Variations in the amount of ribonucleoprotein particles in *Escherichia coli*

The amount of RNA in bacterial cells varies during the growth cycle. It is low in the resting phase, and increases several fold to reach a maximum in logarithmically dividing cells[1-3]. WADE AND MORGAN[4] have shown that this "additional RNA" present in dividing bacteria is found in a cell fraction which sediments at 100,000 × *g* in 4 h and corresponds to the ribonucleoprotein components, with sedimentation coefficients from 20 S to 40 S. This suggested that the number of ribonucleoprotein particles is low in resting cells and high when the organisms are in the exponential phase of growth. In the first series of experiments reported here, the content of ribonucleoprotein particles was traced through a complete growth cycle and compared to the changes in RNA concentration.

DAGLEY AND SYKES[5] have reported that the schlieren ultra-centrifuge diagrams of cell-free extracts are considerably modified after incubation of the bacteria in phosphate buffer for 2 h, an indication that the particles were breaking down under these conditions. In the second series of experiments, we investigated the fate of the particles when intact cells were incubated in various concentrations of buffers.

First series of experiments. E. coli strain B were grown under forced aeration in enriched broth at 37°. After 31-h incubation the culture had reached the stationary phase, with $1.4 \cdot 10^{10}$ bacteria/ml. The cells were small and nearly oval in shape, while logarithmic-phase cells, which divide every 25 min, are larger and rod like. The bacteria were harvested, cooled, washed with ice-cold 0.01 *M* phosphate buffer, pH 7.0, and divided into four equal samples. Sample 1 was analyzed at once (stationary-phase cells), while samples 2, 3 and 4 were incubated at 37° with aeration, as follows: Sample 2 was resuspended in 1 l 0.01 *M* phosphate, pH 7.0, and harvested after 1 h. The bacteria were still small and oval in shape and no division had occurred. Sample 3 was incubated for 1 h in 1 l of fresh broth. No significant division occurred during this time, but the cells became larger and rod like in shape (lag phase). Sample 4 was placed in 1 l of fresh broth and harvested after 3 h, when half of the bacteria had divided. The cells were rod like and were seen to divide under the microscope; thus, they were entering the logarithmic phase of growth. Bacteria from each of the four samples were viable when plated. Extracts were made by grinding the cells with 3 parts of alumina powder and extracting with 6 vol. cold 0.01 *M* phosphate, pH 7.0, containing 0.001 *M* $MgCl_2$ and 5 μg desoxyribonuclease/ml. The mixture was centrifuged at 6000 × *g* for 15 min, and the supernatant, which contains 80–90% of the RNA of intact bacteria, was examined in a Spinco Model E ultracentrifuge. Aliquots were analyzed for RNA by the orcinol method[6] with correction for DNA[7], and for proteins by the biuret test[8]. The results are shown in Table I. The ribonucleoprotein particles appear as two peaks on the schlieren diagrams, with sedimentation coefficients of 32 S and 51 S[9]. While the size of the peaks varied with growth phase, the sedimentation coefficients did not change. The relative concentration of ribonucleoprotein particles in each sample was obtained by adding together the measured areas under these two peaks for each extract. The ratio of this value to that of the area of the 5-S peak was taken as the relative concentration of ribonucleoprotein particles. The 5-S peak, which is formed

Abbreviations: RNA, ribonucleic acid; DNA, deoxyribonucleic acid; Tris, tris(hydroxymethyl) aminomethane.

TABLE I

Variations in the amount of RNA and in the area covered by the ribonucleoprotein components on the schlieren sedimentation diagrams, in cell-free extracts, from bacteria in the three phases of growth and after incubation in phosphate buffer.

	Sample 1 Stationary phase	*Sample 2 Incubation in phosphate*	*Sample 3 Lag phase*	*Sample 4 Logarithmic phase*
mg RNA/mg protein	0.092	0.074	0.322	0.350
Combined areas under the schlieren curves for 32-S and 51-S components (relative to *Sample 1*)	1.00	0.66	2.83	3.33

by the soluble protein fraction of the cell, was thus arbitrarily considered as a standard of reference. The relative concentrations of ribonucleoprotein particles are given in Table I, taking 1 as the ratio of the combined area under the 32-S and 51-S peaks to the urea under the 5-S peak in sample 1.

These results show that the marked changes in the amount of RNA present in the extracts from cells in the various phases of growth are paralleled by similar changes in the amount of ribonucleoprotein particles.

Second series of experiments. E. coli strain B were grown in a minimal medium[10] at 37° under forced aeration. The bacteria were harvested in the early logarithmic phase, when they had reached a concentration of $1–5 \cdot 10^8$ cells/ml, with a generation time of 45 min. They were then cooled, harvested and washed twice with 0.075 *M* phosphate, pH 7.0, which was the phosphate concentration present in the culture medium. A sample was removed to make a cell-free extract for analysis in the ultracentrifuge. The remaining bacteria were resuspended in phosphate or tris(hydroxymethyl)aminomethane buffers, pH 7.0 and 7.4 respectively, varying in concentration from 0.005 *M* to 0.3 *M* and also in 0.15 *M* NaCl containing 0.005 *M* Tris. These cell suspensions were incubated at 37° with aeration for periods ranging from 1 to 18 h, during which cell division did not occur. In 0.005 *M* phosphate for 18 h, the bacteria retained nearly 100% viability when plated. In 0.15 *M* phosphate they were all viable after 4 h. However, after 18 h the viability was reduced to approximately 75%. After incubation the bacteria were harvested, and the cell-free extracts were examined in the analytical centrifuge. The measured areas under the ribonucleoprotein peaks on the schlieren diagrams were compared as described above. The results of these experiments can be summarized as follows: (1) the level of ribonucleoprotein particles decreases only very slightly when logarithmic-phase cells are incubated in 0.005 *M* phosphate or Tris buffers for periods up to 18 h; (2) in 0.075 *M* buffer solutions a significant decrease in the amount of particles is observed after 2 h; after 18 h this decrease reaches several fold; (3) the decrease is proportional to the buffer concentration: with 0.15 *M* phosphate or Tris, it is several fold after 4 h, and after 18 h the peaks on the Schlieren diagrams are barely visible; (4) incubation in 0.15 *M* NaCl containing 0.005 *M* Tris gives results similar to those obtained with 0.15 *M* phosphate or Tris; (5) in some cases a new peak appears in the range 20–22 S; aside from this, the breakdown does not lead to the formation of subunits of high molecular weight visible on the schlieren diagrams.

Table II shows the results of RNA and protein estimations on intact cells and on

TABLE II

Amounts of RNA and relative areas covered by the ribonucleoprotein particles on the schlieren diagrams for bacteria incubated in 0.15 *M* phosphate and Tris for 12 h at 37°.

	Sample 1 *Logarithmic-phase cells*	*Sample 2* *Cells incubated in 0.15 M phosphate for 12 h*	*Sample 3* *Cells incubated in 0.15 M Tris for 12 h*
mg RNA/mg protein { Intact cells	0.232	0.052	0.043
mg RNA/mg protein { Cell-free extract	0.243	0.068	0.056
Combined areas under the schlieren curves for 32-S and 51-S components (relative to *Sample 1*)	1.00	0.23	0.21

cell-free extracts from bacteria before and after incubation in 0.15 *M* phosphate and Tris buffers for 12 h. The relative areas covered by the ribonucleoprotein peaks on the schlieren diagrams are also given, taking 1 as the ratio of the amount of the ribonucleoprotein particles (combined areas of the 32-S and 51-S peaks) to that of the soluble proteins (area of the 5-S peak) in sample 1. After 12-h incubation, the ribonucleoprotein particle concentration, together with the RNA/protein ratio, had decreased by a factor of 4 to 5.

After the bacteria had been harvested, the suspending buffer was collected free of cells and its absorbance was measured in the ultraviolet region; it showed the characteristic absorption spectrum of a mixture of purines and pyrimidines, with a sharp maximum at 258 mμ. This indicates that when the ribonucleoprotein particles break down, the ribonucleic acid is degraded and the breakdown products are liberated into the medium.

In conclusion, when logarithmic-phase cells are incubated at 37° in buffered phosphate, Tris or NaCl solutions at a concentration of 0.075 *M* or more, the ribonucleoprotein particles break down. This effect seems proportional to the salt concentration. RNA is degraded and the breakdown products can be found in the suspending medium.

This work has been supported by a grant from the National Science Foundation.

Biological Laboratories, Harvard University, Cambridge, Mass. (U.S.A.)

J. MENDELSOHN*
A. TISSIÈRES

1 P. C. CALDWELL, E. L. MACKOR AND C. HINSHELWOOD, *J. Chem. Soc.*, (1950) 3151.
2 W. H. PRICE, *J. Gen. Physiol.*, 35 (1952) 741.
3 M. SCHAECHTER, O. MAALØE AND N. O. KJELDGAARD, *J. Gen. Microbiol.*, 19 (1958) 592.
4 H. E. WADE AND D. M. MORGAN, *Biochem. J.*, 65 (1957) 321.
5 S. DAGLEY AND J. SYKES, *Nature*, 179 (1957) 1249.
6 Z. DISCHE, *J. Biol. Chem.*, 204 (1953) 983.
7 Z. DISCHE, *The Nucleic Acids*, Academic Press, Inc., New York, 1955, vol. I, p. 287.
8 A. G. GORNALL, C. J. BARDAWILL AND M. M. DAVID, *J. Biol. Chem.*, 177 (1949) 751.
9 A. TISSIÈRES AND J. D. WATSON, *Nature*, 182 (1958) 778.
10 R. B. ROBERTS, P. H. ABELSON, D. B. COWIE, E. T. BOLTON AND R. J. BRITTEN, *Studies of Biosynthesis in* E. coli, Carnegie Institution of Washington Publication, 1955, p. 4.

Received December 29th, 1958

* Present address: Department of Biochemistry, University of Glasgow, Glasgow, Scotland.