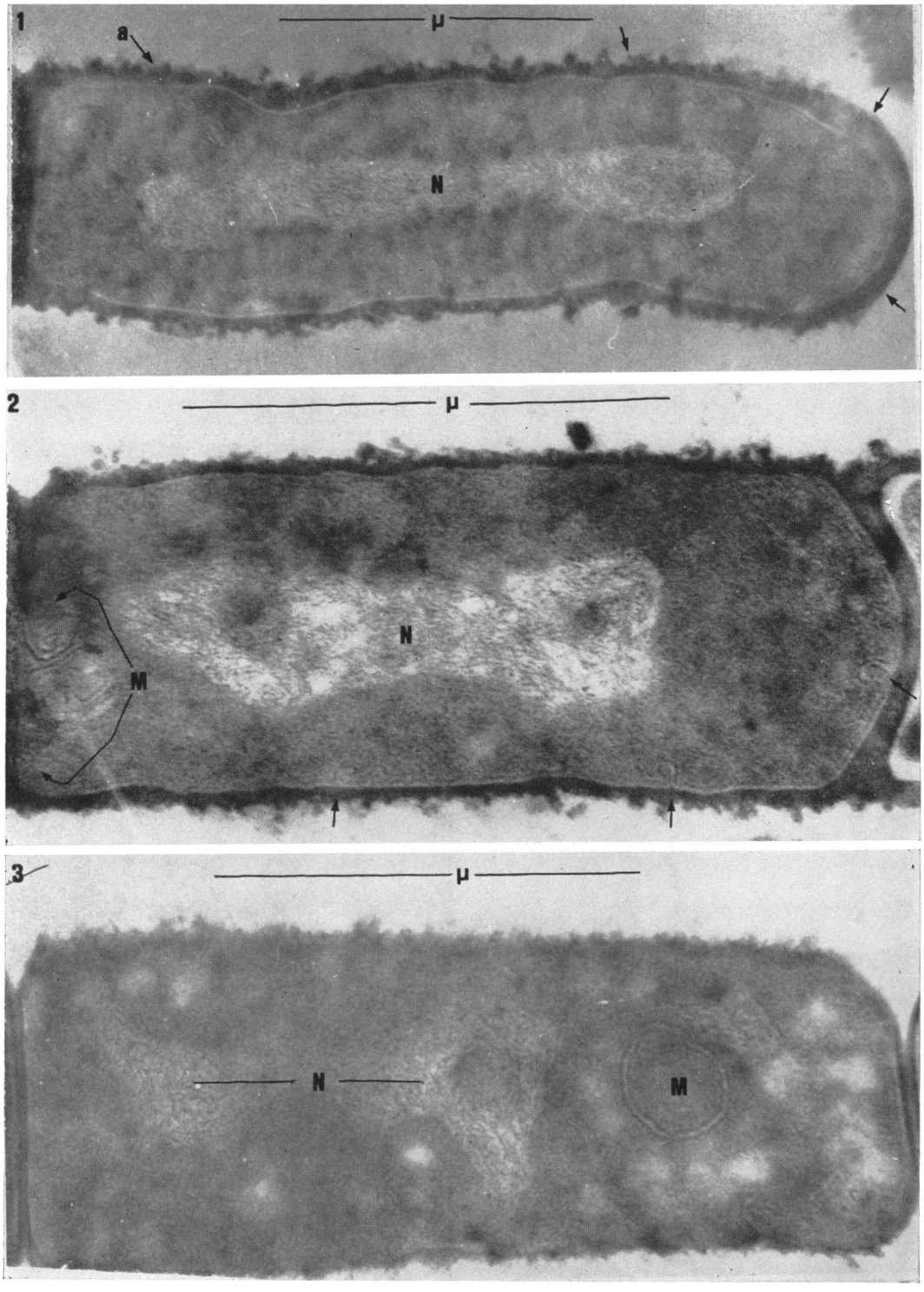

FIG. 1. *Thin section of m-tyrosine-inhibited Bacillus thuringiensis cells (48-hr culture), Ryter-Kellenberger fixation. Several membrane invaginations are indicated by arrows. No evidence of an abortive spore or parasporal inclusion can be found. Nuclear material (N) is in the axial filament stage; attachment to cytoplasmic membrane is at (a).*

FIG. 2. *Thin section of Bacillus thuringiensis inhibited by m-tyrosine (32-hr culture). The mesosome (M) involved in septum formation may be attached to the elongated nuclear material (N), indicating active cell division. Some of the numerous invaginations of the cytoplasmic membrane are indicated with arrows.*

FIG. 3. *Well-defined mesosome (M) in thin section of Bacillus thuringiensis very early in sporulation (13 hr), Ryter-Kellenberger fixation; (N) is nuclear material.*

This present study did not lend support to the suggestion that the cytoplasmic surface of the forespore membrane might function in seeding the initial formation of the parasporal crystal (P. C. Fitz-James, Intern. Congr. Electron Microscopy, 5th, p. R.R. 10, 1962), because, despite the frequent juxtaposition of the parasporal crystal adjacent to the spore, all of our micrographs showed a clear separation between the crystal and the forespore membrane or the exosporium membrane. However, serial sections early during sporulation would be necessary to resolve this question

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