ACTINOMYCIN SENSITIVITY IN ESCHERICHIA COLI PRODUCED BY EDTA

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Actinomycin, which inhibits RNA synthesis both *in vitro* (Hurwitz *et al*, 1962; Goldberg and Rabinowitz, 1962) and, in some organisms, *in vivo* (Kirk, 1960; Reich *et al*, 1961) does not affect normal cells of *Escherichia coli* (Hurwitz *et al*, 1962). Cultures of this organism do become sensitive, however, when converted to spheroplasts by the action of lysozyme and ethylenediaminetetraacetate (EDTA) (Mach and Tatum, 1963; Haywood and Sinsheimer, 1963). The use of such preparations is limited by their osmotic fragility and inability to divide (McQuillin, 1960).

The present experiments show that brief treatment of *E. coli* cells with EDTA renders them completely sensitive to the subsequent action of actinomycin D. The cultures remain viable when treated with EDTA, and are not osmotically fragile.

Materials and Methods - *E. coli* AB 1105*, a β-galactosidase-inducible, permease-negative strain (i+z+y-) which also requires histidine, proline, and thiamine, was grown with aeration at 37° on the medium of Levinthal *et al* (1962b) with peptone omitted and with additions of 2×10^{-3} M K_2HPO_4, 7×10^{-4} M each of histidine and of proline, 2×10^{-6} M thiamine, and 0.25% glycerol. Two hours prior to the beginning of the experiment, the incubation temperature was changed to 25°. All subsequent incubations and manipulations were at this temperature in

*This strain was the gift of Dr. E. Maxwell

order to permit greater precision in kinetic measurements.

β-Galactosidase was induced and assayed[+], and the units of enzyme activity measured, as previously described (Pardee et al, 1959). To determine incorporation of ^{3}H-uracil and ^{14}C-leucine into trichloracetic acid (TCA)-precipitable material, aliquots of the culture were added at 4° to 5% TCA containing a 100-fold excess of unlabeled uracil and leucine, the precipitates collected on Millipore (HA, 0.45 μ) filters, and washed with water. The filters and precipitates were dissolved in the scintillation solvent of Davidson and Feigelson (1957) with the water content reduced to 0.77%, and the two isotopes counted simultaneously. For cell counts, dilutions were made at 4° in the medium of Adams (1959) with added 0.01% $CaCl_2$ and plated on the same medium solidified with 1.5% agar. The cell densities were measured at 530 mμ with a Beckman DU spectrophotometer.

Results and Discussion - As shown in Figure 1, exposure of E. coli to 10^{-3} M EDTA for 2 minutes renders the cells susceptible to the later action of actinomycin. In the EDTA-treated culture, actinomycin halted the incorporation of ^{3}H-uracil almost immediately, the synthesis of β-galactosidase after approximately 7 minutes, and the incorporation of ^{14}C-leucine after approximately 15 minutes (see legend for experimental details). In contrast the control culture was unaffected by the drug. Thus actinomycin acts in EDTA-treated E. coli just as in other sensitive bacteria such as Staphylococcus aureus and Bacillus subtilis (Kirk, 1960; Levinthal, 1962a): RNA synthesis ceases at once, and protein synthesis soon thereafter.

This loss in the ability to synthesize protein probably reflects destruction of preexisting messenger RNA (Levinthal, 1962a). In this particular experiment, extensive breakdown of the labeled RNA did not occur, since only 20% of the ^{3}H-uracil was lost following actinomycin

[+]The isopropylthiogalactoside for induction was synthesized and kindly donated by Dr. G. Crowley.

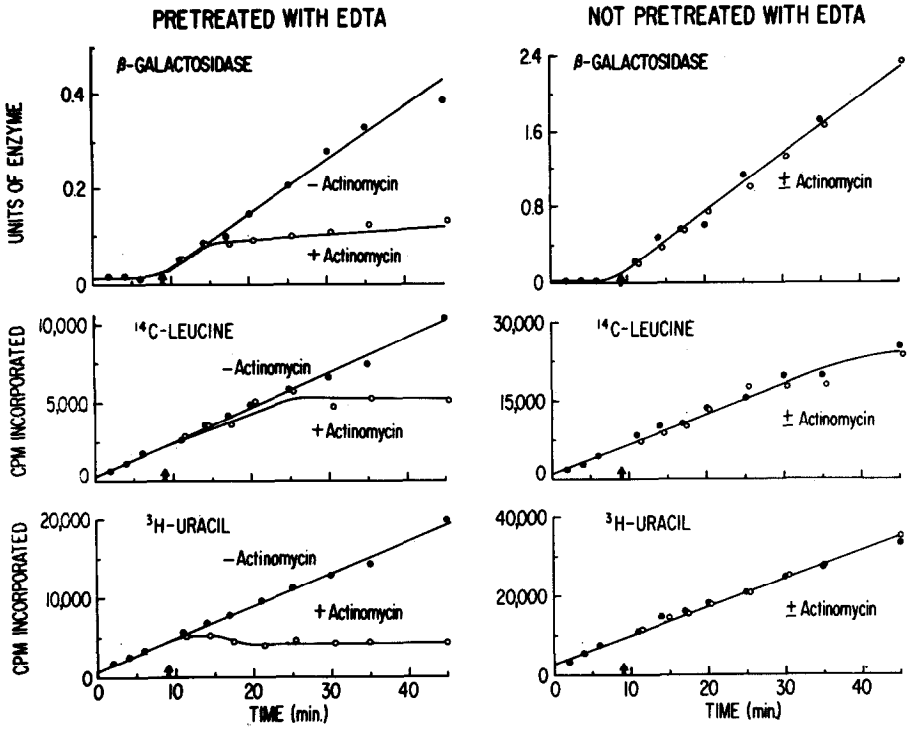

Fig. 1. The action of actinomycin on EDTA-treated E. coli. E. coli AB 1105 was grown as described in the text. The culture was harvested at a density of 2 x 10^8 cells/ml, washed once with 0.01 M Tris-Cl pH 8, and resuspended at a density of 5 x 10^9 cells/ml in 2 ml of 0.033 M Tris-Cl pH 8. The suspension was divided into two equal portions: to one vessel 10^{-3} M EDTA was added; the other served as the control. After a 2 min incubation with shaking 10 ml of growth medium was added to each. Incubation was continued for 13 more minutes, at which time (0 time on the graph) each culture was transferred to a flask containing isopropylthiogalactoside (4 x 10^{-4} M final concentration), ^{14}C-leucine (50 μc/μmole, 20 mM final concentration) and ^{3}H-uracil (1 mc/μmole, 10 mM final concentration). Incubation was continued, samples were taken at the times shown, and were assayed as described in the text. At 9 min (indicated by arrow on each graph) half of each culture was transferred to a vessel containing actinomycin D (10 μg/ml final concentration), and incubation and sampling were continued. Open circles, + actinomycin; closed circles, - actinomycin. All counts and enzyme activities are per 0.25 ml of culture.

addition. In other experiments, considerable variation was observed: when cells were labeled with ^{3}H-uracil for 2% of a generation, either as above or during growth at 37° in a glucose-containing medium, actinomycin addition caused loss of between 20 and 60% of the counts.

The reasons for this variation are as yet unknown and are currently under investigation.

It is interesting that β-galactosidase synthesis terminated sooner than did protein synthesis, as measured by incorporation of ^{14}C-leucine into TCA-precipitable material. This result is consistent with a decay in the activity of β-galactosidase messenger RNA faster than the average rate of decay of other messenger RNA's in the cell.

Several other properties of EDTA-treated cells deserve comment. Cultures treated with EDTA for as long as 15 minutes, and then immediately plated as described above, yielded the same cell counts as did an untreated control, indicating that such cultures are fully viable. Treatment with EDTA does not cause cells to become osmotically sensitive: after dilution into growth medium the optical density of the EDTA-treated culture in Figure 1 was equal to that of the control. After EDTA treatment cells grow more slowly; the figure shows that they incorporate leucine and uracil at about half the control rate. In light of their slowed growth, it is not surprising that their rate of β-galactosidase synthesis, relative to protein synthesis, was lower than in the control. It has been shown previously (Magasanik, 1961; Mandelstam, 1961; Nakada and Magasanik, 1964) that when growth of E. coli is inhibited, β-galactosidase is repressed by carbon sources which do not hinder its formation during normal growth. The glycerol present in this experiment may therefore, in the EDTA-treated cells, have repressed β-galactosidase formation.

Conversion to actinomycin sensitivity occurs very rapidly: after one minute in EDTA the culture is approximately 90% sensitive (as measured by the sensitivity of β-galactosidase synthesis to actinomycin) and after 2 minutes, 100% sensitive. Furthermore, such cells are converted to actinomycin sensitivity during the incubation in buffer containing 10^{-3} M EDTA, and not subsequent to the 11-fold dilution into growth medium, since growing cultures did not become actino-

mycin sensitive when 10^{-4} or 10^{-3} M EDTA was added to the medium. Thus some component of the growth medium, presumably one or more of the divalent cations, antagonizes the effect of EDTA.

Summary - E. coli can be made sensitive to the action of actinomycin by brief treatment with EDTA. Cells thus treated are not osmotically fragile and are viable. It is anticipated that such preparations will be of value in studying RNA metabolism and related problems in E. coli.

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