protein present in the cobra venom incubations stabilises a Zn-DPN complex in the manner described by CALVIN[8]. However, it is of interest that the DPN molecule can theoretically accommodate a tetrahedrally-co-ordinated Zn atom using two phosphate oxygens, adenine amino-N and nicotinamide amide-N.

Evidence is now available for the functional role of Zn in DPN-alcohol dehydrogenase systems[17,18,19]. Other instances have been reported of the effect of Zn and other metals on DPN and dehydrogenases[20,21] and upon general phosphate metabolism[22].

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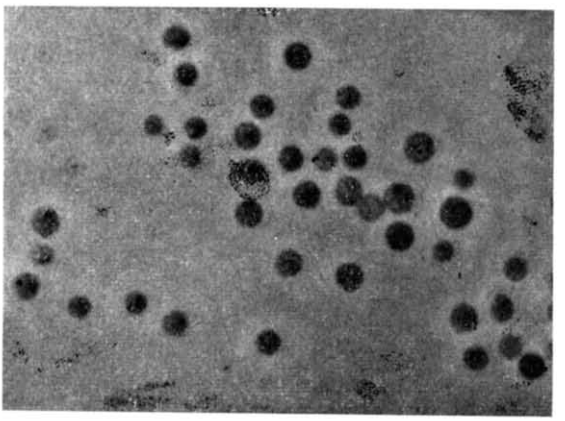
Received September 5th, 1955

Bacterial protoplasts: growth and division of protoplasts of *Bacillus megaterium*

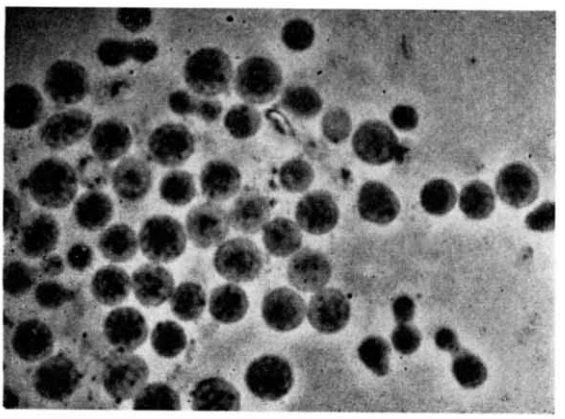
The sub-cellular organelles produced by digesting away the cell walls and cross septa of *Bacillus megaterium* are capable of synthesising protein, nucleic acids and adaptive enzymes (MCQUILLEN[1,2]), of supporting the growth of bacteriophages (SALTON AND MCQUILLEN[3]; BRENNER AND STENT[4]) and of allowing the development of endospores (SALTON[5]). The digestion with lysozyme, if carried out in suitable media of high osmotic pressure, converts the rod-shaped bacilli into from one to four spherical protoplasts (WEIBULL[6,7]; MCQUILLEN[8]). These have now been shown to be able to increase in size and dry weight, and to divide.

Strain KM of *B. megaterium* was grown in a glucose/NH_3/salts medium; the cells were harvested, washed and converted to protoplasts as described earlier (MCQUILLEN[1]) except that in some experiments phosphate buffer (0.5 *M*, pH 7.0) was used as stabiliser in place of sucrose (7.5% w/v). Samples of intact cells and protoplasts were centrifuged, rinsed twice with the stabilising medium and resuspended in the same medium to which had been added glucose (0.5% w/v) and either Bacto peptone (0.1% w/v) or a mixture of 18 amino acids each at a final concentration of 50 μg/ml (MCQUILLEN AND SALTON[9]). 250 ml lots of these suspensions (*ca.* 100 μg dry weight per ml) were incubated at 28° C in rocked Roux bottles fitted with an air vent through a rubber bung. At intervals, 50 ml samples were removed, treated with formaldehyde, centrifuged, washed twice with distilled water and dried to constant weight. Table I records some typical results.

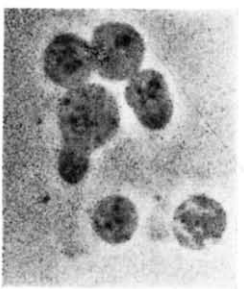
Both the protoplasts and the intact cells more than doubled in dry weight during the first two hours of incubation but whereas the latter continued to grow exponentially, the protoplasts increased more or less linearly. Whether this is significant of a difference in behaviour or whether it is due to lysis of some of the very fragile protoplasts, is not known but similar results have been obtained on many occasions. During the course of such an experiment which was prolonged,



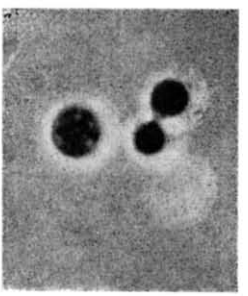
(a)



(b)



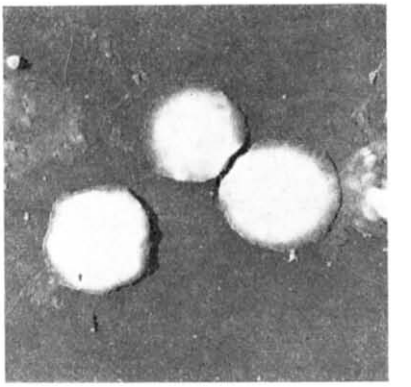
(c)



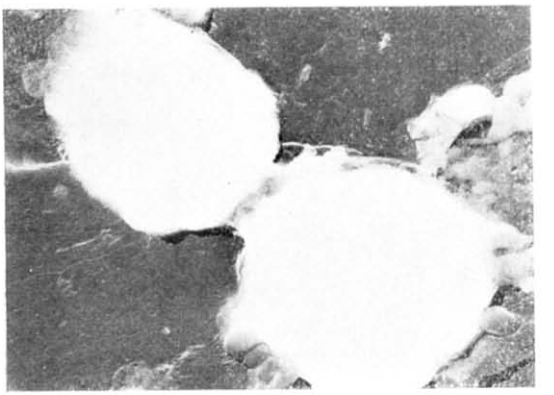
(d)

Fig. 1. Phase contrast photomicrographs of protoplasts of *Bacillus megaterium* (magnification 1100 ×). (a) Protoplasts at beginning of experiment. (b), (c) and (d) Protoplasts after 8–9 hours' incubation.

(a)

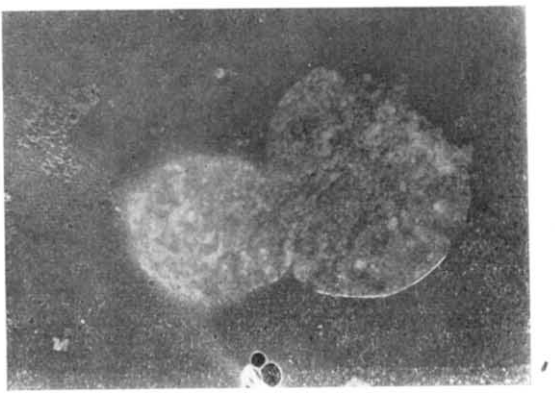


(b)



(c)

(d)



(e)

Fig. 2. Electron micrographs of *Bacillus megaterium* and protoplasts derived therefrom (fixed with formaldehyde; magnification 8000 ×). (a) Whole cells at beginning of experiment. (b) Protoplasts at beginning of experiment. (c) and (d) Protoplasts dividing after 9 hours' incubation. (e) "Ghost" of dividing protoplast (9 hours' incubation).

TABLE I

INCREASE IN DRY WEIGHT OF PROTOPLASTS AND INTACT CELLS

Incubation time hours	*Suspension density*	
	Intact cells µg/ml	*Protoplasts µg/ml*
0	104	82
2	234	172
4	524	248

Suspensions of intact cells or protoplasts were incubated with gentle aeration at 28° C in a medium containing glucose and peptone with sucrose as stabiliser.

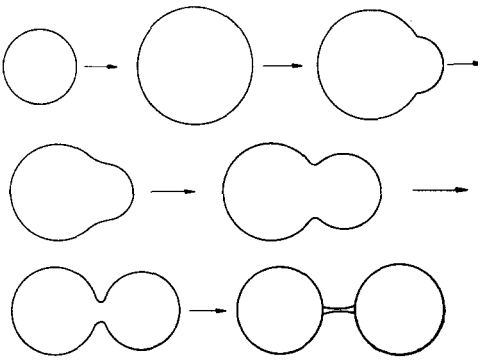


Fig. 3. Probable sequence of forms during the growth and division of protoplasts of *Bacillus megaterium*.

it was noticed that after about 6 hours' incubation there appeared non-spherical forms among the greatly enlarged protoplasts. As time went on these developed into dumb-bell shaped bodies such as appear in Figs. 1 and 2. The probable sequence of forms is represented diagrammatically in Fig. 3. Identical findings have been made on several occasions and there is every reason to believe that these sub-cellular preparations can grow and divide.

A fuller account of this work will appear later.

The author is grateful to Mr. R. W. HORNE for making the electron micrographs.

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Received August 9th, 1955

The binding of sulfate and phosphate ions by salmine

In an earlier report[1] on the molecular weight of the protamine, salmine, it was noted that the sedimentation constant of the phosphate salt is appreciably higher than that of the chloride salt. As reported, this indication of phosphate ion binding was confirmed by conductivity and electrophoresis measurements, and was found to exist in the case of the sulfate salt also. Recently GOLDWASSER AND MATHEWS[2] reported the binding of sulfate ion by mucoproteins and bovine serum albumin. In view of the paucity of sulfate binding data noted by these authors, and the general interest in all types of protein-ion binding, we felt it desirable to report in more detail our preliminary studies of salmine. While the binding of multivalent anions by proteins may be a phenomenon of some importance, the binding by salmine of phosphate, in particular, may have special significance in the problem of the linkage between protamine and nucleic acid in the nucleoprotamine. The physical constants submitted here are not to be accepted as final values, since we have evidence that commercially prepared salmine, at least, is not homogeneous. The problem of the heterogeneity of salmine is currently being investigated in this laboratory.

A commercial sample of salmine sulfate, obtained from Krishell Laboratories, Inc., was dissolved in water and put through an anion exchange column to convert it to the free base. Samples of the base were then titrated to pH 8.5 with 1 *N* HCl, H_2SO_4 and H_3PO_4 to convert them to the corresponding salts. Conductivities of these solutions and the free base were measured