Electron Microscopic Observations of a Remarkable Body in Aged Corynebacterium diphtheriae

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Applying improved electron microscope techniques, Kawata (Japan. J. Microbiol. 5:441, 1961) revealed the fine structure of younger cells of Corynebacterium diphtheriae. The cytoplasm was seen to contain an intracytoplasmic membrane system and metachromatic polyphosphate granules. In addition to these intracytoplasmic structures, a remarkable structure, which has never been reported in any bacterial cells, is presented here in cells from an aged or old culture.

C. diphtheriae C4 (nonlysogenic strain), supplied by Dr. Kurokawa (National Institute of Health, Japan), was grown in Löffler medium or on chocolate agar at 30 C. Sections were prepared by the procedure previously described (Kawata, Japan. J. Microbiol. 5:441, 1961) and poststained with lead hydroxide by the method of Watson (J. Biophys. Biochem. Cytol. 4:727, 1958). Specimens were examined in a Hitachi HU-11 electron microscope, with accelerating voltage of 75 kv.

The apparently heretofore unknown structure appeared subterminally or centrally in the cytoplasm as the polyphosphate granules disappeared. This structure has the form of a sphere or an oval which increases gradually in electron density and in thickness of its outer layer (Fig. 1, p. 1614). The dense outer layer is irregular, and its thickness ranges from about 15 to 50 mµ. A region surrounded by the dense layer becomes filled with fine granules, about 5 to 10 mµ in diameter, and this region develops into a large granulated body about 200 to 1,000 mµ in diameter. The completed body consists of the rough outer layer, the transparent intermediate space and the granulated core (Fig. 2, 4, and 5). Occasionally, the granulated core is not surrounded with the well-outlined outer layer or the intermediate space (Fig. 3). The formation of these bodies in chocolate agar usually takes longer than in Löffler medium. These remarkable bodies gradually decrease in their electron density during 1 to 2 months in Löffler medium, but they remain intact in 2- to 5-month cultures on chocolate agar in which cells still survive (Fig. 4 and 5).

Similar bodies are also produced in aged cells of C. diphtheriae PW-8. A membranous structure, probably consisting of an invagination of the cytoplasmic membrane, remains in the cytoplasm in Fig. 1. Many small vesicles composed of the unit membrane are present between the cell wall and the cytoplasmic membrane in Fig. 4 and 5.

Nakata (J. Yonago Med. Assoc. 7:93, 1956) and Teramoto (J. Juzen Med. Assoc. 69:55, 1963) observed gram-positive granules in older cells of C. diphtheriae, and they, respectively, designated these granules as the “condensed plasm” and the “oval granules.” They considered that the granules might correspond to dormant forms of the organism. It seems possible that the remarkable bodies presented here may play an essential role in the survival of C. diphtheriae, because they are formed in cells from an aged culture. It is perhaps even more likely that the bodies may represent an accumulation of a by-product of metabolism or of a waste product; however, their true nature should be elucidated by further investigation.
**Fig. 1–5.** Electron micrographs of thin sections of *Corynebacterium diphtheriae* C4. Scale mark represents 200 μm in each figure. (1) A cell from an inoculum grown on Löfller medium for 96 hr at 30 C. A large body surrounded by a dense thick layer occurs near one end of the cell. A membranous structure (M) is visible in the periphery of the cytoplasm. ×135,000. (2 and 3) Cells from an inoculum grown on Löfller medium for 120 hr at 30 C. Fig. 2 shows a large body consisting of an irregular outer layer, a lighter intermediate space, and a granulated core. In Fig. 3, a large body is packed thoroughly with fine granules. Fig. 2, ×150,000; Fig. 3, ×100,000. (4 and 5) Cells from an inoculum grown on chocolate agar for 65 days at 30 C. Large dense bodies are shown in the mostly lysed cytoplasm. Small vesicles (V) are visible between the cell wall and the cytoplasmic membrane. ×80,000.