Bacterial "protoplasts": Effects of diaminopimelic acid deprival and penicillin addition compared in *Escherichia coli*

LEDERBERG[1], LIEBERMEISTER AND KELLENBERGER[2] and HAHN AND CIAK[3] have used penicillin, ZINDER AND ARNDT[4] and REPASKE[5] have used lysozyme, to prepare spherical, osmotically shockable forms of Gram-negative bacteria such as *Escherichia coli* and *Proteus vulgaris*. These preparations resemble in many respects the protoplasts formed by digestion of the cell wall of *Bacillus megaterium* and certain other Gram-positive species with lysozyme (WEIBULL[6]; MCQUILLEN[7]). However, whereas in the case of the Gram-positives it is known that the entire mucopolysaccharide wall is absent from the protoplast (and this seems a necessary criterion for strict use of the term), this is not the case with Gram-negatives where the cell wall is a complex of lipoprotein and mucopolysaccharide. It may be that lysozyme and penicillin treatments remove only a mucopolysaccharide stiffening component which gives the wall its rigidity and form.

Diaminopimelic acid (DAP) is a component of cell walls in both Gram-positive and Gram-negative species and is probably present as a structural component only in the mucopolysaccharide of the cell wall (MCQUILLEN[8]; WORK[9]). MEADOW AND WORK[10] reported that the DAP-requiring mutant 173-25 of *E. coli* lysed shortly after reaching maximum density in media containing ample lysine but limited DAP. It occurred to us that protoplast-like forms of *E. coli* lacking all or part of their cell wall structure might be produced if it were possible to grow this mutant in the absence of DAP but in the presence of sucrose which is known to stabilise some osmotically sensitive forms. This proved to be the case. *E. coli* 173-25 was grown overnight at 37° in aerated glucose/NH_3/salts medium (C/G of MCQUILLEN AND SALTON[11]) supplemented with lysine (100 μM) and DAP (100 μM). Samples were transferred to media containing lysine but lacking DAP. In the presence of sucrose (10–20% w/v), continued incubation led to the production of spherical forms similar to those obtained by penicillin treatment. In the absence of sucrose, lysis soon occurred

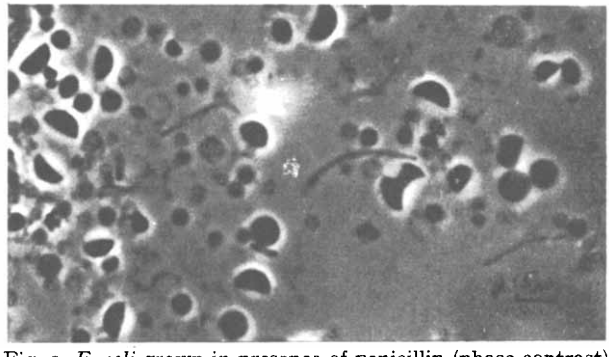


Fig. 1. *E. coli* grown in presence of penicillin (phase contrast).

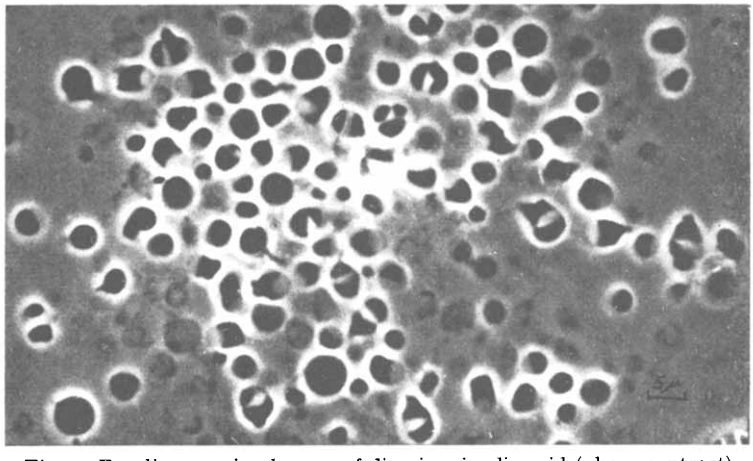
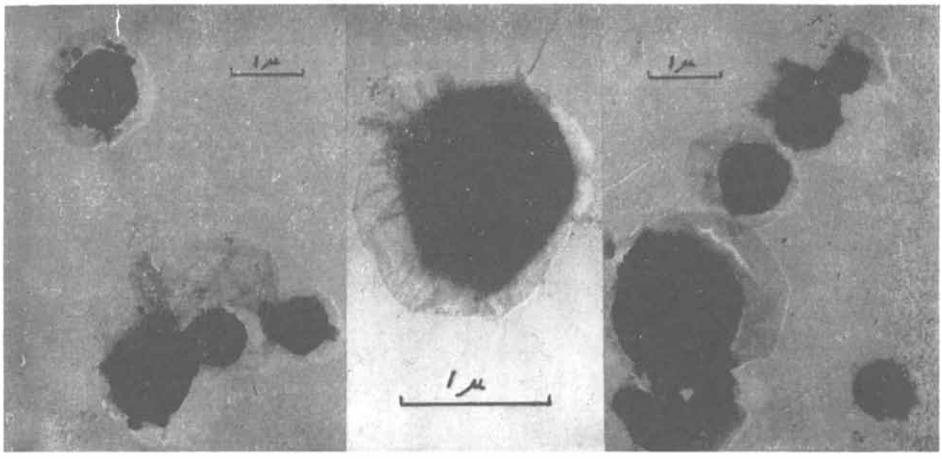


Fig. 2. *E. coli* grown in absence of diaminopimelic acid (phase contrast).



Figs. 3, 4 and 5. Electron micrographs of shadowed, fixed preparations of *E. coli* grown in absence of diaminopimelic acid (Figs. 3 and 5) or in presence of penicillin (Fig. 4).

(lysis has been studied by MEADOW, HOARE AND WORK[12] and RHULAND[13]; see also MCQUILLEN[8]). Good preparations were obtained by using a medium containing peptone (2% w/v), lysine (100 μM) and sucrose (15% w/v).

The original rod-shaped bacteria were about 0.75 $\mu \times 2\ \mu$. Some of the spheres were as much as 4 μ or more in diameter—an increase of 40-times in volume and 10-times in surface area. Often they appeared vacuolated when observed under phase contrast as is the case with penicillin forms. Figs. 1–5 show the appearance of *E. coli* grown in a complex medium containing 15% sucrose but no DAP and in the same medium supplemented with adequate DAP but also containing penicillin (100 μg/ml).

That there is still a well-defined envelope round these preparations is clear. What is not yet known is whether this is a protoplast membrane such as underlies the cell wall in Gram-positives and retains the cytoplasm in protoplasts of those species, or whether it is the cell wall lacking only a mucopolysaccharide component which it is believed to be removed by lysozyme, by certain phage enzymes (WEIDEL AND PRIMOSIGH[14]), prevented from entering by penicillin (PARK AND STROMINGER[15]; MCQUILLEN[8]), and which cannot be synthesised in the absence of its constituent DAP.

Since this work was done, Dr. B. D. DAVIS (personal communication) reports similar findings (see also the note by BAUMAN AND DAVIS[16]).

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