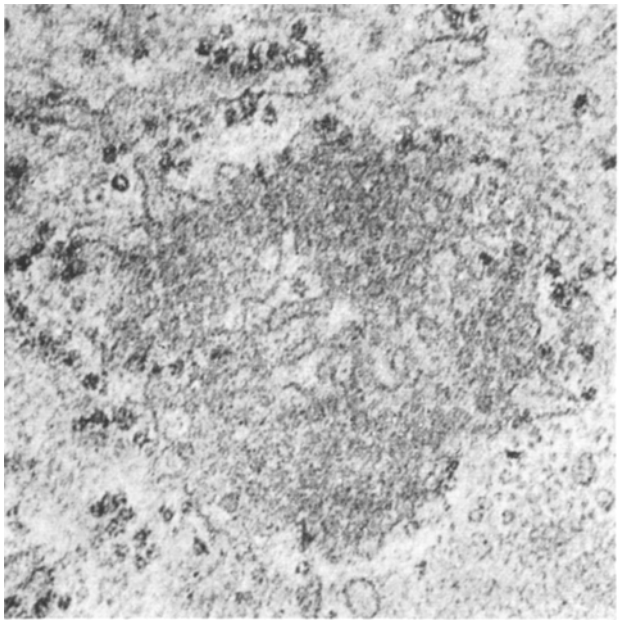

Fig. 1. Portion of endothelial cell from canine aorta. Membrane-bound cytoplasmic structure contains loosely packed microtubules which measure approximately 230 Å in diameter. Most microtubules are seen in cross section, but some are sectioned obliquely. Uranyl acetate, lead citrate. × 120,000.

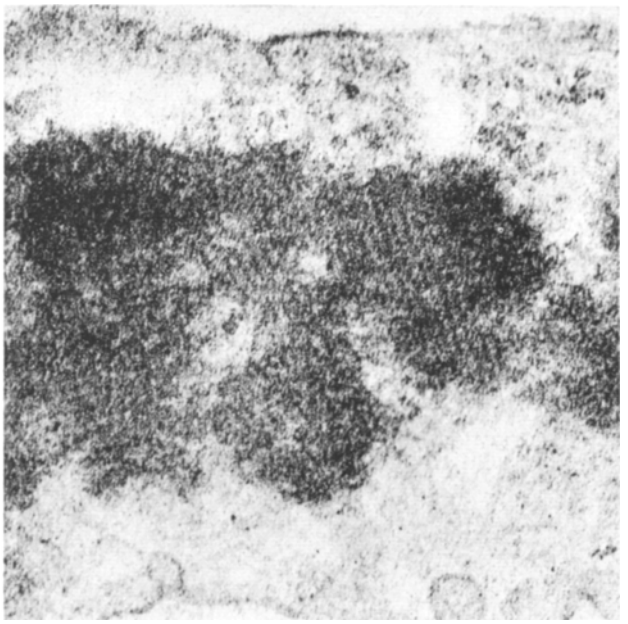

Fig. 2. Portion of endothelial cell from canine aorta. Cytoplasmic structure contains crystalloid material with a prominent period of 130–180 Å in one direction. The bounding membrane of this structure is frequently obscured because of its juxtaposition to the electron-dense crystalloid. Uranyl acetate, lead citrate. × 120,000.

structures and free cytoplasmic microtubules. Similarly, a possible relationship of the microtubule-containing structures we are reporting to both free cytoplasmic microtubules and to the crystalline structures exists.

A third type of structure which we observed, the tubulated body, has been previously described in endothelium of rat lung[6]. This information may well be associated with the other 2 structures described in this communication and with cytoplasmic microtubules in general.

The available data do not allow for definitive interpretation of the morphologic observations, and the function of these apparently related structures remains problematic[7].

Zusammenfassung. Es wurden 2 Typen von membrangebundenen Zytoplasmastrukturen im vorliegenden Endothel von normalen Hundeaorten gefunden. Teilweise handelt es sich um rauhe parallele Mikrotubuli (240 Å) und um kristallines Material mit einer Periode von 130–180 Å. Die funktionelle Korrelation von Mikrotubuli und den kristallinen Strukturen ist noch unklar.

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Department of Pathology,
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[6] E. R. Weibel and G. E. Palade, J. Cell Biol. *23*, 101 (1964).

[7] Supported by USPHS Grants Nos. HE-05435 and HE-11277.

Antigenic Components of Germinated Spores of *Bacillus megaterium*

Our previous studies have demonstrated that the vegetative forms of *Bacillus megaterium* possess at least 2 antigens in common with its spore form[1].

The present work is concerned with the relation between antigenic components of mature spore, germinated spore and vegetative forms of *B. megaterium*.

B. megaterium, Paris strain, was grown on a medium consisting of: Difco peptone, 0.1%; Difco meat extract, 0.3%; Difco yeast extract, 0.3%; manganese sulfate, 0.01%; agar, 1.5%; and 1000 ml of water. Vegetative cells (V) were harvested after 7 h of incubation and were washed 5 times with distilled water. Mature spores (MS) were harvested after 7 days of incubation and were washed 15 times in distilled water. For preparation of germinated spores, washed mature spores were suspended in 0.02 *M* phosphate buffer, pH 7.5, to an OD of 450 nm

[1] P. Mastroeni, A. Nacci and A. Rocca, J. Bact. *94*, 2073 (1967).

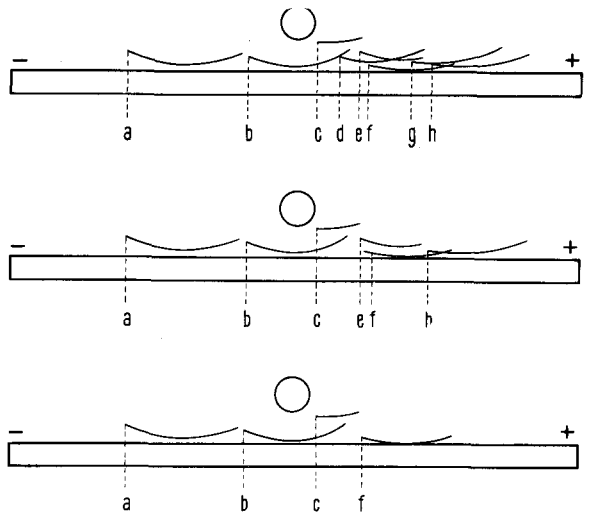

Fig. 1. Immuno-electrophoretic diagrams of: GS vs. antiGS; GS vs. antiGS, absorbed with MS antigens; GS vs. antiGS, absorbed with V antigens.

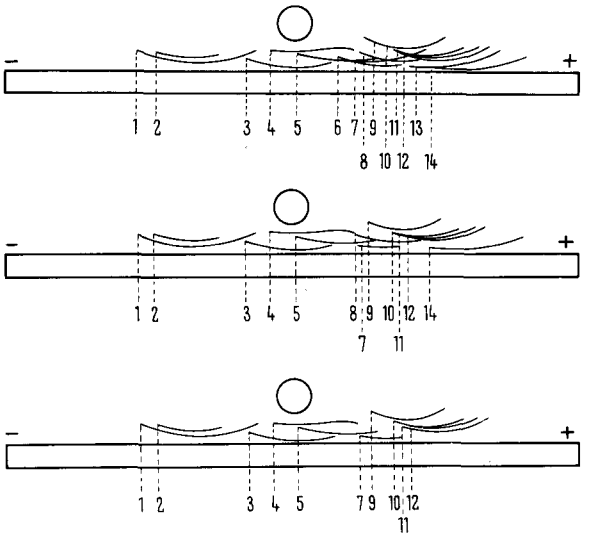

Fig. 2. Immuno-electrophoretic diagrams of: V vs. antiV; V vs. antiV, absorbed with MS antigens; V vs. antiV, absorbed with GS antigens.

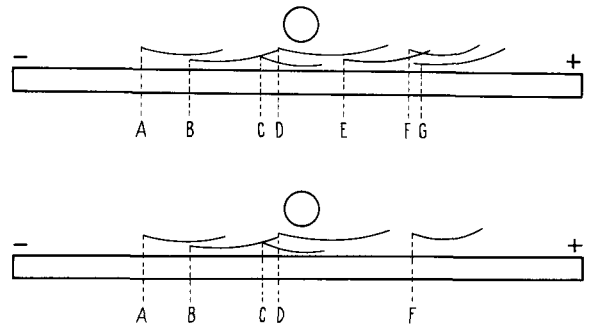

Fig. 3. Immuno-electrophoretic diagrams of: MS vs. antiMS; MS vs. antiMS, absorbed with GS and V antigens.

of 1.0. This suspension, further diluted 1:20 in the same buffer, was germinated by incubation at 37 °C for 60 min with 0.001 *M* inosine. Germinated spores (GS) were harvested by centrifugation and washed 3 times in distilled water.

Cells of the 3 types were disrupted ultrasonically in an MSE Mullard apparatus. Disintegration was complete in 60 min for vegetative forms and in 5 h for spores. The sonicates were clarified by centrifugation at 15,000 *g* for 30 min at 4 °C. The extracts, containing soluble antigens, were adjusted to contain c. 10 mg of proteins/ml[2].

Immune sera against these antigens were prepared in rabbits as previously described[3]. Immunoelectrophoretic analysis was carried out according to the technique of GRABAR.

When tested against their homologous antisera, GS antigens showed 8 precipitation arcs (Figure 1); V antigens showed 14 precipitation arcs (Figure 2); and MS antigens showed 7 precipitation arcs (Figure 3).

Antiserum to GS antigens was absorbed with MS protein and with V protein (Figure 1) by ascertaining possible antigens common to the 3 types of cells. On testing GS against these absorbed sera, it was seen that precipitation arcs representing certain antigens had disappeared and that GS therefore contained 2 antigens (d, g) in common with MS and 4 antigens (d, e, g, h) in common with V. Similarly, tests of V against antisera to V antigens which had been absorbed with MS and with GS proteins (Figure 2) revealed that V and MS had 2 antigens (6, 13) in common, and V and GS had 4 antigens (6, 8, 13, 14) in common.

These results may be summarized as follows: GS contains d, g in common with MS; GS contains d, e, g, h in common with V; V contains 6, 8, 13, 14 in common with GS; V contains 6, 13 in common with MS.

Thus GS, MS and V have antigens d, g, 6 and 13 in common. However, when MS was tested against antiserum to MS which had been absorbed with GS and V proteins, it was seen that only 2 antigens (E, G), not 4, had disappeared (Figure 3), indicating that GS antigens d and g were probably identical with V antigens 6 and 13, and with MS antigens E and G. These identities, are confirmed by the identical electrophoretic mobilities of the d, 6, and E antigens, and of the g, 13, and G antigens.

We may therefore conclude that spores, germinated spores and vegetative cells of *B. megaterium* contain antigens characteristic of themselves, as well as antigens in common with the other forms.

Riassunto. Sono stati studiati gli antigeni di forme vegetative, spore quiescenti e germinate di *B. megaterium*. È stato possibile individuare che le 3 forme possiedono sia antigeni propri sia antigeni comuni.

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23 October 1968.

[2] O. H. LOWRY, N. J. ROSEBROUGH, L. FARR and R. J. RANDAL, J. biol. Chem. *193*, 265 (1951).

[3] P. MASTROENI, A. ROCCA, P. P. GAZZANIGA and F. SONNINO, Atti III Symp. Int. sul Lisozima di Fleming (1964).