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Recent advances in experimental technique (Leive, 1965a,b) have permitted the use of actinomycin for the study of RNA synthesis in *Escherichia coli*. Without special pretreatment this organism is not sensitive to actinomycin (Hurwitz, Furth, Malamy & Alexander, 1962). The lack of sensitivity is believed to be due to the failure of the drug to penetrate into the cells since it has been observed that cell-free preparations from *E. coli* are indeed sensitive (Nisman, Pelmont, Demailly & Yapo, 1963).

A number of authors have noted that in organisms normally sensitive to actinomycin without special pretreatment the syntheses of all proteins are not equally inhibited by the antibiotic (in various species of *Bacillus*: Acs, Reich & Valanju, 1963; Eikhom & Laland, 1965; Harris and Sabath, 1964; Hurwitz, *et al.*, 1962; Kennell, 1964; Levinthal, Keynan & Higa, 1962; Pollock, 1963; in lens: Scott & Bell, 1964; in cotton embryos: Dure & Waters, 1965). A similar observation has now been made with *E. coli*. It has been shown that following the EDTA treatment of Leive (1965a), the cells are particularly sensitive to low concentrations of actinomycin for about 50 min. During this period the differential rates vs. protein synthesis of beta-galactosidase and alkaline phosphatase formation, and of the pulse-labeling of RNA with [^{3}H]uracil, are very severely inhibited. In time, as Leive (1965b) found, the population loses its sensitivity to actinomycin, and we have obtained a close kinetic correlation between the restoration of [^{3}H]uracil incorporation and the onset of beta-galactosidase and alkaline phosphatase synthesis.

Two strains of the organism have been used: J.C.14 (inducible for beta-galactosidase) and C90F1 (inducible for beta-galactosidase and constitutive for alkaline phosphatase). We wish to thank Drs. A.J. Clark, and A. Garen, respectively, for these strains. The cells were grown aerobically at 37° in glycerol-minimal media, supplemented with specific nutrients as required. Beta-galactosidase activity was induced with isopropyl-thio-beta-D-galactopyranoside (IPTG). Portions of the suspensions for enzyme assay were sampled onto

chloramphenicol to stop further protein synthesis, and the cells subsequently treated with toluene. Enzyme activities were determined by the rates of hydrolysis of o-nitrophenyl-beta-D-galactoside for beta-galactosidase and o-nitrophenyl phosphate for alkaline phosphatase (Kepes, 1963).

Pulse-labeling experiments to measure the rates of RNA and protein synthesis were carried out by incubating samples of the cell suspensions with a mixture containing [^{3}H]uracil and [^{14}C]-L-phenylalanine. Following an exposure of 2 min at 37° to the labeled substrates the cells were killed by the addition of ice-cold trichloracetic acid to give a concentration of 5%. After remaining at 0° for 30 min the cells were filtered on a membrane filter, washed, and the whole membrane dissolved in scintillation fluid. The latter was then gelled with Cab-O-Sil thixotropic powder, maintaining the cells in suspension. Radioactivity was measured with a Tri-Carb scintillation counter.

The preparation of the cells for exposure to actinomycin was modified from the procedure of Leive (1965a). Exponentially growing cells were harvested, washed once at room temperature with 0.01 M-tris Cl (pH 8) and resuspended in 0.033 M-tris Cl (pH 8). The cells were then vigorously aerated at 37° for 60 min. A solution of 1 M-K_2EDTA was added to give a final concentration of 10^{-3} M-EDTA. After being aerated for a further 80 min at 37°, the cells were added to four volumes of prewarmed fresh medium containing sufficient IPTG to give a concentration of 5×10^{-4} M after addition of the cells. The experimental flasks contained in addition sufficient actinomycin C (a gift from FarbenfabrikenBayer AG, Leverkusen, Germany) to give a final concentration of 0.0018 mg/ml.

Growth of the cells was measured as the turbidity of the suspension at 6500 A.U. using a Beckman DK-2 spectrophotometer. Turbidity was found to provide a satisfactory measure of total protein both with and without actinomycin.

With the control, exponential growth began soon after the cells were placed in fresh medium, with sometimes a lag of up to 5 min (Fig. 1). In the presence of actinomycin some variability was observed between experiments. Typically, growth at a lower rate than that of the control began immediately or within a few min of the cells being placed in fresh medium. This increase in turbidity, however, was usually linear, not exponential, for the first 50-60 min (Fig. 1). The rate of growth then increased, though after 2 hr was still far short of the control. The initial growth rate in the presence of

actinomycin was usually 50-60% of the control rate. The response to actinomycin was, in our experience, highly concentration-dependent, and this probably accounts for the variability in the growth rates on different occasions.

Beta-galactosidase synthesis in the control started to become significant about 20 min after introduction of the cells to the fresh medium containing inducer. The differential rate of synthesis became constant and maximal by 50-60 min (Fig. 2). The delay in the attainment of a maximal differential rate of beta-galactosidase synthesis may have been due in part, as Leive (1965a) suggested, to catabolite repression as a consequence of starvation in buffer. However, alkaline phosphatase, an enzyme not subject to catabolite repression by carbon compounds (McFall & Magasanik, 1960), also showed a slight lag of 7-10 min before attaining a maximal rate of synthesis. Catabolite repression may therefore not have been the only factor preventing the early full expression of the lactose operon (Moses & Calvin, 1965). In the presence of actinomycin (Fig. 2) the differential rate of beta-galactosidase synthesis was essentially zero for about the first 50 min, gradually increasing after this time, but still typically only 30-40% of the control after 100 min. In a similar experiment alkaline phosphatase showed no synthesis for about 35 min. The differential rate of synthesis of this enzyme then became constant at a maximal level.

The effects of actinomycin on the differential rate of pulse-labeling of RNA compared with protein showed a pattern similar to that for beta-galactosidase (Fig. 2). In the absence of actinomycin the ratio of incorporation of [^{3}H]uracil to [^{14}C]phenylalanine was approximately constant throughout the experiment, but in the presence of the antibiotic RNA synthesis was inhibited about 88% for the first 50-60 min, thereafter increasing to the control level by 100 min. Thus there was a close correlation between the kinetics of the syntheses of RNA and of beta-galactosidase during inhibition by actinomycin.

Preliminary experiments have been performed to investigate the distribution of ^{14}C incorporated from [^{14}]phenylalanine into protein. After passage through a French pressure cell, the broken cells were separated into a large fragment fraction (sedimenting in 30 min at 20,000 x g), a ribosome fraction (90 min at 105,000 x g), and a soluble fraction. Approximately similar findings were obtained with cells growing exponentially, and with EDTA-treated cells growing in the presence or absence of actinomycin. In each case 10-15% of the incorporated radiocarbon was in the ribosomes, 15-20% in the large fragments and 50-75% in the soluble fraction; 15-25% was not accounted for.

Three possible explanations for the differential inhibitory effect of actinomycin on beta-galactosidase might usefully be considered. Firstly, we might suppose that as a consequence of some interference with the overall process of protein synthesis there is a development of catabolite repression (Nakada & Magasanik,1964), which results in the preferential inhibition of the synthesis of beta-galactosidase. Against this hypothesis we must note that alkaline phosphatase is affected in a manner similar to beta-galactosidase, and that during catabolite repression in the presence of carbon sources such as glucose the differential rate of RNA synthesis is usually elevated, not depressed (Sypherd & Strauss, 1963a,b; C. Prevost and V. Moses, unpublished results).

Secondly, we might consider with Pollock (1963) that the transcription of the DNA of some operons is more susceptible than that of others to inhibition by actinomycin; the lactose operon would be particularly sensitive. While we cannot formally disprove this, it must be borne in mind that some 60% of protein synthesis remains unaffected when the differential synthesis of RNA is inhibited by 88%. If the lactose operon is a very sensitive one, there are many others which would also need to be severely inhibited in order to result in such a high degree of inhibition of RNA synthesis.

Even though other types of RNA are probably labeled during a 2 min pulsed incorporation of [^{3}H]uracil, the very great inhibition of uracil incorporation must represent a considerable fall in the rate of messenger RNA synthesis, particularly as actinomycin is believed to inhibit specifically DNA-dependent RNA synthesis (Gellert, Smith, Neville & Felsenfeld, 1965; Cavalieri & Nemchin, 1964).

Lastly, the findings could be interpreted on the basis of a general inhibition of RNA synthesis, with much protein synthesis continuing nevertheless as a result of the stability of many of the species of mRNA present in the cell. Beta-galactosidase synthesis would not be expected in these circumstances since IPTG was introduced to the cells only in the presence of actinomycin. Alkaline phosphatase synthesis is inhibited by actinomycin even though derepression by the absence of inorganic phosphate is not required in the constitutive strain here employed. If mRNA for alkaline phosphatase is unstable, and is not synthesized in the presence of actinomycin, no mRNA would be available as a template. The rapid cessation of repressible alkaline phosphatase synthesis on the addition of inorganic phosphate (Torriani,

1960) would suggest that, like the beta-galactosidase system (Kepes, 1963), the messenger for alkaline phosphatase is functionally unstable. Other evidence has also suggested that beta-galactosidase and alkaline phosphatase mRNA's are functionally much shorter-lived than those for many other protein species (Moses & Calvin, 1965, and references cited therein). The present findings with actinomycin serve to support the suggestion that there may be a wide range of functional stabilities for various mRNA's, and that short-lived messengers might be particularly characteristic of those proteins under the direct control of regulator genes.

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Figure Captions

Fig. 1. Growth of *E. coli* after treatment with EDTA, and the effect of actinomycin. Cells sensitive to actinomycin were prepared by incubation at 37° for 60 min in tris buffer, pH 8.0, followed by a further 80 min in tris buffer containing 0.001 *M*-EDTA. The cells were then added to 4 vol of fresh medium with or without actinomycin C (0.0018 mg/ml). Growth was followed by measuring turbidity at 6500 A.U., a good measure of total protein. Curve *A*, control; curve *B*, in the presence of actinomycin.

Fig. 2. Effect of actinomycin on the differential rates of synthesis of RNA and of beta-galactosidase. Same experiment as shown in Fig. 1. Upper portion: ratio of incorporation of labeled uracil to labeled phenylalanine during 2 min pulsed exposures to a mixture of these two precursors. Curve *A*, no actinomycin; curve *B*, with actinomycin. Lower portion: differential rate of beta-galactosidase synthesis vs. time, calculated as increase of enzyme activity divided by increase of turbidity for successive 10 min intervals. Curve *A*, no actinomycin; curve *B*, with actinomycin.

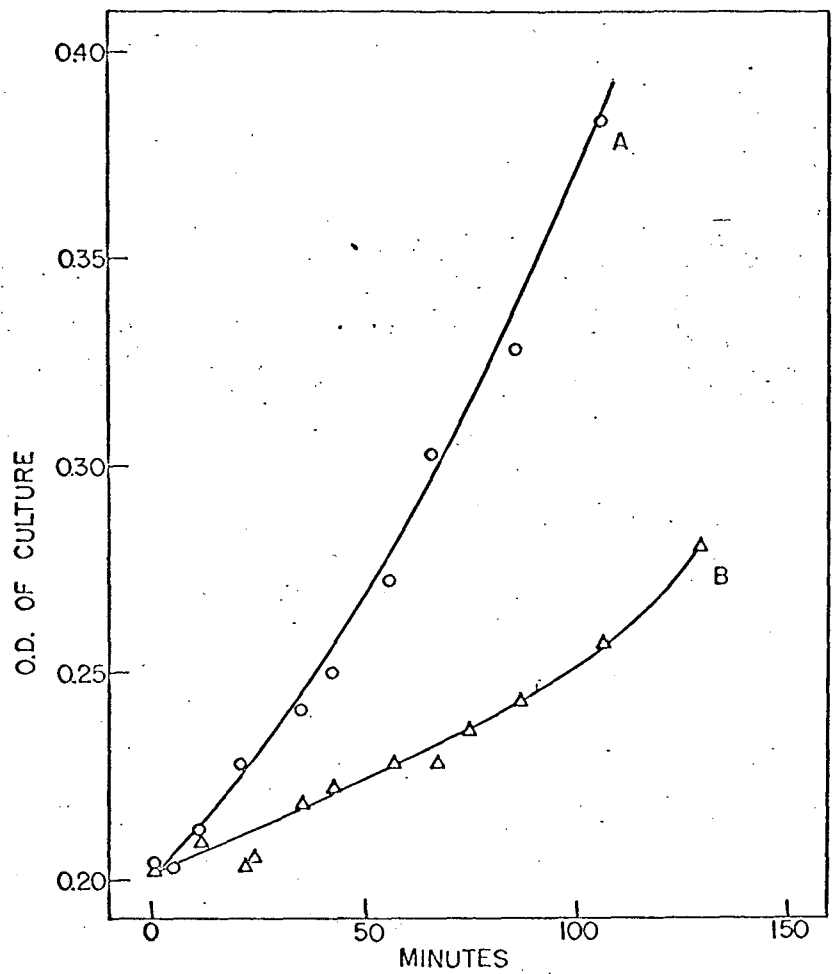

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Fig. 1.

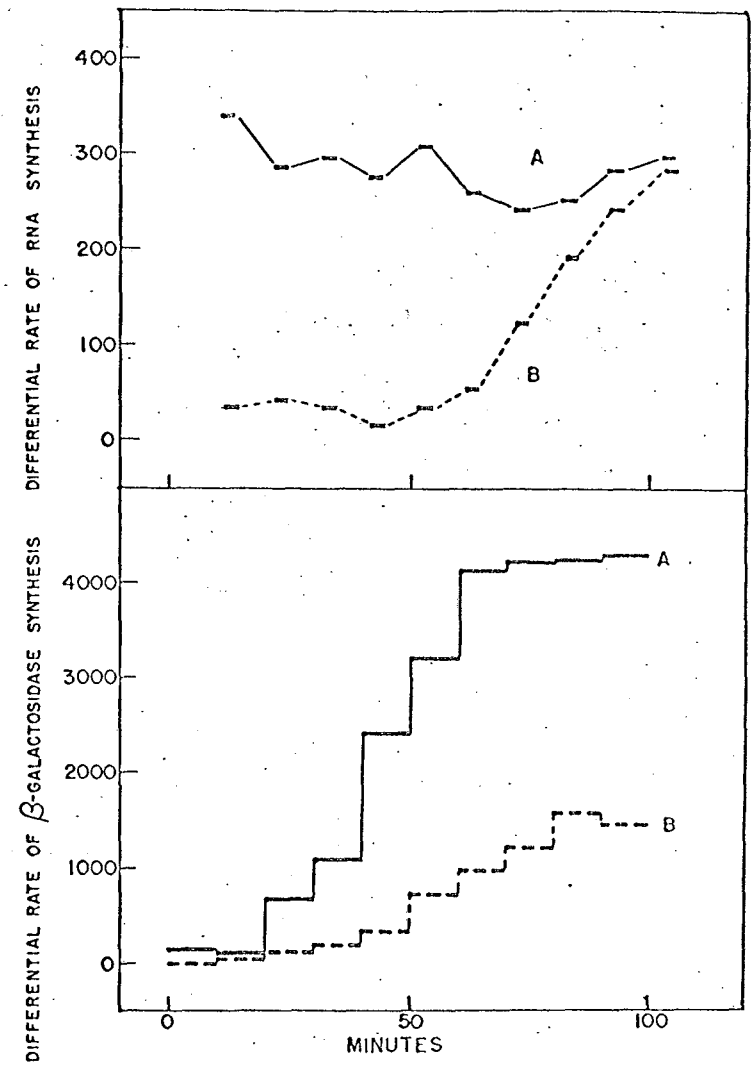

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Fig. 2.