BASE COMPOSITION OF RAPIDLY-LABELLED RNA IN E. coli UNDERGOING THYMINELESS DEATH

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Among the numerous effects of thymine deficiency on thymine requiring mutants (Barner and Cohen 1954, Cohen and Barner 1954), only a few are directly concerned with bacterial death. It has been shown that episome induction, occuring in the absence of thymine (Melechen and Skaar 1962, Korn and Weissbach 1962, Sicard and Devoret 1962, Mennigmann 1964) causes death (Sicard and Devoret 1962, Mennigmann 1964). Strain E. coli 15 T- usually releases a colicine (Ryan et al 1955) probably related to the presence of a defective phage in these bacteria (Endo and Ayabe 1965, Sandoval et al 1965, Mennigmann 1965). When this strain does not produce colicine in the absence of thymine (Sicard and Devoret 1962) or is cured (Ishibashi and Hirota 1965), cultures of bacteria deprived of thymine contain a much greater number of viable cells than when the original strain is used (Sicard and Devoret 1962, Ishibashi and Hirota 1965). Moreover, if a strain is infected with an inducible episome (Sicard and Devoret 1962, Sicard 1964), the dying off observed is much greater and proportional to the number of induced cells (Sicard 1964). Therefore thymineless death results from two processes a) the induction of an episome by thymine starvation and b) a small base line lethal effect is always observed with whatever strain is tested. The explanation of

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the latter is still unknown.

Although some indirect indications, as for example induction, suggest that thymine deficiency must affect DNA, the physical and chemical properties of DNA extracted from thymineless cultures seem to be unchanged (Nakada and Ryan 1961, Luzzati and Revel 1962, Smith and Burton 1965). But some authors have mentioned some difficulties in extracting such a DNA (Dunn and Smith 1958, Mc Fall and Magasanik 1962). Furthermore, Mennigmann and Szybalski (1962) have reported that viscosity and resistance to shearing decrease when bacteria (B. Subtilis) are grown in presence of 5fluorodeoxyuridine which inhibits thymidylate synthetase and, like thymine deficiency, decreases cell viability.

It appears that a direct study of messenger RNA, which could reflect possible modifications in DNA, may be of interest. It has been shown that the rate of messenger RNA synthesis decreases in the absence of thymine (Mc Fall and Magasanik 1962). In order to deal only with the base line lethal effect independent of episome induction, we have chosen a strain which is neither lysogenic nor colicinogenic. We have determined the base composition of rapidly-labelled RNA extracted from starved and unstarved cultures to detect if there is any change during thymine deprivation. No significant differences have been observed.

EXPERIMENTAL

a/ Growth of cells

Cells (Strain E. coli K12 $T^-arg^-B1^-$) growing exponentially in low phosphorus-tris medium supplemented with thymine (2 µg/ml) arginine (30 µg/ml) and vitamine B1 (1 µg/ml), are centrifuged, washed and resuspended in the same medium with or without thymine. After two hours of incubation, the cells are labelled with a 25 sec pulse of ^{32}P (10 µC/ml). Reaction is stopped by addition of the culture to a volume of cold perchloric acid (5N) so that the final concentration is 0,4 N.

b/ Extraction of nucleic acids

Nucleic acids are extracted by the method of Tyner

modified by Volkin and Astrachan (1956). After alcohol precipitation, RNA and DNA are centrifuged,then washed 3 times with cold 5% TCA as described by Midgley and Mc Carthy (1962) to complete the elimination of radioactive soluble material. The precipitate is taken up in distilled water and hydrolysed for 16 hours at 37°C in 0,1 N NaOH.

c/ Nucleotide composition of RNA

Mononucleotides are separated by ion-exchange chromatography. The method is that described by Cohn and Bollum (1961). The optical densities of all fractions (20 ml each) are read at wavelenghs 260 and 280 mμ and their radioactivity is measured. The amount of each nucleotide is determined from the optical density measurements (Volkin and Cohn 1954). Base composition is calculated from the ratio of total activities associated with each nucleotide.

RESULTS and DISCUSSION

The synthesis of messenger RNA decreases with time in the absence of thymine (Mc Fall and Magasanik 1962). In order to assure sufficient killing as well as enough synthesis of messenger RNA, we have chosen an incubation time of 2 hours without thymine during which the viability of the culture is reduced by 50 %. After a 25 sec pulse of ^{32}P and extraction of nucleic acids, RNA is hydrolysed and mononucleotides separated. Separation of RNA nucleotides after elution is satisfactory for RNA extracted from a normal culture (+) as well as for RNA extracted from a starved culture (-) See fig. Specific activity of each nucleotide is quite constant for the series of fractions corresponding to the same elution. The actual radioactivity of each nucleotide is smaller in the absence of thymine indicating that messenger RNA is depressed.

The average variation between base ratios determined in differents experiments is 8%. The base compositions of rapidly-labelled RNA of two cultures are presented in table 1. No significant differences appear between base compositionsof newly formed RNA in bacteria grown in the absence or in the presence of thymine.

Base composition of newly formed RNA in E. coli K12T⁻arg⁻B1⁻

TABLE 1 : grown with (+) or without (-) thymine

		Cytosine	Adenine	Uracil	Guanine
+	total activity (cpm)	175.540	163.140	138.480	167.640
	%	27,2	25,3	21,5	26,0
-	total activity (cpm)	92.920	88.680	85.000	98.520
	%	25,4	24,3	23,3	27,0

Similarly, the base composition of total RNA, calculated from the total amount of each nucleotide recovered after elution, does not vary markedly (Table 2). This is in agreement with the results of Gallant and Suskind (1962) on E. coli B3.

Base composition of total RNA in E. coli K12 T⁻arg⁻ B1⁻

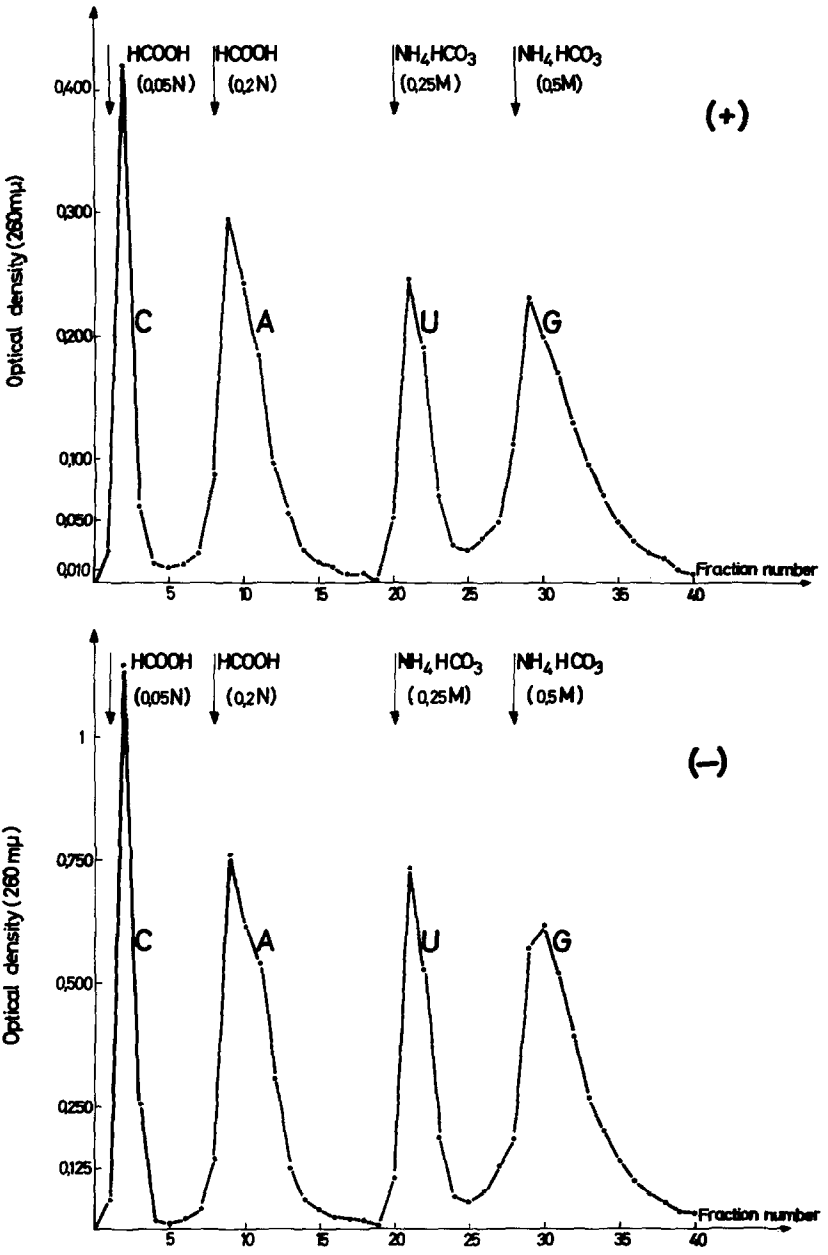
TABLE 2 : grown with (+) or without (-) thymine

%	Cystosine	Adenine	Uracil	Guanine
+	23,4	23,1	21,1	32,4
-	25	21,7	20,8	32,5

It appears that RNA formed during thymine starvation is not abnormal within the limits of sensitivity of this chemical method.

Another attempt has been made to detect modifications of messenger RNA formed in bacteria during thymine starvation, by comparing the distribution of radioactivity in a sucrose gradient of rapidly-labelled RNA extracted from a culture deprived of thymine (labelled with ^{32}P) and a culture grown in its presence (labelled with ^{3}H uridine). The two distribution patterns are identical. There is no specific modification in newly formed RNA coming from thymineless cultures (Sicard and Astrachan unpublished results).

Hanawalt (1963) suggested that messenger RNA is involved in thymineless death but it is not proven that, in the particular case of strain 15 TAU-bar, there is a direct relation between synthesis of messenger RNA and killing rate. Furthermore, such a strain containing an inducible



Separation of nucleotides extracted from unstarved (+ or thymine-starved (-) cultures

Nucleotides of RNA extracted from unstarved or thymine-starved cultures are fixed on a Dowex column (1 8x200-400 mesh), then eluted by solutions indicated by the arrows.
C=cytidylic acid, A=adenylic acid, U=uridylic acid, G=guanylic acid.

prophage is not suitable to study the base line lethal effect.

All the negative results of biochemical experiments do not exclude the possibility that DNA is altered. Biological experiments have already revealed that DNA activity of starved cells is definitely lost. Using B.subtilis grown in presence of 5fluorodeoxyuridine (Mennigmann and Szybalski 1962) or a thymineless strain deprived of thymine (Sicard and Anagnostopoulos 1964), it has been shown that the tranforming activity of the DNA of such treated cells decreases to about 10% of its original value. DNA is therefore inactivated.

Recently, the same conclusion was drawn from experiments testing the ability of DNA to serve as a template for RNA polymerase in vitro (Luzzati, in press).

Mechanism of DNA inactivation by thymine starvation is still unknown. Only hypothesis can be formulated.

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