Polysaccharide Capsule of *Bacillus megaterium*.* (25019)

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By using immunochemically defined antibodies a complex capsule was discovered in a special strain ("M") of *Bacillus megaterium* (1-5). The bulk of the capsular material consisted of glutamyl polypeptide apparently enclosed in a polysaccharide "frame-work," the latter being chemically similar to the cell wall. Baumann-Grace and Tomcsik(7) studied 54 strains of *B. megaterium* belonging to 38 polysaccharide types but found a capsular structure similar to Bacillus "M" in only 10 of them. Polypeptide and polysaccharide components were, however, found in the capsules of all the strains although the location of the specific polysaccharide varied. Tomcsik (6) concluded that bacterial capsule usually differs chemically from the cell wall but that exceptions may occur as, for example in *B. megaterium*. More recently, however, Wilkinson(8) reviewing extracellular bacterial polysaccharides is not inclined to admit that *B. megaterium* is an exception and states rather categorically that "the cell wall and the predominant capsular components are quite distinct." Since Wilkinson did not reach this conclusion on the basis of any of his own experimental work, we wish to report on some new observations which show that under certain conditions strains of *B. megaterium* are able to produce a capsule consisting apparently entirely of polysaccharide serologically indistinguishable from the cell wall in which no trace of glutamyl polypeptide can be detected.

Results. All 54 strains of *B. megaterium* examined produced a complex glutamyl polypeptide-polysaccharide capsule 1-2 years after isolation when cultured on medium such as "G.F." Gladstone Fildes agar(7), which is rich in amino acids. Three to 4 years after isolation, however, most of the strains had lost this ability and even those which continued to produce polypeptide on GF agar failed to do so on Grelet's medium(9). The latter is a synthetic medium recommended for study of the sporulation of *B. megaterium* which contains glucose and nitrate as the sole sources of carbon and nitrogen. In this synthetic medium, although no polypeptide is formed, production of the polysaccharide part of the capsule is unimpaired and in 16 hr shaken cultures often reaches the dimension of that of Mg 45 shown in Fig. 1.

The results listed in Table I were obtained from cultures shaken for 16 hr in T-tubes(10) at 28°C after examination of the cells with both homologous polysaccharide and heterologous glutamyl polypeptide antibody. Older cultures were not usually suitable as sporulation had commenced and the capsules had usually been dissolved. The strains listed here were selected because, although no polypeptide was produced in the ordinary Grelet medium, addition of 0.5% asparagine was sufficient to stimulate its production. The "M" type of *B. megaterium* could not be examined as it failed to grow in Grelet's medium even in the presence of asparagine.

Discussion. Table I shows that same strains of *B. megaterium* produce under certain circumstances a capsule consisting entirely of a single component serologically identical with the cell wall polysaccharide. It is incompre-

TABLE I. Specific Capsular Reactions of 10 *B. megaterium* Strains Grown in Grelet's Synthetic Medium in Presence and Absence of Asparagine.

Medium:	With asparagine		Without asparagine	
Strain	Ps*	Pp†	Ps	Pp
Mg 6	+	+	+	—
" 7	+++	++	++	—
" 11	+++	+	+++	—
" 13	+	+++	+	—
" 16	+++	+++	+++	—
" 20	—	++	—	—
" 25	+	+++	+	—
" 26	—	+	—	—
" 29	++	+	+++	—
" 45	+++	+++	+++	—

* Ps = homologous polysaccharide type specific antibody.
† Pp = glutamyl polypeptide antibody.

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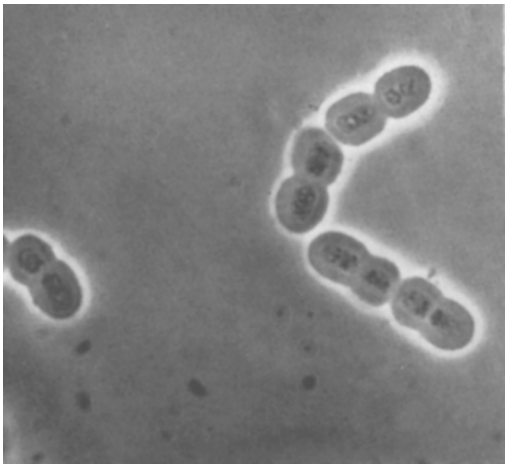

FIG. 1.

hensible that Wilkinson(8) based his assumption that the cell wall and capsular material are always chemically distinct, to some extent on the observations made by Jacox (11) as he postulated a non-specific salt-like linkage between excess cell-wall polysaccharide and polypeptide. Jacox(11) actually discovered a non-specific "swelling" reaction of the pneumococcal capsule which was only positive at pH 4.0 in presence of proteins and a NaCl concentration of less than 0.02 M. A similar reaction was not obtained with any other encapsulated organisms but only pH 4.0 was examined. Subsequently, however, Tomcsik and Guex-Holzer(12) described the pH dependent salt-like combination between bacterial capsular substances and various proteins.

The results here described also show that certain strains of *B. megaterium* do not produce glutamyl polypeptide in a synthetic medium if nitrate is the sole source of nitrogen and addition of asparagine is necessary for its production. This observation, however, requires confirmation with freshly isolated strains which produce capsules composed predominantly of polypeptide in media rich in amino acids.

Summary. Certain strains of *B. megaterium* produce capsules consisting of polysaccharide and lacking polypeptide if cultured in a synthetic medium containing glucose and nitrate as sole sources of carbon and nitrogen. The capsules give a specific capsular reaction with the same homologous antibody as that which reacts with the cell wall.

1. Tomcsik, J., *Experientia,* 1951, v7, 459.
2. Tomcsik, J., Guex-Holzer, S., *Schweiz. Z. Path. Bakt.,* 1952, v15, 517.
3. ———, *ibid.,* 1954, v17, 221.
4. ———, *J. Gen. Microbiol.,* 1954, v10, 317.
5. Tomcsik, J., Grace, J. B., *ibid.,* 1955, v13, 105.
6. Tomcsik, J., *Bacterial Anatomy,* University Press, Cambridge, England, 1956, pp41-67.
7. Baumann-Grace, J. B., Tomcsik, J., *J. Gen. Microbiol.,* 1957, v17, 227.
8. Wilkinson, J. F., *Bact. Rev.,* 1958, v22, 46.
9. Grelet, N., *Ann. Inst. Pasteur,* 1950, v78, 423.
10. Kay, D., Fildes, P., *Brit. J. Exp. Path.,* 1950, v31, 338.
11. Jacox, R. F., PROC. SOC. EXP. BIOL. AND MED., 1947, v66, 635.
12. Tomcsik, J., Guex-Holzer, S., *J. Gen. Microbiol.,* 1954, v10, 97.

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Mode of Action of Trypsin on *Bacillus megaterium.** (25020)

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We described(1-3) our findings of the action of trypsin on cells of *B. megaterium.* Our results differed from those obtained by most other workers and suggested that the cytoplasm of the majority of strains of *B. megaterium* could be slowly but completely digested with trypsin at 37°C without previous heat treatment (curve 3). Several crude commercial preparations of trypsin were compared, but owing to the necessarily long in-

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