This Week's Citation Classic®

Lederberg J & Zinder N. Concentration of biochemical mutants of bacteria with penicillin. *J. Amer. Chem. Soc.* 70:4267-8, 1948.
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This article and the accompanying one by Bernard Davis reported the simultaneous, independent discovery of an efficient method of isolating auxotrophic mutants of bacteria. Davis and I agreed to publish these commentaries back-to-back in this issue of *Current Contents®*. [The *SCI®* indicates that the Lederberg-Zinder paper has been cited in over 175 publications since 1955; the Davis paper has been cited in over 220 publications since 1955.]

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In 1941 G.W. Beadle and E.L. Tatum[1] introduced the experimental production of nutritional mutants in fungi as a way of exploring gene expression and biochemical pathways. This was followed by similar experiments with bacteria;[2,3] these systems represented the beginning of modern biochemical genetics.

Until 1948 such mutants could be obtained only with tedious manual effort, entailing the isolation and testing of many thousands of individual spores or colonies. It was easy to enrich for the occurrence of rare prototrophic, or nutritionally wild-type, variants by inoculating large populations into restricted nutrient media: if one could only do the converse! But how would you select for organisms that grow poorly as compared to the wild type?

Exhausted by the tedious task of mutant isolation, I recalled a lecture at Columbia University Medical School by Gladys Hobby, in which she remarked that penicillin killed only rapidly growing cells, leaving stationary cells alive.[4] She had also reported that penicillin was active on *Escherichia coli*, albeit at 100-fold higher concentrations than needed to inhibit staphylococci.[5] Her observations suggested that penicillin, if applied to growing bacteria in a restricted medium, might leave the auxotrophic mutants still living. The penicillin could then be diluted away, or inactivated, permitting the recovery of the mutants as viable clones. Norton Zinder, who was then working in my laboratory on his dissertation on *Salmonella* transduction,[6] was delighted to discover that this scheme worked very well indeed.

At one point, S.E. Luria (then at the University of Illinois) visited our laboratory. We discovered from him that B.D. Davis had independently developed the identical method. The three of us (Davis, Zinder, and I) promptly agreed to publish jointly. Our first journal preference was the *Journal of Biological Chemistry*, but we were rebuffed with the comment that the reports had too little "chemistry" in them. We were pleased that the *Journal of the American Chemical Society* took a broader view of chemistry and promptly accepted the papers.

The penicillin method has lightened the labors of thousands of investigators who use auxotrophic mutants in a wide range of organisms.[7] There are many variants that take advantage of other reagents that preferentially attack growing cells.

Researchers may find themselves in a quandary over whether to cite Lederberg and Zinder, or Davis, or both. The majority of authors probably cite neither paper, especially in recent years. The need to use selective methods has been mitigated by the development of powerful chemical mutagens (such as nitroso-methyl-guanidine) and of other tricks like replica-plating.[8] Nevertheless, a great many mutant strains, including some of substantial industrial importance, were born in a penicillin bath.

Further inquiry into the mechanism of differential killing of growing cells by penicillin has contributed to understanding the biochemical mechanism of action of this antibiotic. Unbalanced growth of the cytoplasm while the wall is inhibited is overlain by the triggering of cell wall-autolytic enzymes.[9] Penicillin-treated cells can be protected from lysis by immersing them in hypertonic media, thus permitting the emergence of spheroplasts and L-forms.[10]

1. **Beadle G W & Tatum E L.** Genetic control of biochemical reactions in *Neurospora*. *Proc. Nat. Acad. Sci. USA* 27:499-506, 1941. (Cited 205 times since 1955.)
2. **Roepke R R, Libby R L & Small M H.** Mutation or variation of *Escherichia coli* with respect to growth requirements. *J. Bacteriology* 48:401-12, 1944.
3. **Gray C H & Tatum E L.** X-ray induced growth factor requirements in bacteria. *Proc. Nat. Acad. Sci. USA* 30:404-10, 1944. (Cited 70 times since 1955.)
4. **Hobby G L & Dawson M H.** Effect of rate of growth of bacteria on action of penicillin. *Proc. Soc. Exp. Biol. Med.* 56:181-4, 1944.
5. **Hobby G L.** The antibacterial action of penicillin against gram-negative organisms. *Science* 100:500-1, 1944.
6. **Zinder N D & Lederberg J.** Genetic exchange in Salmonella. *J. Bacteriology* 64:679-99, 1952. (Cited 335 times since 1955.) [See also: **Zinder N D.** Citation Classic. (Barrett J T, ed.) *Contemporary classics in the life sciences. Volume 1: cell biology*. Philadelphia: ISI Press, 1986. p. 30.]
7. **Lederberg J.** Isolation and characterization of biochemical mutants of bacteria. *Meth. Med. Res.* 3:5-22, 1950. (Cited 410 times since 1955.)
8. **Lederberg J & Lederberg E M.** Replica plating and indirect selection of bacterial mutants. *J. Bacteriology* 63:399-406, 1952. (Cited 960 times since 1955.)
9. **Tomasz A.** Mode of action of beta-lactam antibiotics—a microbiologist's view. (Demain A L & Solomon N A, eds.) *Handbook of experimental pharmacology*. New York: Springer-Verlag, 1983. Vol. 67/I. p. 15-96.
10. **Lederberg J & St Clair J.** Protoplasts and L-type growth of *Escherichia coli*. *J. Bacteriology* 75:143-60, 1958. (Cited 215 times.)

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