A "PACE-SETTING" PHENOMENON IN DEREPRESSED ENZYME FORMATION

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Recent findings on the repression of acetylornithinase and acetylornithine permease (Vogel, 1957, 1960) have encouraged a study of the kinetics of enzyme formation as a function of conditions of prior repression. Such a study has now been made of the acetylornithinase (Vogel, 1953a, b) of Escherichia coli. The strain used was 39A-23R3, which has an early block in the arginine path and gives a growth response to N^{α}-acetyl-L-ornithine or to L-arginine (or to arginine precursors following acetylornithine in biosynthetic sequence).

Mixed supplements of acetylornithine and arginine, in suitable proportions, give diphasic growth: arginine is used preferentially, at wild-type growth rate, in the first phase, and acetylornithine, due to restrictive uptake, is used at a slower exponential growth rate in the second phase (Vogel, 1953b, 1957, 1960). In the first phase, acetylornithinase is produced under repressive conditions; in the second phase, this enzyme is produced under derepressive conditions, i.e., conditions of repression release (Vogel, 1957). The change from repression to derepression occurs relatively abruptly and nearly

simultaneously with the change from first-phase to second-phase growth. The use of this mutant strain thus permits a swift transition from repression to derepression, without interruption of cultivation, and the point of transition is easily fixed by adjustment of the initial arginine concentration in the mixed supplement used.

After preliminary cultivation in a glucose-salts medium, supplemented with a growth-limiting amount of arginine, strain 39A-23R3 was grown in a glucose-salts medium containing N^{α}-acetyl-L-ornithine, 50 μg/ml, and L-arginine hydrochloride, either 5 μg/ml (Culture A) or 15 μg/ml (Culture B). The two cultures were incubated anaerobically at 37°C and, after establishment of second-phase growth, were sampled at intervals. Organisms from the samples taken were harvested, suspended in 0.1 M phosphate buffer (pH 7) containing 1.0 mM glutathione, and disrupted in a Mullard Ultrasonic Disintegrator. The resulting extracts were assayed for acetylornithinase (Vogel and

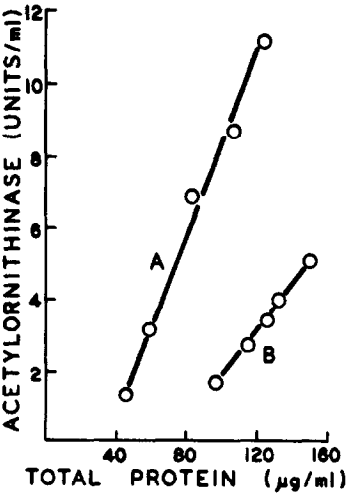

Figure 1. Formation of acetylornithinase following early (A) or late (B) onset of derepression (see the text). The differential rates of synthesis of the enzyme for A and B are 0.129 and 0.063 units per μg protein, respectively.

Bonner, 1956); protein (corrected for glutathione) was determined by the method of Lowry et al. (1951).

The results obtained are shown as plots of acetylornithinase versus total protein, both per ml of the respective cultures (Fig. 1). In each case, the enzyme and the total protein are seen to be formed in a constant ratio; for Culture B, this ratio is smaller than it is for Culture A. Since Culture B had a higher initial arginine concentration than did Culture A, Culture B exhibited the later onset of derepression. Thus, the lower "differential rate" of acetylornithinase synthesis is associated with the longer cultivation in the presence of added arginine and the later termination of repression. The constant differential rates of derepression can be shown to start, within the accuracy of the methods used, from the respective points of onset of derepression and can be viewed as reflecting a "pace-setting" phenomenon.

Several interpretations of this phenomenon are presently possible. An adequate working hypothesis would seem to be that, in addition to repression of acetylornithinase formation by arginine, there is a repression-like antagonism (at the primary or secondary template level) to the formation of a component, or components, of the corresponding enzyme-forming system. It is assumed that the differential rates of acetylornithinase formation (Fig. 1) reflect the number of corresponding functional enzyme-forming sites available, per unit mass of the bacteria, at the point of onset of derepression. During growth on arginine, under the conditions used, this number would decrease and, on subsequent derepression, the pace-setting effect would become apparent.

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