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Fine Filaments on the Outside of the Exosporium of *Bacillus anthracis* Spores

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Recently, P. Gerhardt and E. Ribi (J. Bacteriol. **88**:1774, 1964) reported that the exosporium of the spore of *Bacillus cereus* consisted of a nap of hairlike projections and a basal membrane. Similar structures were also seen in *B. anthracis* spores. This paper reports the ultrastructures of both resting and germinating spores of *B. anthracis*, particularly the presence of the nap, as revealed by electron microscopy.

B. anthracis AKASHI was used throughout this study. The organism was cultured on meat extract-agar at 37 C for 1 week. Spore crops were harvested and suspended in distilled water. The suspension was centrifuged at 1,500 × *g* for 5 min to separate vegetative cells and sporangial remnants. The spores collected by centrifugation from the supernatant fraction were washed several times with distilled water. Germination took place in a medium containing 0.5% adenosine, 0.5% L-alanine, 1% yeast extract, and 1% Casamino Acids (*p*H 7.2). After incubation at 37 C for either 30 or 150 min, the cultures were prefixed with 2% cold osmic acid for 30 min. The germinating spores were harvested by centrifugation and embedded in an agar block. The block containing the specimen was refixed with 1% buffered osmic acid for 18 hr at 3 C, dehydrated in alcohol, embedded in Epon 812, and sectioned with a LKB ultrotome. The sections stained with lead citrate and uranyl acetate were examined with a Hitachi 11B electron microscope.

A nap of fine filamentous structures was clearly seen on the outside of the exosporium (Fig. 1, 3, and 4). These filaments were about 720 A in length and 100 A in diameter (Fig. 2). The micrographs show the filaments coming from the basal membrane of the exosporium.

As shown in Fig. 1, the resting spore had a wide space between the exosporium and the spore coat consisting of two osmiophilic thin membranes. The cortex was thick and consisted of the electron-opaque and the electron-dense layers. As A. Ryter (Ann. Inst. Pasteur **108**:40, 1965) suggested, the dense layer in the cortex seemed to be the cortex material. The spore membrane (alias spore wall) did not show a clear structure. In the germinating spores, the aforementioned space became narrower as the core developed (Fig. 3 and 4). The thickness of the cortex was reduced, and a thin membranous layer became visible (Fig. 3). Therefore, there were three thin membranes (two membranes of the spore coat and a newly appeared membranous layer) in the space between the exosporium and the spore membrane in the germinating spores (Fig. 3 and 4). These were clearly shown in the "shell" from which a young vegetative cell had slipped out (Fig. 4). In some cells, the structures which were called "inclusions" by P. Gerhardt and E. Ribi (J. Bacteriol. **88**: 1774, 1964) were found in the space between the exosporium and the spore coat (Fig. 3). The fine structure of the inclusion resembles that of the spore coat seen in the same figure. At the late stage of germination (Fig. 4 and 5), the spore membrane became thicker and more osmiophilic, and showed the appearance of the cell wall of the young vegetative cell (Fig. 5).

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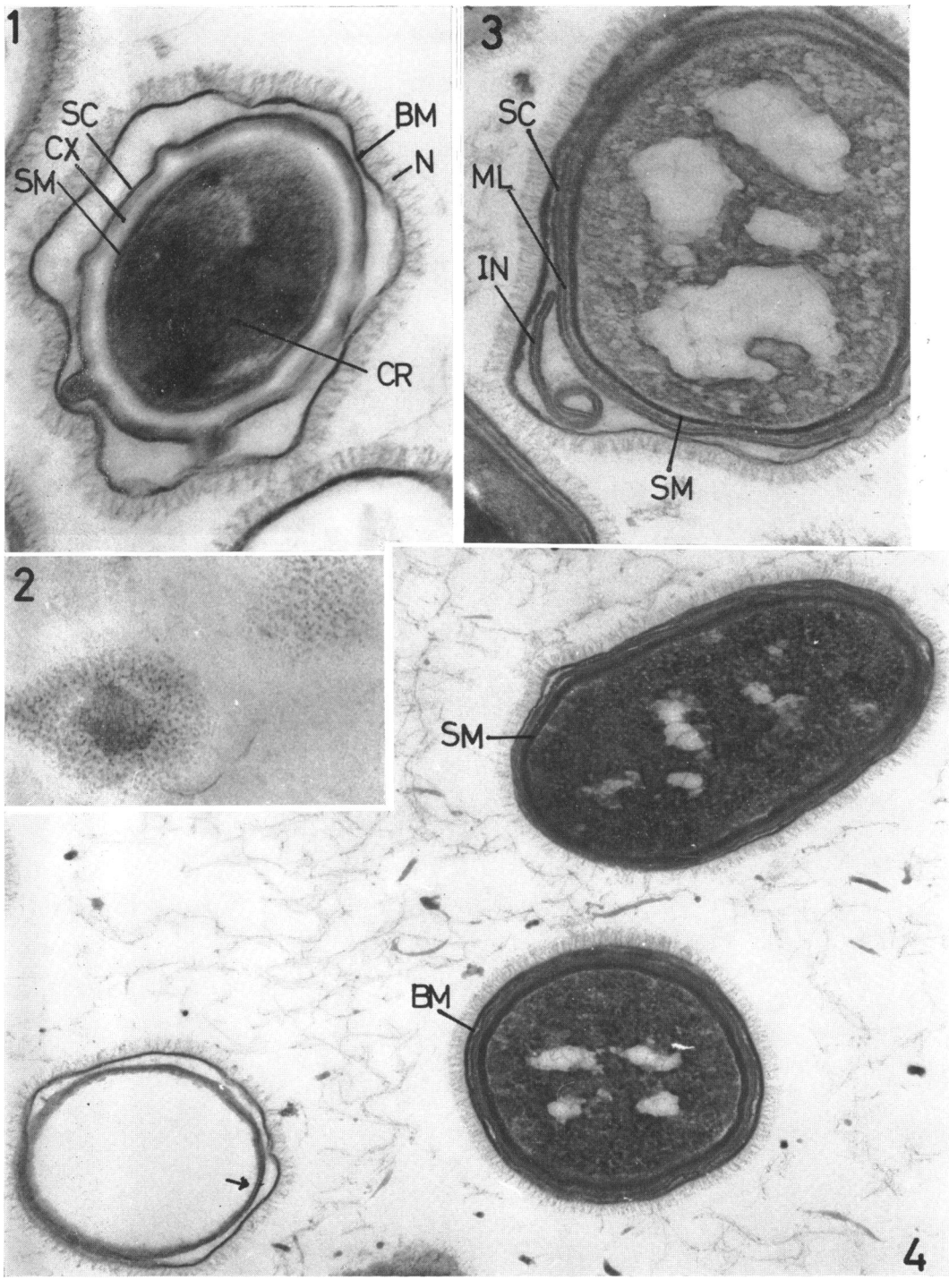

FIG. 1. *Section of the resting spores of Bacillus anthracis.* × *52,000. The exosporium consists of a nap (N) and a basal membrane (BM). The spore coat (SC), the cortex (CX), the core (CR), and the spore membrane (SM) are shown. The spore coat consists of two osmiophilic thin membranes.*

FIG. 2. *Cross section of the nap.* × *45,000.*

FIG. 3. *Section of the spore incubated for 30 min.* × *50,000. The core elongates and the spore membrane becomes thicker and more osmiophilic. A thin membranous layer (ML) is found after disappearance of the cortex. Inclusion (IN) is similar in structure to the spore coat.*

FIG. 4. *Section of the spores incubated for 150 min.* × *39,000. The arrow shows three membranes of the "shell" from which young vegetative cell slipped out. The spore membranes show the appearnce of the cell wall of the young vegetative cell.*

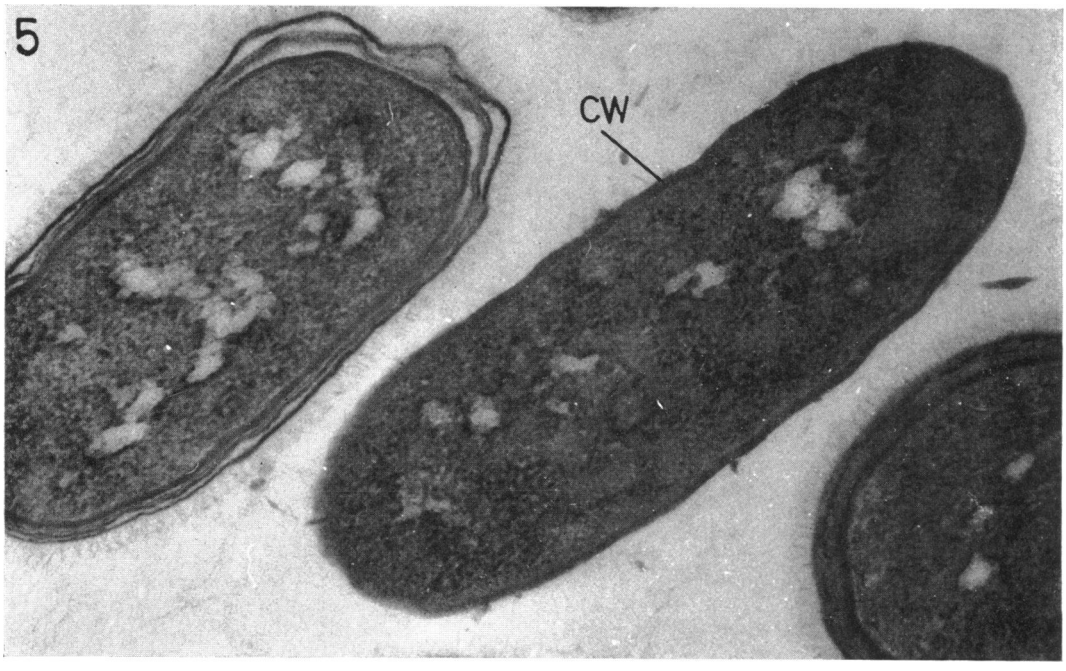

FIG. 5. *Section of the young vegetative cell.* × *50,000. The cell wall (CW) of the young vegetative cell is shown. Left and right cells are the germinating spores.*