INDUCTION BY AN ENZYME REPRESSOR IN "NONREPRESSIBLE" STRAINS

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Certain "nonrepressible" strains of Escherichia coli have been reported in which L-arginine does not have its usual repressive effect on the formation of enzymes of arginine synthesis. Such non-repressible strains include mutant isolates derived from the normally repressible W (Cocito and Vogel, 1958) and K-12 (Maas, 1960) strains, as well as wild-type organisms, like the B strain (Ennis and Gorini, 1959; Albrecht and Vogel, 1960).

In a survey of the repressibility of acetylornithinase by arginine, Cocito and Vogel (1958) encountered considerable variations among a number of enterobacteria examined. For a relatively small group of organisms, including strains of Escherichia, Aerobacter, and Serratia, it was found that arginine not only failed to repress acetylornithinase, but even appeared to stimulate the latter's formation. In the B strain of E. coli, Gorini (1960a,b) has noted an arginine-stimulated formation, suggestive of induction, of ornithine transcarbamylase. One obstacle to the interpretation of such stimulations in terms of enzyme induction was the possibility that the

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arginine supplied, rather than having a regulatory (inducing) function in the synthesis of enzymes of its pathway, is required at relatively high levels merely as a structural component in some phase of the formation of these enzymes. In the present investigation, evidence has been provided that arginine can indeed act as an inducer in non-repressible strains. This evidence came from a comparison of the formation of two enzymes, acetylornithinase and acetylornithine δ-transaminase (Vogel, 1953; Albrecht and Vogel, 1960).

For the study of the effect of arginine on the two enzymes, strain B of E. coli was grown in liquid glucose-salts medium, either supplemented with 0.1 mg/ml L-arginine hydrochloride or unsupplemented. The two cultures were incubated anaerobically at 37°C and were sampled at intervals. Organisms from the samples taken were treated and disrupted as described (Vogel, 1960), and extracted acetylornithinase (Vogel and Bonner, 1956), acetylornithine δ-transaminase (Albrecht and Vogel, 1960, 1961), and protein (Lowry et al., 1951) were determined.

The results obtained are shown as plots of the enzymes versus total protein, all per ml of the respective cultures (Fig. 1). In each case, the enzyme and the total protein are formed in an approximately constant ratio, i.e., the enzyme is produced at approximately constant "differential rate." It can be seen that added arginine stimulates the formation of acetylornithinase, but has little, if any, effect on the formation of the transaminase.

Similar experiments were performed with strain B4S-7 grown in unsupplemented medium. This strain was isolated as a mutant of

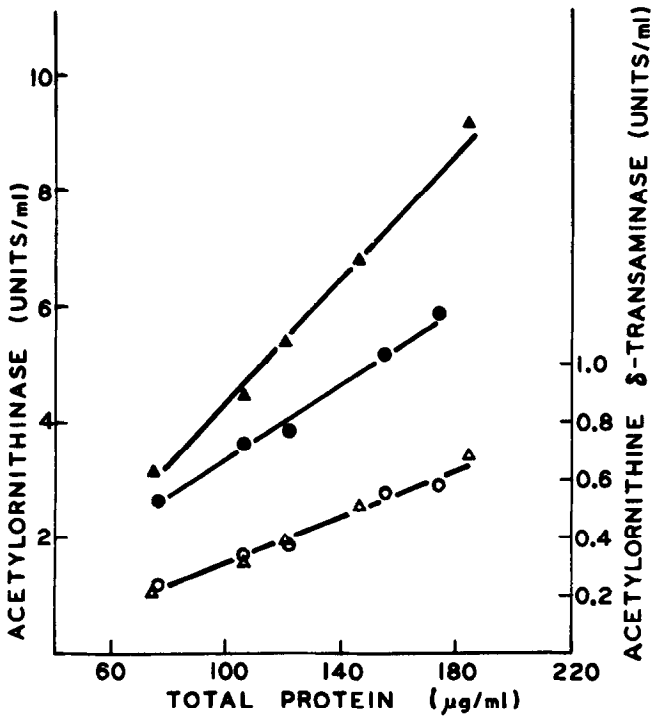

Figure 1. Effect of arginine on the formation of acetylornithinase (solid symbols) and acetylornithine δ-transaminase (open symbols) in strain B. Circles, cultivation without added arginine; triangles, cultivation with added arginine. The differential rates of synthesis of acetylornithinase are 0.032 and 0.053 units per μg protein for cultivation without and with arginine, respectively.

strain B by L. Gorini and W. Gundersen; the mutant exhibits relatively high specific activities of ornithine transcarbamylase and of the argininosuccinate-splitting enzyme (Ennis and Gorini, 1961). We found that strain B4S-7 synthesizes acetylornithinase and acetylornithine δ-transaminase at differential rates approximately four times as high as the corresponding rates for the parental B strain.

It appears likely that the increased differential rates of

formation of the enzymes in the arginine pathway of strain B4S-7 are due to a single characteristic of this mutant. Since the differential rate of acetylornithine δ-transaminase formation in strain B does not seem to be appreciably responsive to added arginine, it is inferred that this characteristic of the mutant is not an increased level of arginine synthesis. Accordingly, the endogenous supply of arginine achieved by strain B probably is not restrictive for the availability of this amino acid as a structural component in the formation of acetylornithinase. Thus, arginine (as such, or in a modified or "active" form) is indicated to function as an inducer of acetylornithinase in the B strain. Such induction, by its nature, would differ from induction through repression release (Vogel, 1957; Pardee et al., 1958; Gorini, 1960a), and could be "positive" induction (Vogel, 1961), although certain other possibilities have not been excluded.

The differential inducibility behavior indicated for acetylornithinase and acetylornithine δ-transaminase suggests individual susceptibility (or insusceptibility) of the corresponding synthesizing systems to arginine.

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