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CONCENTRATION OF BIOCHEMICAL MUTANTS OF BACTERIA WITH PENICILLIN[1]

Sir:

Existing methods for isolating biochemical mutants are still tedious, although mitigatory procedures have been described.[2] We have found that penicillin can be used to augment the proportion of mutants in a culture, greatly facilitating their isolation.

The method depends on the finding that penicillin lyses only growing cells with little permanent effect on resting suspensions.[3] This suggested that, if allowed to act on a mixture of mutant and non-mutant cells in a synthetic medium, penicillin might concentrate the mutants which are unable to grow in this medium.

These expectations were first tested in reconstruction experiments. Y-53 is a mutant of *Escherichia coli* requiring threonine, leucine and thiamin, and is lactose-negative; K-12 is its lactose positive wild type ancestor. Suspensions were assayed for mutants by planting on EMB-lactose agar[4] and counting the dark and light colonies as K-12 and Y-53, respectively. After preliminary study of various conditions, the following were adopted: Washed suspensions of young cells harvested from a complete medium were mixed in the ratio K-12:Y-53 of 100 – 1000:1. These mixtures were inoculated into a synthetic medium[4] containing 300 O.U./ml. penicillin G to give an initial cell concentration of 10^8–10^9/ml. The suspensions were incubated with shaking for four hours, when there were about 10^4–10^5 viable cells. In a number of experiments, assays of these survivors have given ratios of K-12:Y-53 ranging from 2:1 to 1:100, and representing amplifications of mutants of several hundred to several thousand-fold. No change from the initial ratio was observed when the growth factors required by Y-53 were added to the treatment medium.

New mutants have also been obtained from several *Salmonella* strains, by treatment of cells grown from irradiated inocula. Approximately half the colonies surviving the treatment failed to grow when inoculated in synthetic medium. However, only about two-thirds of these proved to be stable mutants when subcultures on nutrient agar slants were retested. No satisfactory explanation of this behavior has been found.

The mutants so far characterized require a variety of growth factors, including histidine, methionine, tryptophan, leucine, threonine, proline, phenylalanine and tyrosine.

The method would lend itself to the isolation of specific mutants by the addition of irrelevant growth factors to the treatment medium. The method should be applicable to other bacteria, but the optimal conditions will have to be worked out for each organism.

Parallel experiments with streptomycin and streptothricin[5] gave no alteration in the mutant ratio. Very few antibiotics are reputed to have the differential activity on growing cells needed for this method.

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(2) J. Lederberg and E. L. Tatum, *J. Biol. Chem.*, **165**, 381 (1946).

(3) G. L. Hobby, *Proc. Soc. Exptl. Biol. Med.* **56**, 181 (1944).

(4) J. Lederberg *Genetics*, **32**, 505 (1947).

(5) Kindly provided by Merck and Co.

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