EFFECT OF PENICILLIN ON THE UPTAKE OF AMINO ACIDS IN BACTERIA

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In the course of investigations on the mechanism of feed-back inhibition of enzyme formation (repression) in the biosynthesis of arginine (Gorini and Maas, 1957) a study of enzyme formation was begun using osmotically sensitive spheres (OSS) (Sistrom, 1958) prepared with penicillin in the presence of 20 percent sucrose (Lederberg, 1956). It was noted that the addition of penicillin to an exponentially growing culture abolished the repression ordinarily exerted by arginine on the synthesis of ornithine transcarbamylase, which converts ornithine to citrulline. About 60 minutes after the addition of penicillin to cells growing in a yeast extract-casein hydrolysate medium the level of the enzyme began to rise and increased 20-fold within 30 minutes.

A possible explanation for the release of repression is a defect in the concentrating mechanism for arginine (Schwartz, Maas and Simon, 1959) so that the cell cannot maintain a large enough concentration gradient. A similar effect of penicillin on the uptake of glutamic acid had been noted previously by Gale and Taylor (Gale and Taylor, 1947). The uptake of radioactive arginine was therefore measured in cultures growing in 20 percent sucrose in the presence of penicillin. Chloramphenicol was added before arginine to prevent incorporation of the amino acid into protein.

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As shown in Table I, penicillin markedly inhibits the ability of the cell to concentrate arginine. The inhibition is maximal when penicillin is added concomitantly with chloramphenicol and decreases with increasing time intervals between the addition of chloramphenicol and penicillin. When penicillin is added 30 minutes after chloramphenicol the action of penicillin is slow, the effect being slight at 60 minutes after addition of arginine, but pronounced after 240 minutes (Table I). Under these conditions (20 percent sucrose and chloramphenicol) the cells are protected from the lytic action of penicillin.

The effect of penicillin on the concentrating mechanisms for a number of other amino acids (lysine, glycine and D-serine, see Schwartz, Maas and Simon, 1959) was found to result in a similar decrease in uptake. Furthermore, penicillin inhibited the uptake of arginine in the gram-positive organism Bacillus megaterium strain KM (Table I).

Experiments with OSS have indicated that an intact cell wall is necessary for penicillin to inhibit uptake of amino acids. These forms were prepared either with lysozyme(Sistrom, 1958) or penicillin (Lederberg, 1956) or by omitting diaminopimelic acid (DAP) from the medium of a DAP-requiring auxotroph (Meadow, Hoare and Work, 1957). Even in the absence of penicillin, in each type of OSS arginine is concentrated only to 1/4 to 1/3 the level reached in whole cells. This is one of a few instances in which OSS behave differently from intact cells. The lowered level of arginine uptake is not depressed further in the presence of penicillin (tested up to 10,000 units per ml). Thus, altering the integrity of the cell wall has resulted in an impairment of the concentrating mechanism for amino acids as well as a loss of penicillin's capacity to interfere with this concentrating mechanism.

Apparently protein synthesis is also required for the inhibition of uptake by penicillin. In various amino acid auxotrophs

TABLE I

Organism	Interval between addition of chloramphenicol and penicillin, minutes	Uptake, μg/g wet weight bacteria + penicillin	no penicillin
E. coli, strain W	0	403	2100
"	10	935	3260
"	30	1400 (885)*	1800 (1790)*
B. megaterium, strain KM	0	488	985

Chloramphenicol (final concentration 200 μg/ml) was added to cells growing exponentially at 37° in an arginine-free, enriched medium (AF) (Gorini and Maas, 1958) with 0.5 percent lactate as carbon source. Penicillin (final concentration 1000 units/ml) was added at times indicated. Five minutes later, C^{14}- arginine, randomly labelled, was added (final concentration 20μg/ml) and incubation continued for 60 minutes. Aliquots of the cultures were then collected on membrane filters and their radioactivities determined (Atkinson and McFadden, 1956). The intracellular concentrations of arginine were calculated from the specific activity of the preparation.

* Uptake of C^{14} arginine measured 240 minutes after its addition.

(lysine, isoleucine, arginine) penicillin does not prevent the concentration of radioactive arginine after exhaustion of the required growth factor. However, the addition after exhaustion of a small amount of the required amino acid in the presence of penicillin will restore the inhibition of arginine uptake. Under these conditions a small increase in mass occurs, but there is no lysis, even in the absence of sucrose or chloramphenicol. Thus the effect of penicillin described here is one of the early manifestations of the antibiotic. It may be the trigger action which sets off further changes, such as inhibition of cell wall synthesis (Park and Strominger, 1957)the observed unbalance in the formation of repressible enzymes, and finally cell lysis.

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