MORPHOLOGY OF SPORES OF *BACILLUS APIARIUS* KATZNELSON[1]

PHILIP C. FITZ-JAMES[2]

Department of Bacteriology and Immunology, and Department of Biochemistry, University of Western Ontario, London, Canada

Received for publication May 10, 1959

The peculiar rectangular outline of the spores of *Bacillus apiarius* Katznelson, observed while measuring spores in the electron microscope (Fitz-James and Young, 1959), prompted this study of the structure of these spores. Electron microscopy of thin sections and of carbon replicas revealed that the odd horned and rectangular outline was the result of a remarkably thick and ridged spore coat.

METHODS

The culture of *B. apiarius* used and the procedure for obtaining spores has been fully described (Fitz-James and Young, 1959).

Fixation and embedding for electron microscopy. The usual procedures of osmium fixation proved unsuitable for the spores of this bacillus. Even overnight exposure at room temperature to 1 per cent osmium left the interior of the spore unfixed. Reasonably good fixation and subsequent embedding was achieved either by first boiling the spores for 30 min or by partly disrupting them in a Mickle (1948) disintegrator while suspended in fixative. Fixatives used were either osmium sucrose (Caulfield, 1957) or osmium-$CaCl_2$ (Bradbury and Meek, 1958) solutions.

Fixed spores were washed first in their respective buffers, followed by 50 per cent alcohol and then dehydrated and methacrylate impregnated by standard procedures. Prepolymerized butyl and methyl methacrylate (10:1) dissolved to a thick syrup in the same monomers were used to transfer the samples to gelatin capsules. Polymerization was completed by 4 to 6 hr exposure to ultraviolet light (Hanovia, Slough, England). Sections were cut with glass knives in a Porter-Blum microtome onto either acetone water, 0.5 per cent lanthanum nitrate, or 1 per cent phosphotungstic acid. The sections were flattened with the aid of xylol vapor picked up on carbon grids and washed with one drop of distilled water. They were examined in a Philip 100A electron microscope fitted with a 25 μ objective aperture and used at an accelerating voltage of 60 kv.

Carbon replicas were made by the procedures described by Bradley and Williams (1957) and shadowed with tungsten oxide at an angle of 22 degrees.

OBSERVATIONS AND DISCUSSION

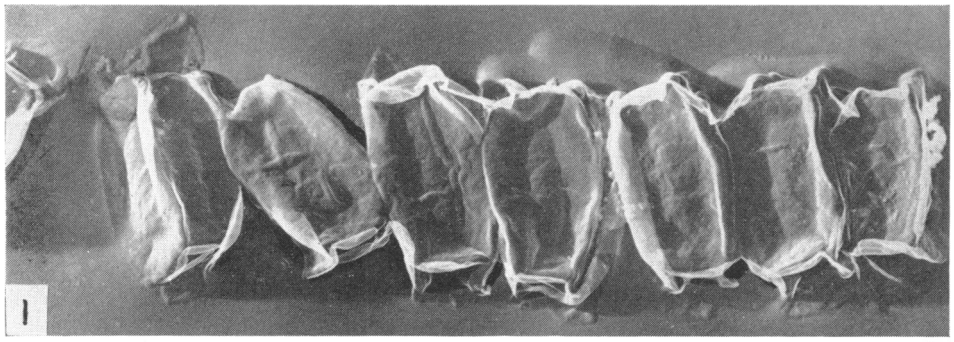
In his original description, Katznelson (1955) noted the rectangular shape of the free spores of this species and suggested that this was due to the attachment of sporangial remnants. However, both carbon replicas (figure 1) and thin sections reveal that the spore coat is responsible for this peculiar shape. Cross sections were often nearly square in outline (figure 2) and longitudinal cuts rectangular (figure 3).

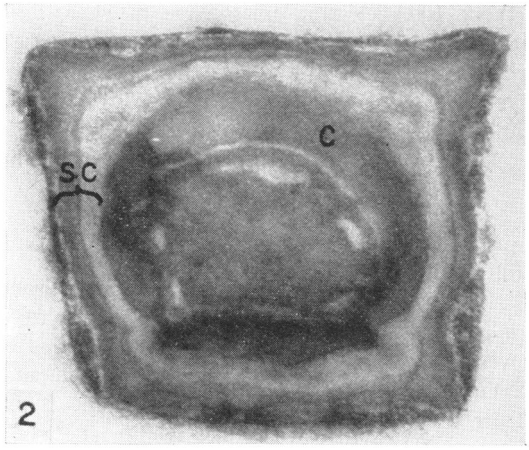
The ripe spores of *B. apiarius* invariably remained within the remnant of the sporangium. That is, the terminal lysis of spore formation did not remove the cell wall. In fact, two thin outer layers covered each spore. Occasionally these were seen closely applied to the heavy spore coat but, when fixation of the spore body was achieved, they were usually lifted out by the methacrylate (figure 3). In replicas these outer membranes were seen folded together against the spore coat (figure 1). The outermost layer, from its shape and density, appears to be derived from the old cell wall. The inner loose layer (figure 3) often reflects the shape of the spore.

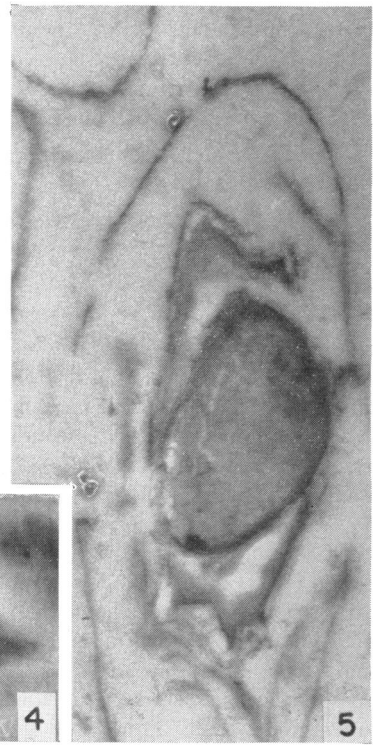
In spite of the rectangular outline of the spore coat, the spore body and the surrounding cortex are spheroidal. The difference in shape between coat and cortex is made up by the inner less dense layer of the spore coat. The cortex of both the boiled and disrupted spores took up both lanthanum and phosphotungstate. A somewhat similar staining behavior of the cortex of acid-

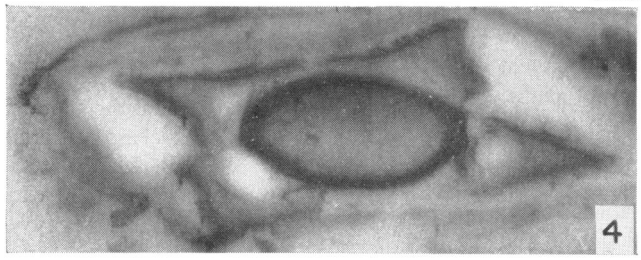
[1] Supported by a grant-in-aid from the National Research Council of Canada.

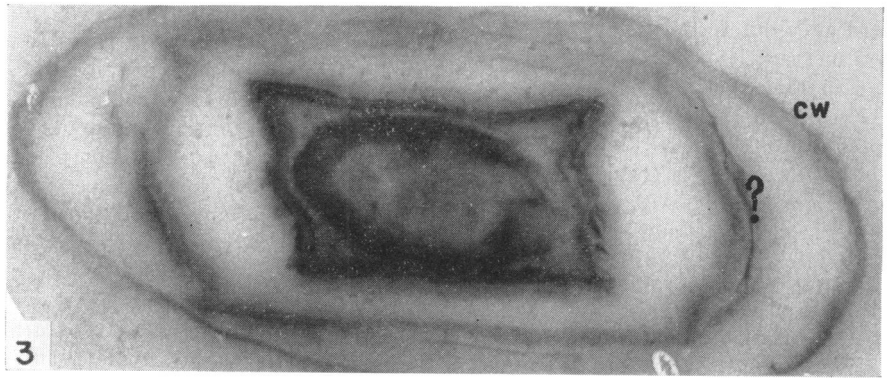
[2] Medical Research Associate, National Research Council of Canada.











FIGS. 1-5

treated spores of *B. megaterium* was observed by Mayall and Robinow (1958).

In spores slightly damaged during disruption-fixation, the heavy, ridged outer coat and the lighter zone just under it usually held together as a single layer. Although some dissolution of the undercoat was occasionally seen, a plane of cleavage was not observed between it and the dense inner coat. On the other hand, cleavage often occurred around the outer surface of the cortex (figures 4 and 5). With more severe damage the continuity of the cortex was broken and the contents of the spore body displaced. The densely staining cortex of partly damaged spores (figures 4 and 5) was found in more severe states of disruption to open out into a mass of tortuous strands or lamellae (figures 6 and 7) not unlike the striations seen in sections of acid treated spores of *B. megaterium* (Mayall and Robinow, 1958) and of forming and resting spores of *B. cereus* (Young, 1958). The cortical strands were seen best in sections cut onto phosphotungstic acid or lanthanum. (See page 768 for figures 6 to 9.)

The formation of the rectangular spore coat on the oval spore body can be readily observed in the phase-contrast microscope (figure 8). On the ripe spores during acid hydrolysis this thick coat retained and partly obscured the nuclear body which was displaced against its inner surface (figure 9).

SUMMARY

Thin section electron microscopy and carbon replicas reveal that the peculiar rectangular shape of the spores of *Bacillus apiarius* Katznelson is a function of the spore coat only. The spore body and its covering cortex are, like other spores, spheroidal in shape.

REFERENCES

BRADBURY, S. AND MEEK, G. A. 1958 The fine structure of the adipose cell of the leech *Glossiphoria complanata*. J. Biophys. Biochem., **4**, 603–607.

BRADLEY, D. E. AND WILLIAMS, D. J. 1957 An electron microscope study of the spores of some species of the genus *Bacillus* using carbon replicas. J. Gen. Microbiol., **17**, 75.

CAULFIELD, J. B. 1957 Effects of varying the vehicle for OsO_4 in tissue fixation. J. Biophys. Biochem. Cytol., **3**, 827–829.

FITZ-JAMES, P. C. AND YOUNG, I. E. 1959 Comparison of species and varieties of the genus *Bacillus*. Structure and nucleic acid content of spores. J. Bacteriol., **78**, 743–754.

KATZNELSON, H. 1955 *Bacillus apiarius* n. sp. an aerobic spore forming organism isolated from honeybee larvae. J. Bacteriol., **70**, 635–636.

MICKLE, H. 1948 A tissue disintegrator. J. Roy. Microscop. Soc., **68**, 10–12.

MAYALL, B. H. AND ROBINOW, C. F. 1958 Observations with the electron microscope on the organization of the cortex of resting and germinating spores of *Bacillus megaterium*. J. Appl. Baceriol., **20**, 333–341.

YOUNG, I. E. 1958 Chemical and morphological changes during sporulation in variants of *Bacillus cereus*. Ph.D. thesis, Medical School Library, University of Western Ontario, London, Canada.

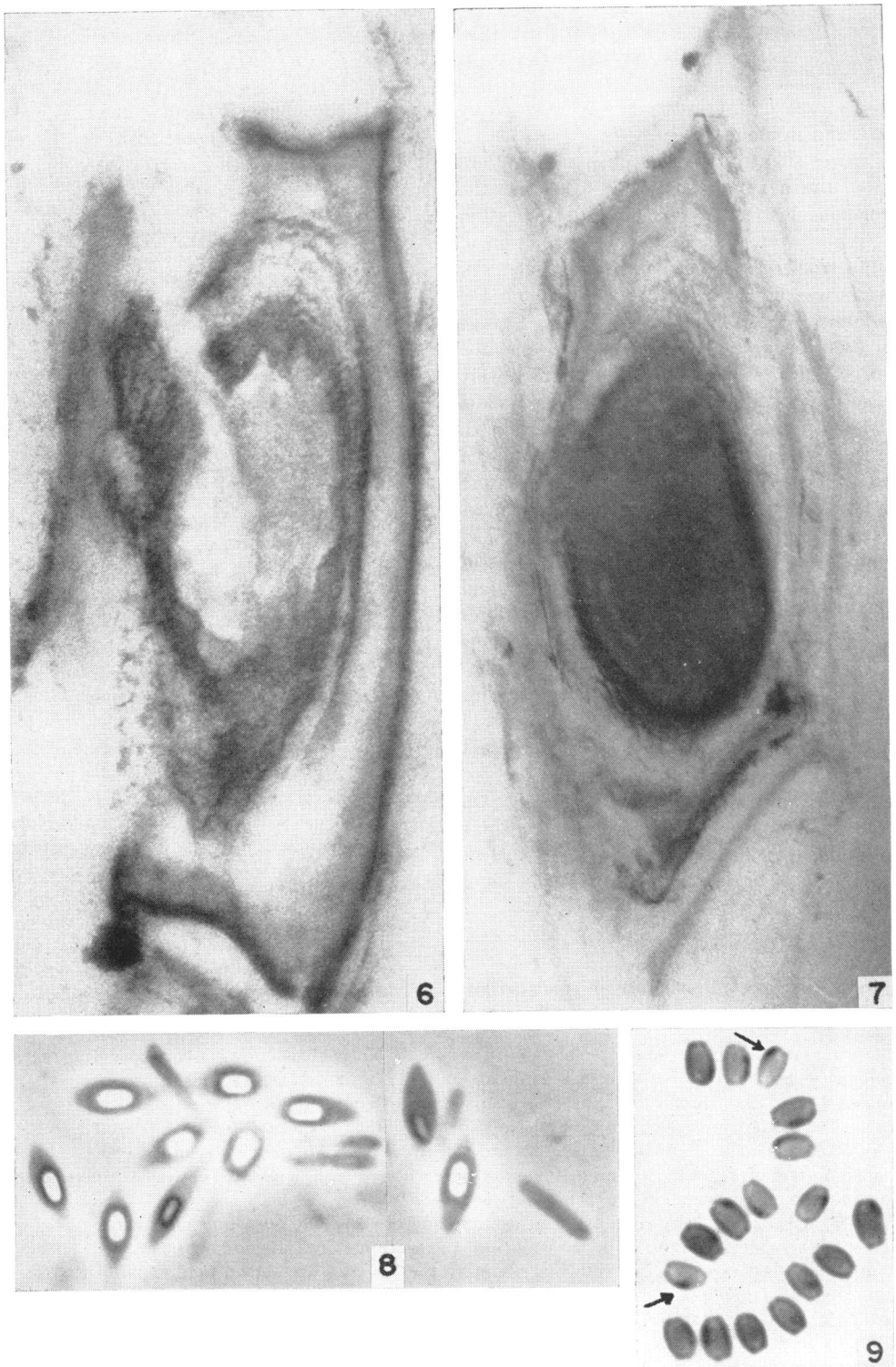
Figure 1. Carbon replicas of spores of *Bacillus apiarius* Katznelson showing their peculiar rectangular geometry. Shadowed with tungsten oxide at an angle of 22 degrees (×13,000).

Figures 2 to 5. Electronmicrographs of a thin section of spores of *B. apiarius*.

Figure 2. A boiled spore fixed in osmium and cut onto lanthanum nitrate at right angles to its long axis, showing the square outline of the coat and the circular profile of the contained spore. The heavy coat is composed of at least two major layers of different electron density. The well-stained cortex (*c*) assumes the shape of the spore, not the coat (*sc*) (×85,000).

Figure 3. A spore fixed by disruption in osmium-sucrose and cut onto phosphotungstic acid in the long axis of the spore. In spite of poor resolution, the section shows the odd shape of these spores. The two outer loose layers which invariably accompany these spores are shown here expanded by the embedding plastic. The outer one is derived from the old cell wall (*cw*), the other (*?*) usually reflects the shape of the spore (×52,000).

Figures 4 and 5. Disruption-fixed spores (sucrose-osmium) cut onto phosphotungstic acid showing the coat separating from the deeply stained cortex (figure 4, ×55,000; figure 5, ×35,000).



Figures 6 and 7. Electron micrographs of sections of spores of *Bacillus apiarius* fixed by disruption in osmium sucrose. The more severe damage has displayed the fibrillar structure of the cortex. Figure 6 (×69,000) was cut onto phosphotungstic acid, figure 7 (×70,000) onto acetone-water.

Figure 8. Dark phase-contrast photomicrograph showing various stages of spore formation of *B. apiarius* after 5 days on agar medium (×4000).

Figure 9. Spores of *B. apiarius* treated with N HCl (60 C; 9 min) stained with Giemsa. The chromatin is seen as a small dot or streak presumably pushed against the inside of the very thick spore coat (×3600).