THE SIMULTANEOUS ACCUMULATION OF RNA AND OF A REPRESSOR OF β-GALACTOSIDASE SYNTHESIS

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The repression of β-galactosidase formation seems not to involve the synthesis of protein (Pardee and Prestidge, 1959; Yanagisawa, unpublished) nor the synthesis of DNA (Yanagisawa, unpublished). RNA is, as a consequence, implicated. Borek, et al (1955) have shown that in Escherichia coli K 12, strain 58-161, which requires methionine and is therefore met⁻, starvation for that amino acid allows the synthesis of RNA but not that of DNA or of protein. They also have observed that there is a long delay in β-galactosidase formation after methionine starvation. Stent and Brenner (1961) have proposed that this specific accumulation of RNA is due to the relaxation of control of its synthesis by amino acids, a phenomenon attributable to mutation in a single gene (RC) which segregates from that concerned with methionine synthesis.

E. coli 58-161 was starved for methionine for 60 minutes in the presence and the absence of glycerol and the inducer IPTG. As observed by Borek et al, there was a long delay in the for-

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mation of β-galactosidase after methionine starvation in the presence of glycerol (Figure 1, curve 4). In the absence of glycerol, β-galactosidase was formed even during methionine starvation if IPTG is present. This delay in enzyme formation was not observed after methionine starvation of E. coli 15 histidineless, methionineless and inducible for β-galactosidase, in which RNA is stringently controlled by amino acids (Fig. 1,curve 5). E. coli K 12 Ya 2, methionineless and inducible for β-galactosidase (kindly supplied by Dr. Stent) was obtained by crossing Hfr Cavalli (a derivative of 58-161) and F^- W945. Ya 2 synthesizes RNA during methionine starvation, but it is much less compared to 58-161. In this strain, β-galactosidase is synthesized even during methionine starvation in the presence of glycerol and an inducer (Fig.1, Curve 6). Upon restoration of methionine, however, the maximum rate of β-galactosidase synthesis was not resumed as fast as in E. coli 15, suggesting that there is some repression. In spite of the delay in β-galactosidase synthesis, in E. coli 58-161 total protein increased immediately upon restoration of methionine under the same condition, as was shown by C^{14}-leucine uptake (Fig. 2) Thus it seems that this lag is specific for a particular enzyme (or enzymes) and not a general characteristic of all proteins. It would also mean that the delay in the enzyme synthesis is not caused by damage to protein synthesizing apparatus. It is most likely, therefore, that the observed lag is a result of a repression of enzyme synthesis with which the RNA synthesized during methionine starvation has something to do.

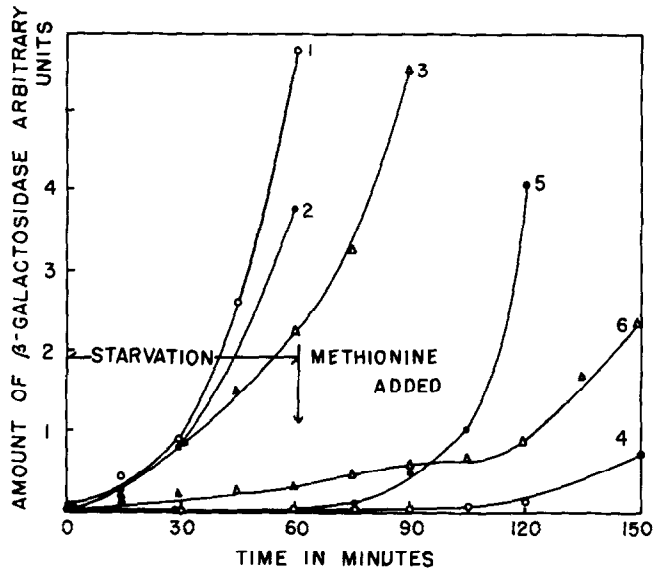

Fig. 1. Effect of methionine starvation on the formation of β-galactosidase.
Curves 1-3. Control: glycerol and methionine were present from O time.
1. E. coli (K-12) 58-161.
2. E. coli 15 his⁻met⁻
3. E. coli (K-12) Ya 2
Curves 4-6. Starved for methionine for 60 minutes in the presence of inducer IPTG.
4. E. coli (K-12) 58-161.
5. E. coli 15 his⁻ met⁻.
6. E. coli (K-12) Ya 2.

Several experiments have been performed with E. coli 58-161 to discover the nature of this repression. The cells were suspended in starvation medium with glycerol and the inducer IPTG. At different times methionine was restored and aliquots were withdrawn for β-galactosidase assay. A linear relationship was found between the starvation time and the lag in the production of β-galactosidase (Fig. 3). This result strongly suggests the accumulation of a rather stable substance during starvation in the presence of glycerol.

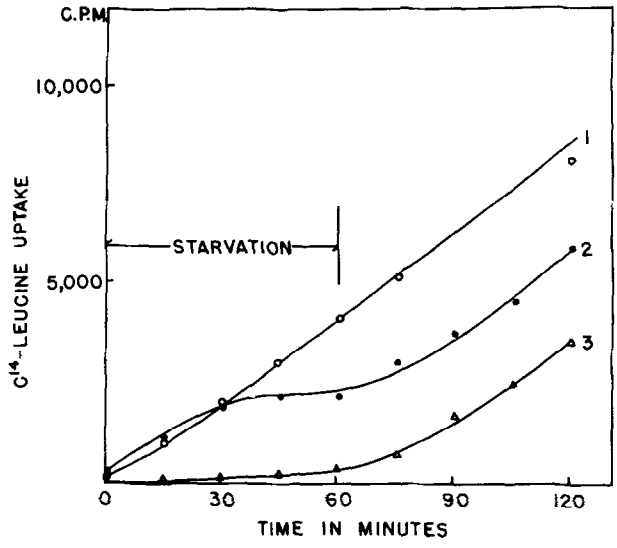

Fig. 2. C^{14} -leucine uptake by E. coli 58-161.
1. Control: glycerol and methionnne were present from O time.
2. Starved for methionine for 60 minutes.
3. Starved for methionine and glycerol for 60 minutes.

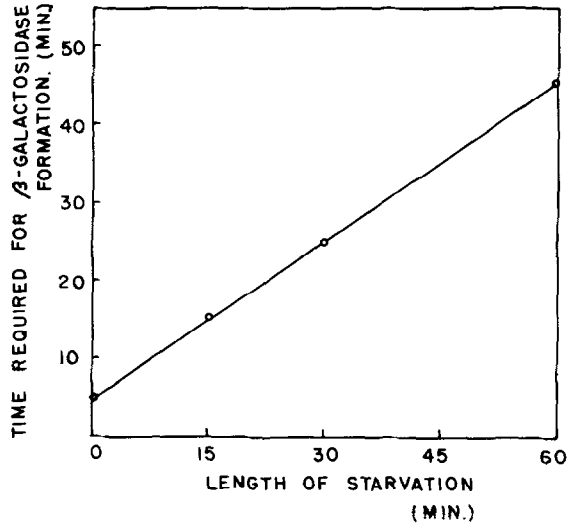

Fig. 3. The relationship between starvation time and the delay in β -galactosidase formation in E. coli 58-161.

A constitutive mutant of E. coli 58-161 was isolated by the method of Cohen-Bazire and Jolit (1953). The mutant did not show any delay in β-galactosidase formation after the restoration of methionine. Thus, the repression seems to be a characteristic of the inducible strain. On the other hand, the inducer IPTG did not reverse the delay in β-galactosidase synthesis at a concentration of 10^{-3}M.

The substance which accumulates concomitantly with RNA during methionine starvation could be a repressor itself or the precursor of a repressor. It should also be repressor-forming machinery which forms repressor at much higher rates upon restoration of methionine than under normal conditions. It takes 40-45 minutes for a measurable amount of β-galactosidase to be synthesized after 60 minutes starvation, suggesting that the repressor is a rather long-lived substance. This, together with the fact that the repression is not reversible by the inducer suggests that this repression is not of the same type as observed by Jacob & Monod (1961). Recently, Sypherd et al (1962) have shown that induced enzyme synthesis is very sensitive to chloramphenicol. The RNA accumulated during chloramphenicol treatment is similar to that formed by methionine-starved E. coli 58-161 (Dagley et al., 1962). The RNA formed under both of these conditions probably plays some role in the repression of induced enzyme synthesis.

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