3.5 percent in the three layers, respectively. Apparently the maximum penetration into the mud cakes was only slightly more than 1 mm even in a dry atmosphere. No DDT was found in layers below a depth of 1.5 mm.

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Selection of Auxotrophic Bacterial Mutants through Diaminopimelic Acid or Thymine Deprival

The significance of unbalanced growth as a cause of cell death has been brought out by Cohen and Barner (*1*), who worked with a thymine-requiring mutant of *Escherichia coli*. When this organism was incubated in a culture medium that lacked thymine, it could not form deoxyribonucleic acid but continued to synthesize its other cell constituents, and, as a result, the viable count fell rapidly. Recently, Lederberg (*2*) found that unbalanced growth similarly underlies the bactericidal action of penicillin. When a suitable protective agent (such as hypertonic sucrose) was added to the growth medium, penicillin no longer caused lysis but, instead, led to protoplast formation. It could be concluded from this observation, as from the independent biochemical findings of Park and Strominger (*3*), that penicillin interferes selectively with bacterial cell-wall formation.

These developments suggested the possibility of replacing penicillin in the selection of auxotrophic mutants of bacteria. In the penicillin method (*4*), a mixed population is exposed to this drug in minimal medium, whereupon the wild-type cells grow and are lysed, while auxotrophic cells, unable to grow, are spared. We now wish to report two modifications of this procedure which utilize a genetic block rather than penicillin to make growth fatally unbalanced.

The first method closely resembles the use of penicillin in that it also involves the cell wall. The method is based on the availability of a mutant (173-25 of the W strain of *E. coli*) that is blocked in the synthesis of *meso*-α,ε-diaminopimelic acid (DAP) (*5*). In certain bacterial species this compound is a cell constituent (*6*) as well as a precursor of lysine (*5, 7*), and analyses of bacterial fractions have suggested that its incorporation as a constituent may possibly be limited to the cell wall (*8*). This suggestion is supported by the results of incubating mutant 173-25 in media that contained lysine but no diaminopimelic acid: the cells lyse in a medium of ordinary tonicity (*9*) but form protoplasts when 20 percent sucrose is also present (*10*). Deprival of diaminopimelic acid thus results in selective interference with cell-wall formation.

To use this "suicidal" property of mutant 173-25 in the selection of mutants with additional requirements, a procedure similar to the penicillin method (*4*) was followed. The bacteria were irradiated with ultraviolet light to about 1 percent survival. For phenotypic expression of the resulting induced mutations, large inocula were cultivated overnight in minimal medium A (*11*) enriched with 10 μg of diaminopimelic acid (*12*) per milliliter and with 0.2 percent tryptic casein hydrolysate (Sheffield NZ–Case) and 0.2 percent yeast extract (Difco). For selection of auxotrophic mutants, the cells were then washed and incubated (10^5 to 10^7 cells per milliliter) for 14 hours at 37°C in medium A supplemented with 20 μg of L-lysine per milliliter (*13*). Survivors were recovered by plating in the enriched medium described, solidified with 1.5 percent agar. Mutant colonies were recognized by the inability of subinocula to grow on minimal medium supplemented with only the compounds required by the parental strain (*14*).

The second method involves the metabolic imbalance originally described by Cohen and Barner and utilizes the same thymine auxotroph (15T⁻). The procedure used was the same as that described in the preceding paragraph except for appropriate changes in the composition of the media. Thus, selection was carried out in minimal medium A, and all other steps were carried out in this medium supplemented with thymine (20 μg/ml), together with other supplements as needed.

In a small-scale experiment, strain 173-25 yielded mutants with various additional requirements: cystine, methionine, *p*-aminobenzoic acid, arginine plus uracil, and an unidentified factor. Strain 15T⁻ yielded offspring with additional requirements for arginine, methionine, phenylalanine, and a mixture of aromatic metabolites.

The alternative methods that have been described may have advantages, in certain circumstances, over the use of penicillin. In particular, it is known that "thymineless death" can be produced by a method that is capable of quite general application: by using a sulfonamide antagonist of *p*-aminobenzoic acid to prevent the synthesis of a group of products of one-carbon metabolism and simultaneously providing all these products except thymine (*1, 15*). The present work (*16*) suggests that production of thymine deficiency in this way might be useful for selecting mutants of organisms (such as yeasts) that are susceptible to sulfonamides but indifferent to penicillin.

The use of penicillin for the selection of auxotrophic mutants of bacteria can be replaced by taking advantage of the fact that a DAP-requiring or a thymine-requiring strain is suicidal when it grows in media that lack the required compound (*17*).

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12. For samples of *meso*-α,ε-diaminopimelic acid we are indebted to Charles Gilvarg, to Lemuel Wright of Merck, Sharp and Dohme, and to Chas. Pfizer and Co. This supplement is necessary since the complex enrichments used do not contain detectable amounts of DAP.
13. Presumably, as in the penicillin method [see J. Lederberg, *Methods Med. Research* 3, 5 (1950)], longer or shorter periods of incubation would also be satisfactory.
14. The supplement contained L-lysine (20 μg/ml) as well as DAP (10 μg/ml), since strain 173-25 has a relative requirement for lysine as well as an absolute requirement for DAP (*5*).
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16. This work was aided by research grant RG-4235 from the U.S. Public Health Service.
17. A similar method has proved useful in the isolation of mutants of the molds *Ophiostoma* sp. and *Aspergillus* sp., since the survival of certain auxotrophs of these species, when incubated in minimal medium, is significantly prolonged by the presence of various additional metabolic blocks. [See N. Fries, *Hereditas* 34, 338 (1948); G. Pontecorvo, *Advances in Genet.* 5, 141 (1953)].

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