50

MECHANISM OF ACTION OF PENICILLIN

JOSHUA LEDERBERG
Department of Genetics, University of Wisconsin, Madison, Wisconsin

Reprinted from JOURNAL OF BACTERIOLOGY
Vol. 73, No. 1, p. 144, January, 1957
Printed in U.S.A.

Reprinted from JOURNAL OF BACTERIOLOGY
Vol. 73, No. 1, p. 144, January, 1957
Printed in U.S.A.

NOTE

MECHANISM OF ACTION OF PENICILLIN[1]

JOSHUA LEDERBERG

Department of Genetics, University of Wisconsin, Madison, Wisconsin

Received for publication October 1, 1956

"The mechanism whereby penicillin exerts its cytotoxic effect remains obscure" (Eagle and Saz, Ann. Rev. Microbiol., **9,** 173, 1955) notwithstanding the universal use of this antibiotic in chemotherapy. However, there has been a concordance by many workers on the development of protoplasts or L-forms of bacteria (for review see Liebermeister and Kellenberger, Z. Naturforsch., **11,** 200, 1956). These observations support the argument that penicillin inhibits cell-wall synthesis, and thereby provokes osmotic fragility in the excoriated bacteria (Cooper, Bacteriol. Revs., **20,** 28, 1956).

The argument may be illustrated with observations on *Escherichia coli* strain K-12 (Lederberg, Proc. Natl. Acad. Sci. U. S., **42,** SM4, 1956 —where the point was not amplified). Cells actively growing in customary broth media will lyse after one to two hr exposure to penicillin. In a protective hypertonic medium, i. e., one supplemented with M/3 sucrose plus M/100 $MgSO_4$, the treated cells do not lyse but instead they balloon into spherical "protoplasts." Direct microscopic observations showed a one-for-one conversion of rods into protoplasts. The protoplast suspension is osmotically fragile and lyses when diluted into water or ordinary broth. In the protective medium, however, the protoplasts remain almost fully viable, and will revert to typical (colony-forming) rods when diluted in protective media lacking penicillin. Therefore, the bactericidal effect of penicillin in ordinary media is sufficiently explained by the induced osmotic fragility. As non-growing cells are not killed by penicillin, new wall-formation, rather than the existing wall, is the probable target.

Lower concentrations of penicillin provoke the formation of long filamentous forms and may have a bacteriostatic effect by virtue of the inhibition of cell division. In the production of protoplasts, it was observed that dividing cells usually swelled first from the point of incipient separation. This suggests that the division-septum is especially sensitive to penicillin. Filamentous forms would arise when septum-formation was blocked without impairment of synthesis of the outer wall.

It remains to define the target in biochemical terms. The simplest speculation is that penicillin inhibits a specific wall-building polymerase. The chemotherapeutic specificity would then follow from the unique makeup of bacterial cell walls. Park (J. Biol. Chem., **194,** 877, 1952) reported the accumulation of uridine-pyrophosphate derivatives of amino-sugars and various amino acids in penicillin-treated staphylococci. These derivatives may represent the activated forms of the residues for their polymeric condensation, which accumulate owing to the block in this reaction.[2] However, more remote influences on cell-wall formation cannot be precluded. At any rate, further studies of antibiotic effects must be conducted with protected protoplasts, rather than with lysed or lysing cells in which the ramification of secondary lesions is an inevitable complication.

The viability of penicillin-treated cells in protective media is further indicated by their proliferation in penicillin-containing agar (but not in broth) to form L-colonies. That is, the "protoplast" is equivalent to the initial stage, the large body, of the L-cycle of Klieneberger-Nobel, Dienes, and others. Certain bacteria, such as Proteus, form protoplasts which are unusually resistant to osmotic shock, and have therefore been more amenable to previous experimentation on L-forms.

[1] Paper No. *641* of the Department of Genetics. This work has been supported by a research grant (C-2157) from the National Cancer Institute, Public Health Service.

[2] For a more complete statement and substantiation of the same proposal, see Park and Strominger, Science, (*accepted for publication*), 1956.