Production of surplus DNA in *E. coli*, and its effects on bacterial syntheses

Although several methods have been used to inhibit the synthesis of bacterial deoxyribonucleic acid (DNA) relative to other cell syntheses[1], no means of achieving a surplus of DNA has been reported. It is possible to increase the cellular content of DNA relative to protein and ribonucleic acid (RNA) by use of the amino-acid analog β-2-thienylalanine (βTA). When *Escherichia coli* strain B was grown aerobically in a salts–glycerol medium (the media and analytical techniques have been listed elsewhere[2,3]) plus various concentrations of βTA, an increasingly disproportionate content of DNA was produced in 1 h, up to a concentration of 1 μg/ml βTA (Fig. 1). Beyond this level no additional effects were observed: DNA synthesis was depressed about 35% while RNA and protein syntheses were inhibited by about 85%. Surplus DNA could bring the total concentration to almost twice normal.

Cellular division did not take place during 4 h in the presence of 20 μg/ml βTA, except for a 50% increase in colony-forming units in the first 40 min. All the original cells remained capable of colony formation, and no synchrony of division was observed when the inhibition was reversed after 1 h by 10 μg/ml tyrosine. The production of T2 bacteriophage in 40 min was completely inhibited by 20 μg/ml βTA added to *E. coli* prior to 15 min after infection. However, the ability of these bacteria to form plaques on broth-agar plates was not impaired. The enzymes for oxidation of glycerol or glucose, β-galactosidase, or D- and L-serine deaminase did not increase in activity in the presence of 5 μg/ml βTA.

The disproportionate increases in DNA were not observed with two other strains of *E. coli* —the radiation-resistant B/r or the thymineless mutant 15T—(E. McFALL, personal communication). These bacteria contained a ratio of DNA to RNA that is twice that of *E. coli* strain B.

The presence of intact DNA appears to be required for bacterial syntheses[3] (although the synthesis of new DNA is not). In order to discover whether the cellular content of DNA was directly related to synthetic abilities, surplus DNA was accumulated in the presence of 5 μg/ml βTA over a 2-h period. At intervals, inhibition was reversed in aliquots of the culture by the addition of 1 mg/ml yeast extract, 2 mg/ml acid-hydrolysed casein, 13 μg/ml tryptophan, and also the inducers DL-serine (0.4 mg/ml) and lactose (2.9 mg/ml) were added. At each time of reversal and 30 min later, the content per ml of DNA, RNA, protein, and the enzymes β-galactosidase and D- and L-serine deaminase were determined (Fig. 2). In the presence of βTA (solid curves), the DNA had approximately doubled by 90 min and the rates of synthesis of the other materials became slightly more rapid with time but were not proportional to the content of DNA. The rates of synthesis of these components after reversal of inhibition remained near the initial levels. Thus, the surplus DNA did not proportionately increase these rates.

The two most probable explanations for this negative result are that (1) the content of DNA per cell does not limit the rate of synthetic processes of the bacterium, or (2) that surplus DNA formed in the presence of βTA is not normal cellular material but is defective in its combination

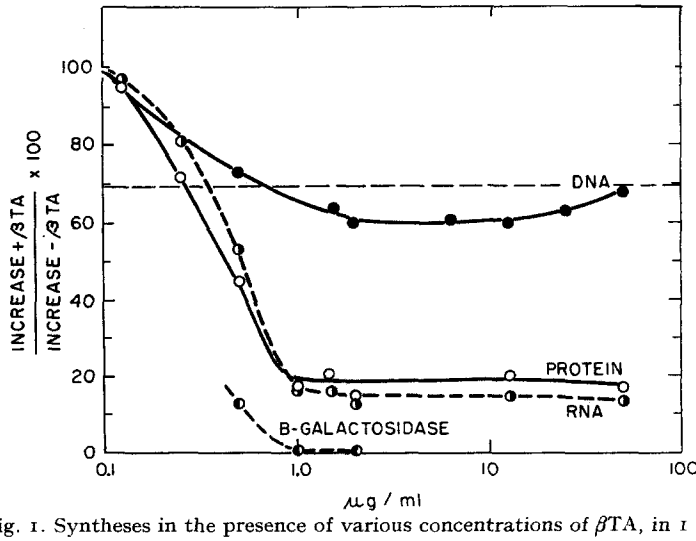

Fig. 1. Syntheses in the presence of various concentrations of βTA, in 1 h.

with protein, its base content, or its structure (as has been shown to be the case with RNA made in the presence of chloramphenicol[2]). The gross properties of the DNA made in the presence of βTA, however, were like those of normal DNA. It was precipitable by trichloroacetic acid, and not hydrolysed by 1 *M* KOH at 37° or 1 *M* $HClO_4$ at 4° in 15 h. The increased content of deoxyribose was proportional to the increased base content (measured as material in the isolated DNA absorbing light at 260 mμ) and also proportional to the increased content of thymine as determined by paper chromatography.

Another approach was to determine whether the surplus DNA was useful in preventing lethal damage by agents which act on cell DNA. In one set of experiments the resistance to ultraviolet light of *E. coli* grown for 90 min in the presence of 20 μg/ml βTA was determined. These were appreciably more resistant than normal cells: the slopes of their survival curves (log of survivors against dose) were about twice that of the control. This result would indicate that the surplus DNA is functional; but such curves are notoriously dependent on experimental conditions.

Similarly, a study was made of the effect of ^{32}P decay on cell viability. Lethal action is caused by decay of the ^{32}P incorporated into the cell DNA[3,4]. *E. coli* growing exponentially in a low phosphate medium were exposed to 8.6 mc/mg P for 60 min in the presence or absence of 20 μg/ml βTA, and then aliquots were frozen in liquid nitrogen, and were thawed at intervals and assayed for viability. The results (Fig. 3) show that phosphate incorporated into the bacterial DNA in the presence of βTA is as lethal as that incorporated into DNA of normally growing cells.

These findings suggest that the DNA is functional but does not act as a direct rate-limiting catalyst in the formation of other macromolecules. Such a conclusion is of course to be anticipated if it is recalled that the growth rate of bacteria depends strongly on nutritional conditions, in spite of a presumably constant DNA content per cell.

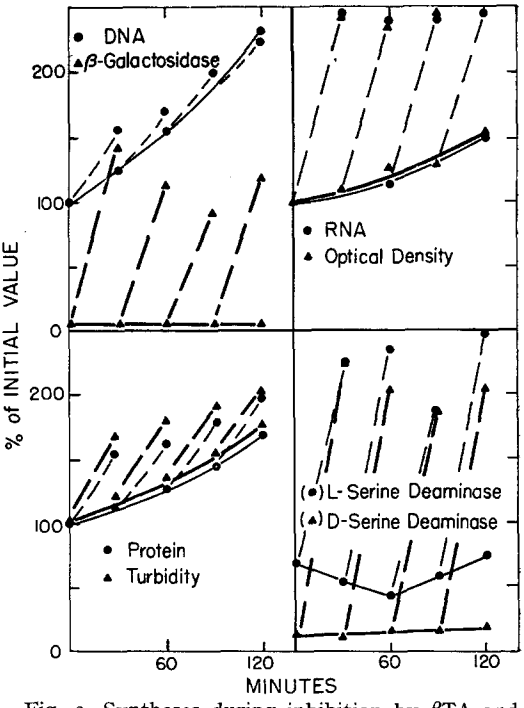

Fig. 2. Syntheses during inhibition by βTA and after reversal.

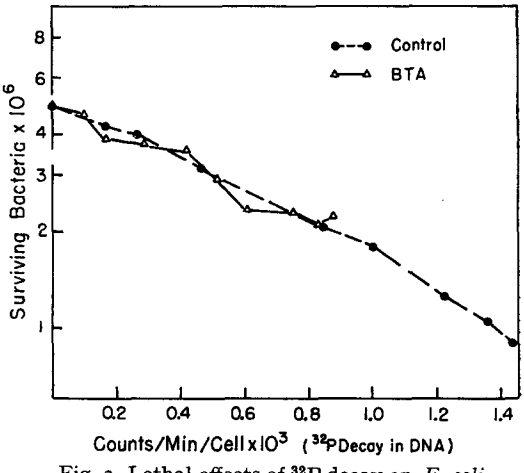

Fig. 3. Lethal effects of ^{32}P decay on *E. coli*.

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