BIOPHYSICS

Involvement of Synthesis of RNA in Thymineless Death

THE phenomenon of thymineless death in bacteria¹ is of particular interest because of its apparent relationship to DNA replication, as shown by Maaløe and Hanawalt². Only those bacterial cells which are synthesizing DNA at the time thymine is removed from the culture medium appear to be susceptible to thymineless killing under conditions of protein synthesis inhibition³. The 97 per cent susceptible fraction from an exponentially growing culture of strain *TAU* (*E. coli* 15T-A-U-, requiring thymine, arginine and uracil) succumbs to the lethality of thymine deficiency at about the same rate whether arginine and uracil are present or not². Thus, little correlation exists between thymineless killing and cytoplasmic synthesis. Using *E. coli* B3 Gallant and Suskind⁴ also reported thymineless death under conditions of protein synthesis inhibition, but they found further that phosphate starvation prevented killing. They implicated RNA synthesis on the basis of this result and the finding that the inhibitory effect of 5-methyl tryptophan could be partially reversed (with respect to RNA synthesis and thymineless killing) by chloramphenicol. The investigations to be reported here provide evidence that messenger-RNA synthesis in particular is necessary for the killing event in thymine-deficient cultures.

Our previous work with strain *TAU* has indicated that uracil deficiency does not prevent thymineless killing². However, this strain is known to be able to synthesize up to 2 per cent of its uracil requirement⁵. Also, *TAU* succumbs to thymine deprivation in a phosphate-deficient medium⁶, as consistent with the findings of McFall and Magasanik⁷ for *E. coli* 15T- but at variance with the observations on *E. coli* B3 (ref. 4). However, B3 is normally much less sensitive to the lethal effects of thymine deprivation than 15T- and strains derived from it. Perhaps phosphate is necessary for killing but it is made available in the *TAU* system by RNA turnover. A low level of chloramphenicol (1 µg/ml.) which slightly stimulated RNA synthesis in *TAU* (as determined by incorporation of uracil labelled with carbon-14 into RNA) had no striking effect on rate of thymineless killing although the rate of killing was slightly enhanced in a few experiments. A level of chloramphenicol (10 µg/ml.) which inhibited growth and overall RNA synthesis still permitted killing to a 3 per cent survival-level.

A new polyauxotroph of *E. coli* 15T-, designated *TAU-bar*, was obtained through the courtesy of Dr. Richard Wax. This strain requires arginine, methionine, proline, and tryptophan, and it shows a more stringent uracil requirement than strain *TAU*. Experimental procedures used were the same as reported previously², where membrane filtration was used for the rapid collection and washing of bacteria to remove supplements from the synthetic glucose-salts medium, and where viable cell count was determined by the pour-agar technique on fully supplemented nutrient agar plates. When thymine (T) and uracil (U) were removed from an exponentially growing culture of *TAU-bar* there was a very slow loss in viability as shown in Table 1. However, the removal of any one (or all four) of the required amino-acids, as well as thymine and uracil resulted in an increased killing rate and allowed the entire susceptible fraction, 97 per cent, to succumb in 3 h. Now, it is known that amino-acid deficiency in a stringent bacterial strain⁸ represses ribosomal RNA and transfer RNA synthesis but has little effect on messenger RNA synthesis⁹. Thus, a logical explanation of the result would be that the inhibition of ribosomal and transfer RNA synthesis (which together account for 98 per cent of the cellular RNA) allows more of the available uracil as released by RNA turnover to be incorporated into messenger-RNA. This messenger-RNA

could be directly involved in the killing event (for example, by specifying a colicine to be synthesized during subsequent growth on nutrient agar¹⁰). Or else it could leave the unreplicating DNA in a labile state in which it would be susceptible to thermally dependent denaturation or disaggregation.

Table 1. SUSCEPTIBILITY OF *TAU-bar* TO THYMINELESS DEATH

Experiment	Condition	Percentage of initial viable count at times shown				
		1 h	2 h	3 h	5 h	6 h
1	T + U + amino-acids	15	1	0.01		
2	T + U - amino-acids	20	5	4	4	
3	T - U + amino-acids	90	81	68	58	30
4	T - U - arginine	73	12	3	3	3

A possible hypothesis would be that the synthesis of a complementary messenger-RNA normally precedes DNA replication. One might envisage a model in which the DNA strands separate for messenger-RNA synthesis but are left in the separated state unless DNA replication of the free strands follows immediately. However, no detectable amount of single-stranded or denatured DNA has been isolated (in caesium chloride density gradient) from bacteria grown in the thymineless condition¹¹⁻¹³. Mennigman and Szybalski¹⁴ have recently reported evidence for damage to the molecular structure of DNA from thymineless cultures. They found a loss in viscosity and an increased sensitivity to shear which they attributed to single strand breaks. Perhaps these 'single strand breaks' represent portions of the genome which have released newly synthesized messenger-RNA but have not afterwards reformed the complementary DNA strands.

The evidence presented suggests that messenger-RNA synthesis is involved in the killing process in thymineless bacteria. However, one must distinguish between the event which causes killing and the required event which converts a cell from the immune state to the susceptible state². This latter aspect of the problem will be treated in a subsequent communication.

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BIOCHEMISTRY

Paper Electrophoresis of Xanthine Dehydrogenase from *Drosophila*

XANTHINE dehydrogenase from *Drosophila melanogaster* is of particular interest since mutants at two separate loci lack this enzyme¹ while a mutant at a third locus produces about 25 per cent of normal activity². In an effort to detect strains of flies with genetically altered forms of xanthine dehydrogenase we devised a technique involving paper-strip electrophoresis in which this enzyme is located after electrophoresis by spraying the strip with the