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SHORT COMMUNICATION

ELECTRONMICROSCOPIC OBSERVATION ON THE PARACRYSTAL FRACTION OF SPORE COATS OF *BACILLUS MEGATERIUM*

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The spore coat is an initial site of contact for germinative substances. It is well known that the permeability of the spore coat changes in the initial stage of germination and this is followed by release of spore constituents. This suggests that chemical or physical modification of the spore coat structure must occur in the first step of germination. Therefore, the chemical and physical constitution of the spore coat is probably closely connected with the mechanism of germination. In the previous paper, it was reported that the spore coat of *B. megaterium* QM B1551 consisted of at least three components; a middle, paracrystal fraction, which consisted mainly of keratin like substance, is sandwiched between or cemented with the alkali soluble fraction on one side and the resistant residue layer on the other (KONDO & FOSTER, 1967). It was also found that the paracrystal fraction was the only one of these spore coat fractions which was physically and chemically active. The present paper describes electronmicroscopic studies on the paracrystal fraction.

Spore coats were prepared by mechanical rupture of spores in a Nossal disintegrator (NOSSAL, 1953). Washed and dialyzed spore coats were suspended in M/100 Tris buffer (pH 7.8) and treated with lysozyme (final concentration 200 μg/ml) at 37°C over night (with toluene added as preservative). Lysozyme digested coats were suspended in 0.006N NaOH solution (O.D. 0.8) and maintained at 50°C for 30 min. This treatment eliminates the alkali soluble fraction from the purified spore coats. After extraction with NaOH, coats were suspended in deionized water and the suspension was treated with a Branson Sonifier for 5 min. The paracrystal fraction is released from the coats by this treatment and a slightly opalescent paracrystal solution was obtained by centrifugation of the suspension at 10,000 $\times g$ for 20 min.

The morphological changes of the spore coats were observed under a model EMU 3-G RCA electronmicroscope during the treatments. When coats which had been extracted with NaOH were treated with a Sonifier, loose particles were seen on or around the coats in the initial stage of the treatment (Fig. 1). The number of these particles increased during further treatment and after treatment, agglutinated material composed of discrete particles was seen around the coat residues (Fig. 2). This represented the major portion of the paracrystal fraction which was recovered as a paracrystal material by concentration *in vacuo* (KONDO & FOSTER, 1967).

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During the concentration and drying of a dilute, optically clear solution of the paracrystal fraction on mica films for electronmicroscopy, the particles associated in a regular manner as contoured layers (Fig. 3). Occasionally, crystalloid structures were detected at the edges of the sheets where presumably local conditions were conductive to their formation (Fig. 4). These crystalloid structures are flat and elongated with a basically hexagonal form. They also exhibit contour lines suggestive of a vertical buildup from horizontal, one particle thick strata. The paracrystal material dissociated into smaller subunits when treated in a Sonifier in sodium lauryl sulfate solution (Fig. 5).

The results presented here indicate that the paracrystal fraction, which was mainly composed of a keratin like substance, occurred as one or more layers of subunits which were solubilized by sonication. This fraction can be dissociated into a solution of subunits which under certain conditions associate and there is evidence for a tendency toward crystallinity. The characteristic nature of this fraction indicates that the coat layer consists of a paracrystal fraction with a specific morphological nature. Recently, it has been reported that the spore coat of *B. megaterium* QM B1551 includes the membranous integument, fibrillar integument and outermost integument (RODE & WILLIAMS, 1967). There seems to be a close connection between the fibrillar integument and the paracrystal fraction. Studies on the identities of these materials are in progress.

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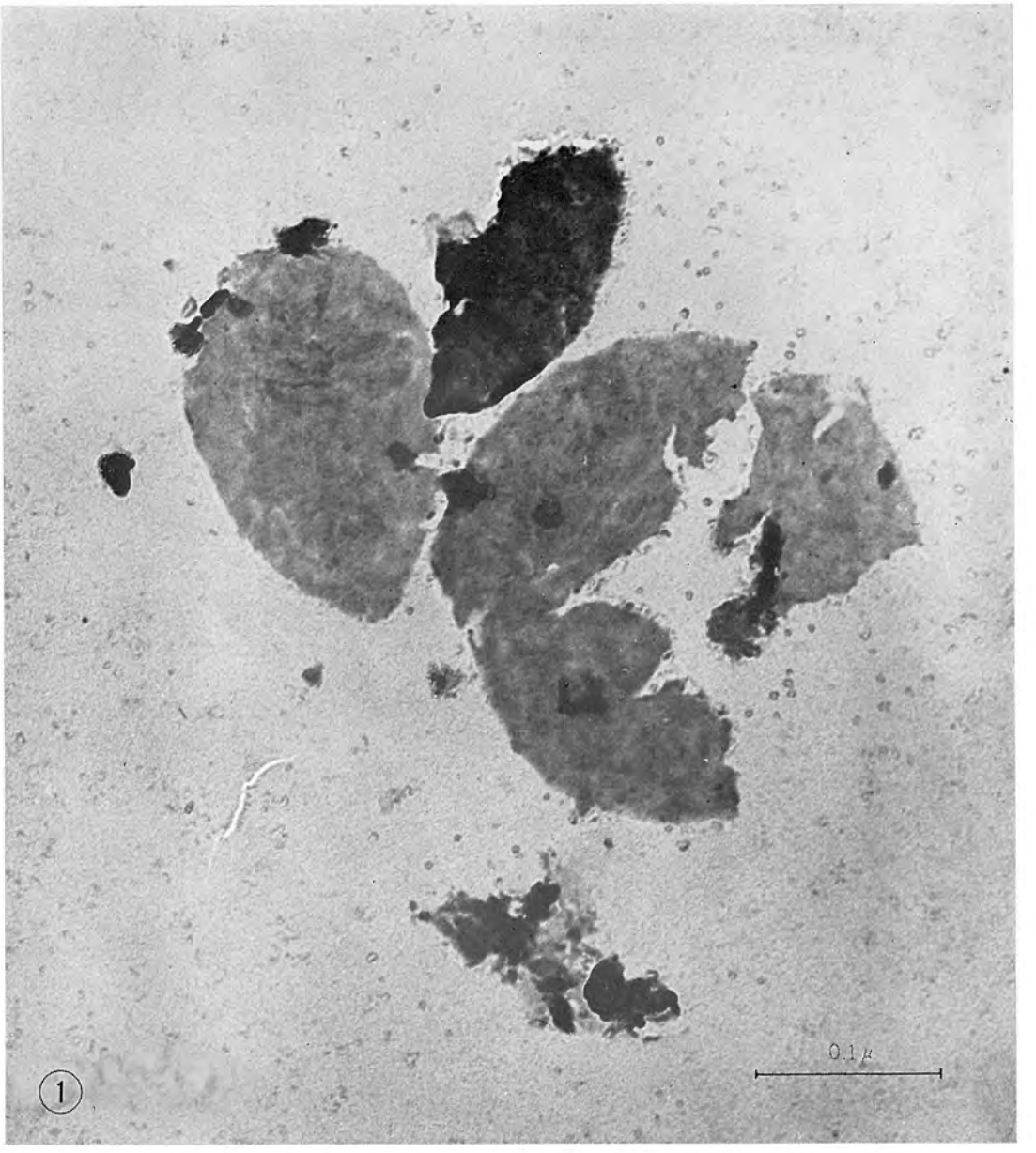
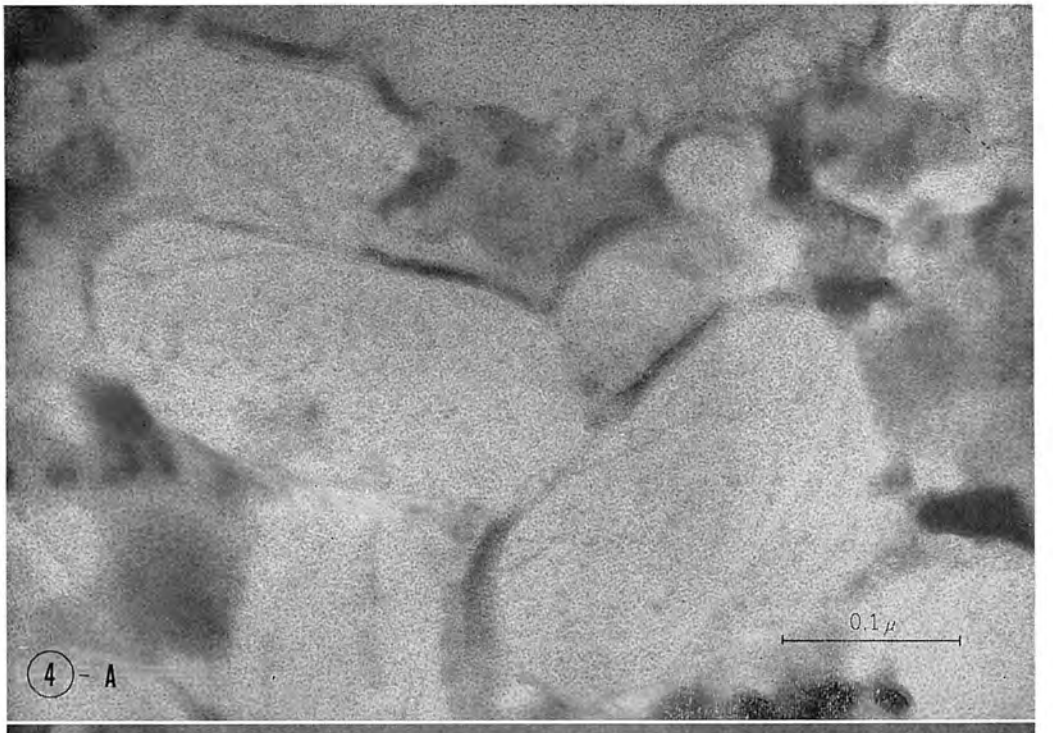


FIGURE 1 Spore coat after extraction with NaOH in the final stage of sonic treatment
Shadowed at 45° Magnification : ×100,000

FIGURE 2 Spore coat after extraction with NaOH in the initial stage of sonic treatment.
Specimens were freeze-dried on freshly cleaned mica, shadowed with platinum-palladium (80 : 20 w/w) and coated with carbon. The carbon-coated preparation was floated from the mica on distilled water onto a 200-mesh wire grid.
Shadowed at 45° Magnification : ×34,800.

FIGURE 3 Concentrated and dried paracystal material.
Shadowed at 45° Magnification: ×112,200

FIGURE 4 Crystalloid structure of paracrystal material.
Shadowed at 45° Magnification : A—×220,000
B—×100,000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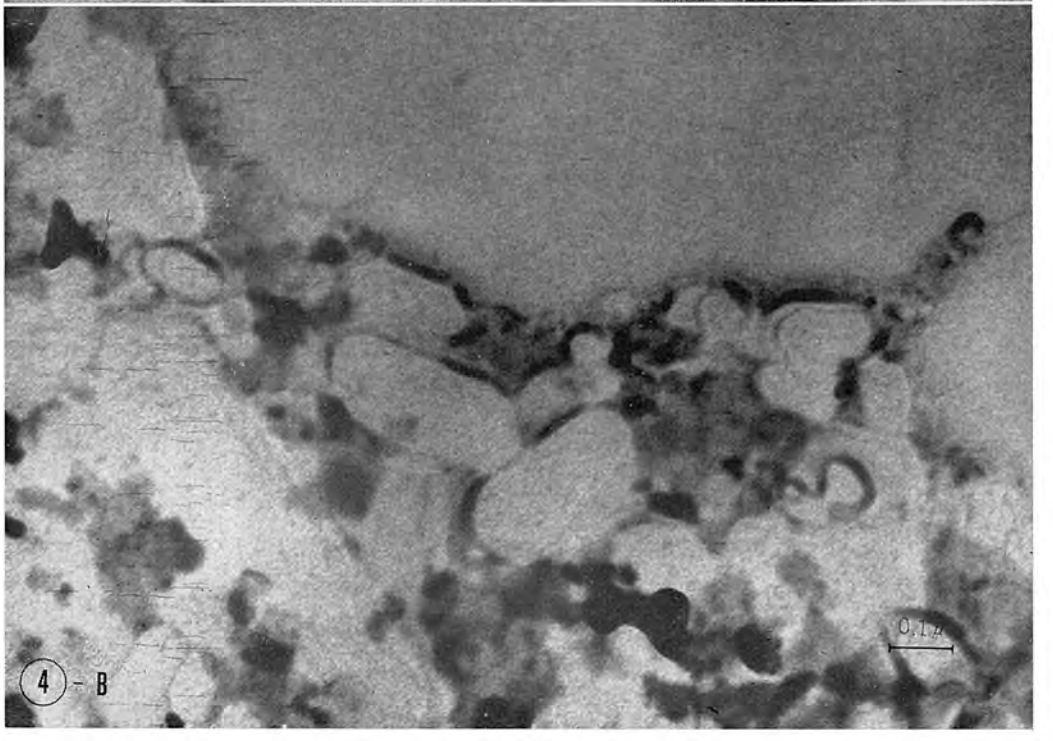
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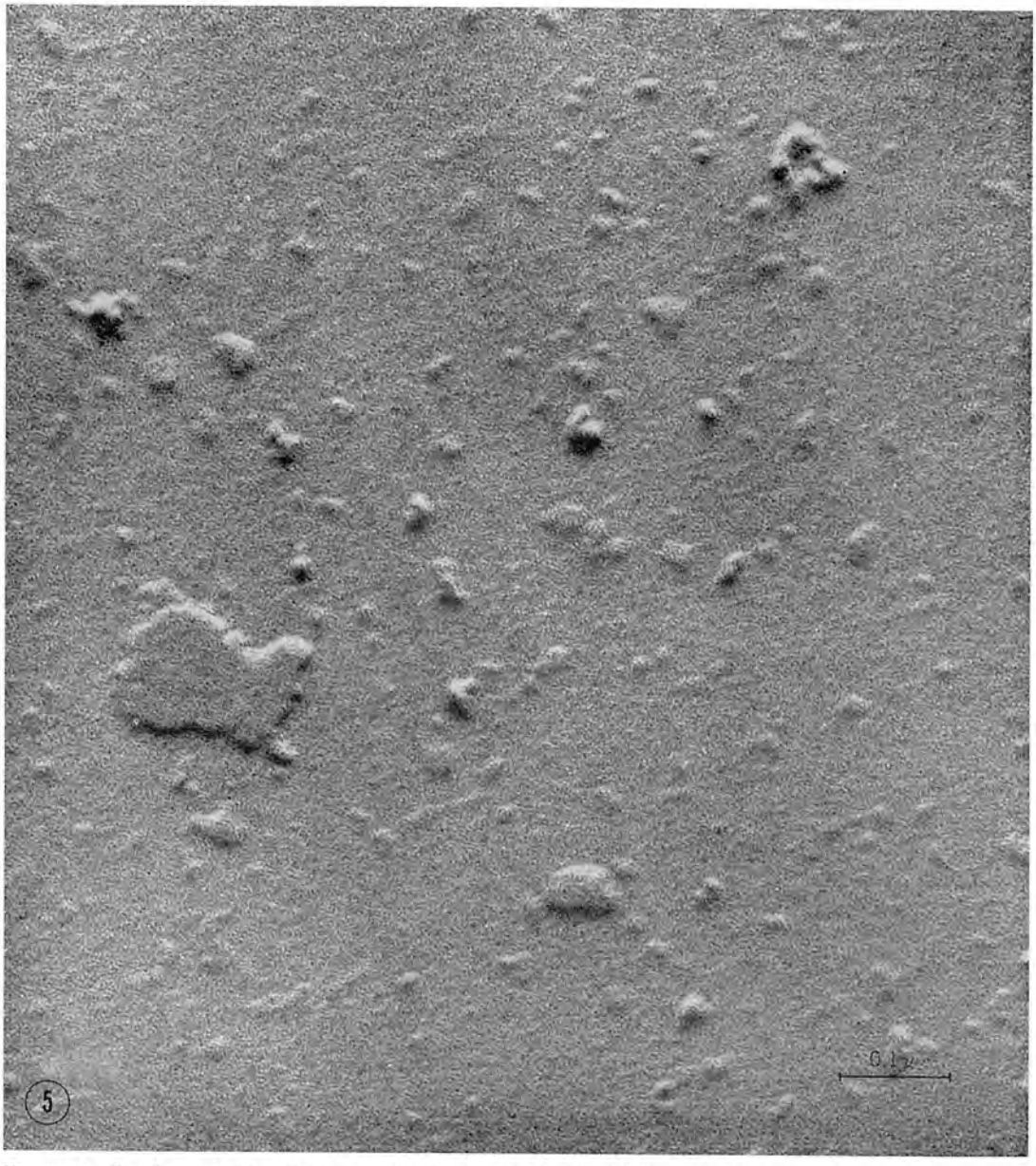

FIGURE 5 Smaller subunits dissociated from paracrystal material by sonic treatment.
Shadowed at 45° Magnification : ×200,000

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