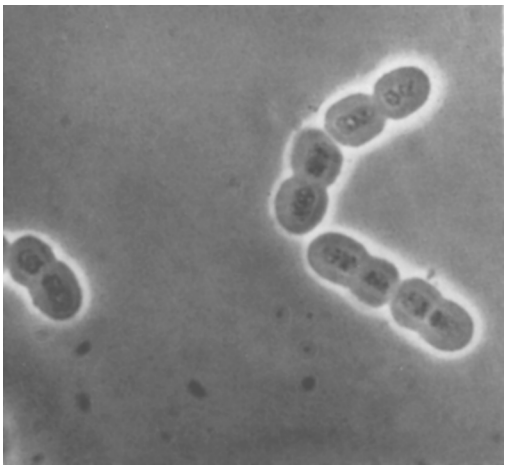

FIG. 1.

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.

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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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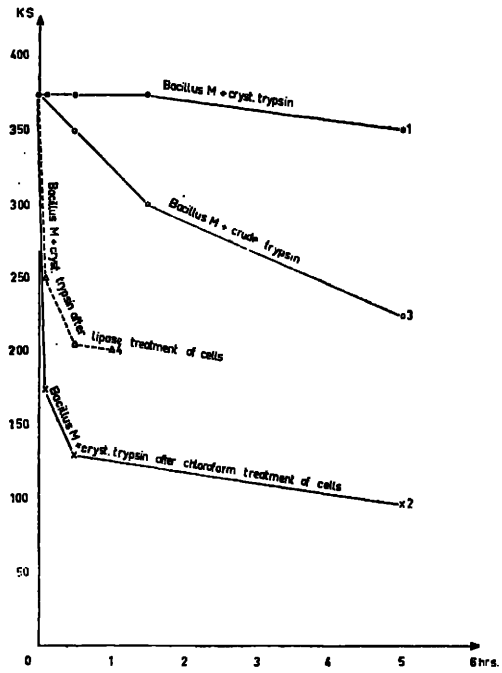

FIG. 1. Klett-Summerson readings (blue filter No. 42) of a suspension of a *B. megaterium* strain (Bacillus M), time calculated after addition of trypsin.

cubation time, chloroform was usually added as a preservative. The possibility that the chloroform played a role in the proteolytic digestion of the cells was not then suspected as control experiments with crude trypsin in which the chloroform was omitted yielded the same results. The small amount of pure crystalline trypsin which was then available to us (and for which we express our thanks to Mrs. E. Work, London) was examined only in the presence of chloroform. Since the publication of these results we have had the opportunity to study in more detail the action of crystalline trypsin (Mann) as well as of a purified preparation of the proteolytic enzyme elastase† on the cells of *B. megaterium* strain "M".

Methods. A moderately thick suspension of cells from a 16-hour culture of Bacillus *M* (Klett Summerson reading 375, with blue filter No. 42) in phosphate buffer M/30 pH 8.0 containing 1:16,000 dilution of either of these purified enzymes when incubated at 37°C showed no drop in turbidity even after 5 hours incubation. The addition of one drop of chloroform/ml of suspension, however, was sufficient to produce a marked drop in turbidity within 30 min. on further incubation and a microscopical examination showed an advanced digestion of the cytoplasm. Since the presence of chloroform interfered with the K.S. readings, the effect of pretreating the cells with chloroform before incubating with the enzymes was examined. One volume of cell suspension in buffer was shaken mechanically at room temperature with 1 vol. of chloroform. After removal of the chloroform layer and addition of enzyme to the cell suspension a rapid fall in turbidity (curve 2) and a marked digestion of the cytoplasm occurred within 5 min. and had almost reached a maximum in 30 min. Control cells treated with chloroform alone remained stable and showed no signs of cytoplasmic degeneration. Subsequent experiments showed that as little as one minute contact with chloroform was sufficient and that other organic solvents such as ether, butanol, 95% alcohol, acetone and toluol were equally effective. Chloroform could also be replaced, though less effectively, with 5% Tween.

Results. Reagents such as chloroform and butanol probably act directly on some lipid component of the cell surface, damaging it in such a way as to allow access of trypsin to the protoplast. The chloroform-treated cells were, in fact, found to be non-viable and on subsequent addition of lysozyme in buffered sucrose could not be transformed into spherical protoplasts but remained fixed in the rod form, suggesting that the cytoplasmic membrane had been damaged(4). Since, in contrast to crystalline trypsin, crude trypsin slowly digested the cytoplasm of untreated cells, it seemed probable that it was contaminated with a substance which acted in a similar way to chloroform. A spot test(5) for presence of lipase was weakly positive with the crude trypsin, so the combined action of lipase and crystalline trypsin was then examined.

The following 4 lipase preparations (Mann's Research Chemicals) were available: a relatively pure lipase from wheat germ, 2 crude

† We would like to thank Dr. I. Balbó for kindly providing us with a sample of elastase and for co-operating in preliminary experiments.

pancreatic lipases and a crude lipase of microbiological origin. The wheat germ lipase was, unfortunately, found to be unusable as it contained a lysozyme-like enzyme which dissolved the isolated cell walls of *Bacillus M.* The results obtained with the pancreatic lipases were interesting, however, as it was shown that both lipases slowly digested the cytoplasm without previous or subsequent treatment of the suspension. Cells in phosphate buffer pH 6.6 containing 1:1,000 dilution of the lipase showed a gradual drop in turbidity of the suspension and microscopic examination after 5½ hours incubation at 37°C revealed almost complete digestion of the cytoplasm. Subsequent treatment of these lipase treated cells was, therefore, not possible but examination of the pancreatic lipases by the technic of Kolmer and Boerner (6) showed that both contained large quantities of trypsin. The lipase of microbiological origin, however, proved to be more suitable for experiments involving subsequent treatment of the cells with trypsin.

Bacillus "M" was incubated in phosphate buffer at pH 6.0 containing 1:1,000 microbial lipase for 30′, at 37°C. No drop in turbidity or alteration in cell structure could be observed after this time and the cells were centrifuged down and resuspended in the same amount of phosphate buffer at pH 8.0 containing 1:10,000 crystalline trypsin. Within 5 min. there was a noticeable drop in turbidity which 60-120 min. had fallen by more than 40% (curve 4). Microscopically the cells showed a marked, although not complete digestion. A more prolonged incubation with trypsin or a longer treatment with lipase were not possible owing to instability of the controls.

Discussion. These results show that both crude lipase containing a high concentration of trypsin, as well as impure trypsin which probably contaminated with lipase can bring about digestion of the cytoplasm of untreated cells of *Bacillus "M"*. Pure crystalline trypsin alone, however, had no detectable effect, but preliminary treatment of the cells with an organic fat solvent such as chloroform rendered them immediately susceptible. In addition, a lipase of microbiological origin which was ineffective alone, behaved similarly to chloroform. It seems probable, therefore, that treatment of the cells of *Bacillus "M"* with lipase or with a fat solvent removes some lipoid material from the surface of the cell and allows subsequent access of trypsin to the protoplast. A further study of the problem would necessitate an examination with a purified preparation of lipase.

Summary. Our previous results, demonstrating with crude trypsin the digestibility of *B. megaterium* and the non-digestibility of *B. cereus,* could be confirmed with crystalline trypsin only by pretreating the bacteria with fat solvents or by adding lipase to it. The surface of *B. cereus* is different from that of *B. megaterium*; a lipid material can be removed from the surface of the later one which renders the bacterial protoplasts accessible to the action of proteolytic enzymes.

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