The effect of thymidine upon the incorporation of uracil into *Bacillus cereus*

In a recent publication[1] 6-azauracil was reported to inhibit the transformation of orotic acid into uracil in the organism *Bacillus cereus*. As a result of this lowered uracil production, the synthesis of RNA and of DNA was decreased. By the addition of thymidine to the treated bacteria complete restoration of DNA synthesis could be effected. Under the same conditons RNA synthesis was increased but, nevertheless, remained below the normal level. This latter observation suggested that the thymidine might be converted to a uracil-containing material.

This observation has been further investigated. An experiment was performed in which the incorporation of [2-^{14}C]uracil into the total nucleic acids of the organism was measured in the presence and absence of thymidine. The procedure used was as follows: aliquots (1 ml) of the bacterial suspension in logarithmic growth were taken after varying periods of incubation time and added to 1 ml of cold 10 % trichloroacetic acid, containing a small amount of the unlabelled precursor. The mixture was allowed to stand for 30 min in the cold and then was filtered through a Millipore filter and washed 4 times with 5 % trichloroacetic acid. The filtered material plus the filter paper were placed on copper planchettes, dried and counted. The results shown in Table I indicate that the thymidine considerably decreases the incorporation of the uracil into the total nucleic acid (60 % decrease after 20 min), although total nucleic acid synthesis continues uninhibited.

TABLE I

EFFECT OF THYMIDINE UPON THE INCORPORATION OF [2-^{14}C]URACIL INTO THE NUCLEIC ACIDS OF *B. cereus*

2-^{14}C]Uracil (237 μg/ml and 1.5 μC/ml), 0.7 ml; thymidine (10 mg/ml), 0.6 ml; bacteria, 20 ml.

Incorporation time (min)	*Total counts/min*	
	Labelled uracil	*Labelled uracil + thymidine*
10	37	12
20	114	45
40	378	126
60	776	293

Experiments were performed in order to test the possibility that the thymidine was contaminated with uracil, which would also explain the above results. A uracil-less mutant, *Aerobacter aerogenes* P-12, was kindly provided by Dr. B. MAGASANIK and D. KARIBIAN of the Harvard Medical School. This mutant grows well on either uracil or uridine[2]. A culture of this bacteria was incubated in the presence of thymidine to determine whether it was contaminated with a uracil-containing material. Fig. 1 illustrates the effect on growth of different concentrations of uridine with the effect produced by thymidine. The results indicate that the thymidine does not contain a quantity of uracil (or uracil derivative) which can account for the decreased incorporation in the above experiment. The maximum amount of uracil present in our sample of thymidine cannot exceed 1 μg/300 μg thymidine and in order to produce the dilution

Abbreviations: RNA, ribonucleic acid; DNA, deoxyribonucleic acid.

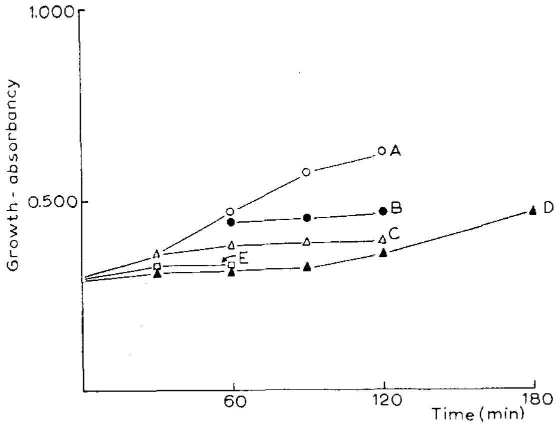

Fig. 1. Growth of *A. aerogenes* P-12 in the presence of uridine and thymidine. Curve A, uridine, 12 µg/ml; B, uridine, 6 µg/ml; C, uridine, 3 µg/ml; E, uridine, 1.5 µg/ml; D, thymidine, 300 µg/ml.

of uracil incorporation recorded in Table I almost 14 µg/ml uracil would have to be added (calculated after 20 min incubation).

There are several possible explanations for the above results the most evident of which is that the thymidine is transformed into a uracil derivative. However, up to the present time no such mechanisms have been observed although reactions in the reverse direction are well known[3]. The work of FRIEDKIN *et al.*[4] on chick embryos and of REICHARD[5] on liver tissue has shown that thymidine apparently is not metabolized to give rise to material which enters RNA. In *E. coli* and *Salmonella typhimurium*[6] similar observations have been made. One further point although it supports this interpretation does not explain all the facts. In the 6-azauracil-treated *B. cereus* thymidine restores only partially the formation of RNA[1]. If thymidine (300 µg) can provide 14 µg uracil this amount would certainly seem ample to restore RNA synthesis completely, unless azauracil affects the conversion of thymidine.

The explanation that thymidine exerts a sparing action on uracil also seems unlikely to account for the available data. In *B. cereus* almost 90 % of the total nucleic acid is in the form of RNA[7]. Another possibility is that the presence of thymidine may affect the penetration of uracil into the cell or the formation of its riboside. Experiments are planned in order to clear up these points.

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