ASPECTS OF THE INVARIABILITY OF A BACTERIAL RIBONUCLEIC ACID*

ANNA LOMBARD** AND ERWIN CHARGAFF

Cell Chemistry Laboratory, Department of Biochemistry, College of Physicians and Surgeons, Columbia University, New York, N.Y. (U.S.A.)

This paper deals with the *total* ribonucleic acid of *Escherichia coli*. In considering the global distribution of the several nucleotide constituents of the polymer, we leave aside the question whether the cell contains many different ribonucleic acids or ribonucleoproteins, though we are not unaware of this possibility; nor can we be concerned here with changes in nucleotide sequence or in molecular size. We may define our title by saying that it refers to the absence of compositional variation, *i.e.*, of changes in the internucleotide ratios of the total polymer. In the sense in which we use it here, this term must, therefore, be distinguished from the concepts of constancy, which we shall take to denote the absence of a change in quantity, and of stability, which will stand for resistance to decomposition.

The purpose of this study was to ascertain whether it is possible to compel a change of the normal composition of the ribonucleic acid of *E. coli* by displacing the proportions of nucleic acid constituents in the presence of an excess of a single nucleotide precursor. These experiments were carried out both under conditions of simultaneous synthesis of proteins and nucleic acids and of nucleic acid production alone. We shall discuss the conclusions to be drawn from our work, together with some of what has been done before, later in this paper.

EXPERIMENTAL

Material and procedures

Bacterial cultures. The strains used were *E. coli*, Strain B, and the lysine-deficient mutant of *E. coli*, M 26 26, obtained through the courtesy of Dr. B. D. DAVIS of New York University. The organisms were grown in a glucose-containing synthetic medium[1] at 37° with aeration by shaking, in the case of the mutant supplemented with 20 mg of L-lysine per liter. The cells were collected in the early logarithmic phase of growth, washed with fresh medium with the omission of glucose, resuspended in the medium containing the desired supplements, and again incubated at 37° for 1 to 2 hours depending upon the experiment.

In some of the initial experiments the washed bacterial preparations were, before analysis, broken by treatment in a sonic oscillator (Raytheon, 10 kc, 10 minutes) or by being shaken with ballotini (90 minutes); but since undisrupted cells yielded the same results, they were employed in the majority of the experiments.

Substances. Commercial preparations of the ribonucleosides were used (Schwarz Laboratories, Mount Vernon, N.Y.). Of the preparations labeled with ^{14}C, adenosine was labeled in the 8 position, guanosine and uridine were uniformly labeled. Chloramphenicol (chloromycetin) was obtained

* This work has been supported by research grants from the National Institutes of Health, U.S. Public Health Service, from the American Cancer Society (on recommendation of the Committee on Growth, National Research Council), and the Rockefeller Foundation.

** Holder of a Quincy Ward Boese Postdoctoral Fellowship of Columbia University, 1955–6.

References p. 554.

through the courtesy of Dr. J. J. PFIFFNER of Parke, Davis & Co., Detroit, and used in a concentration of 30 mg per liter.

Analytical procedures. The growth of the cultures was in all cases followed turbidimetrically and in many by the determination of total ultraviolet absorption and of protein (biuret). The latter procedures were also used in the cell preparations treated with chloramphenicol.

The nucleotide composition of the ribonucleic acids was determined as described previously[2,3]. Paper electrophoresis, though useful for the separation of pure components, could not be applied for analytical purposes, since the prerequisite depression of the high salt concentration of the hydrolysates led to variable losses of nucleotides[3]. In cases in which it was desirable to separate guanylic and uridylic acids, which are not separated in the buffered ammonium *iso*butyrate-*iso*butyric acid solvent[4] usually employed, use was made either of the *iso*propanol-HCl solvent[5], in which uridylic acid moves as the fastest component (18 h), or of *iso*propanol-ammonia[6], in which guanylic acid is separated as the slowest zone from the other nucleotides (40 h).

In the experiments with radioactive nucleotides, each separated zone containing 0.1 to 0.2 μmole of nucleotide was extracted with 3 ml of dist. water overnight. One ml portions of these extracts, as well as of extracts from corresponding blank segments, were uniformly evaporated to dryness under a heating lamp in planchets and counted at infinite thinness with a thin end-window counter tube. All determinations were made at least in duplicate. Other portions of the same extracts were used for the measurement of ultraviolet absorption.

Nature of sugar component

The sugar of the pentose nucleic acid of *E. coli* does not appear to have been identified previously. As is customarily done in this laboratory, we have performed a tentative identification by means of paper chromatography in several solvents. The nucleotides contained in an alkaline hydrolysate of the nucleic acid prepared in the usual way[2] were separated as bands in the NH_3-*iso*butyric acid solvent, the areas containing the purine nucleotides were cut off, the fractions were concentrated in the tips of the paper strips by ascending chromatography with water and then extracted with distilled water. The combined extracts were frozen and evaporated *in vacuo* and the residue was hydrolyzed with N H_2SO_4 at 100° in a sealed tube for one hour. The solution was neutralized with solid barium hydroxide and clarified. Portions containing 10 to 20 μg of sugar were chromatographed on Whatman paper No. 1 in four solvents, *viz.*, (a) *n*-butanol–ethanol–water (4:1:5, v/v, upper layer)[7]; (b) *n*-butanol (saturated with water)[8]; (c) *iso*butyric acid (saturated with water)[7]; (d) ethylacetate–pyridine–water (2:1:2, v/v)[9]. In some cases circular chromatograms were also prepared. The adsorption zones were demonstrated by means of an aniline diiodosalicylate spray (0.5 g diiodosalicylic acid, 0.3 ml aniline in 100 ml *n*-butanol saturated with water), developed in this laboratory by Dr. A. ROSENBERG. After the papers are heated at 120 to 140° for 5 minutes, pentoses appear as brick red spots, hexoses are tan colored.

In all solvents, the preponderant component was a pentose coincident in position with that of authentic D-ribose; its R_F relative to arabinose also was that of ribose. A minor slower component, most probably a hexose, was also seen. Butanol-water, when used, as was done here, in long runs (72 h), permits the separation of arabinose, xylose, lyxose and ribose. One may conclude tentatively that ribose is the sugar constituent of the pentose nucleic acid of *E. coli*.

RESULTS AND DISCUSSION

Nucleosides as precursors of ribonucleic acid

The experiments presented in Table I deal with the incorporation of labeled nucleosides into the ribonucleic acid of *E. coli* cells, either under conditions in which the simultaneous synthesis of new ribonucleic acid and protein molecules occurred or in a system in which, by the addition of chloramphenicol, the formation of new protein was suppressed while that of ribonucleic acid continued[10,11]. That nucleosides are utilized efficiently for nucleic acid synthesis in *E. coli* has been shown repeatedly by different techniques[12,13].

The purpose of the present studies was to compare the relative efficiencies of incorporation of a nucleoside in the absence and in the presence of an agent preventing protein synthesis. The cells were grown as described above. In Experiments 1 and 2, logarithmically growing cultures of *E. coli*, Strain B, were divided into equal parts, harvested, resuspended in fresh medium (one third of the initial volume) containing

References p. 554.

TABLE I

NUCLEOSIDES AS PRECURSORS OF *E. coli* RNA FORMED IN THE PRESENCE AND ABSENCE OF SIMULTANEOUS PROTEIN SYNTHESIS*

Experiment No.	Nucleoside as precursor in experiment**	Component	Nucleotides of ribonucleic acid: Specific activity		Proportion of nucleotide derived from precursor***: % of component in total RNA		% of component in newly formed RNA	
			N	*Chl*	*N*	*Chl*	*N*	*Chl*
1	^{14}C-Uridine (0.07 μmole/ml; specific activity, 6600)	Adenylic acid	200	200	(3)	(3)	(8)	(5)
		Guanylic acid	300	300	(5)	(5)	(12)	(8)
		Cytidylic acid	1150	1450	17	22	42	39
		Uridylic acid	1350	1425	20	22	49	38
2	^{14}C-Uridine (0.09 μmole/ml; specific activity, 6600) Guanosine (0.3 μmole/ml)	Adenylic acid	300	275	(4)	(4)	(7)	(7)
		Guanylic acid	200	125	(3)	(2)	(5)	(4)
		Cytidylic acid	2000	1825	30	28	47	47
		Uridylic acid	2150	1875	32	28	50	48
3	^{14}C-Adenosine (0.03 μmole/ml; specific activity, 10,700)	Adenylic acid	2400	2450	22	23		47
		Guanylic acid	2600	2400	25	22		45
4	^{14}C-Guanosine (0.014 μmole/ml; specific activity, 11,800)	Adenylic acid	500	400	(4)	(3)		(7)
		Guanylic acid	1350	1700	11	14		27

* See the text for experimental conditions. The experiments 1 and 2 were performed with *E. coli*, Strain B; 3 and 4 made use of the *E. coli* mutant M 26–26. The specific activity is expressed as counts per minute per micromole of substance. *N* designates normal synthetic conditions in a complete medium, *Chl* signifies the suppression of protein synthesis by the addition of chloramphenicol (30 γ/ml).

** Uridine (Expts. 1 and 2) and guanosine (Expt. 4) were uniformly labeled with ^{14}C, adenosine (Expt. 3) was labeled in the 8 position.

*** Values based on low radioactivity counts are placed in parentheses. In the calculation of the figures contained in the last two columns, the assumption has been made that only the RNA portion formed after the addition of the precursor took up the latter. The proportion of ^{14}C recovered in the total RNA, as per cent of ^{14}C applied as precursor, was as follows: Expt. 1, *N* 25, *Chl* 27; Expt. 2, *N* 27, *Chl* 20; Expt. 3, *N* 35, *Chl* 34; Expt. 4, *N* 20, *Chl* 30.

the labeled nucleoside with or without chloramphenicol, and kept at 37° for 90 minutes. In Experiments 3 and 4, the additions were made directly to portions of cultures of the lysine-requiring mutant, and the experiments were terminated 1 h later. The culture volumes were so chosen as to give final batches of 20 to 50 mg dry weight of cells. For analysis, the cells were collected at the end of the experiments and dried in a vacuum.

It will be noticed: (a) that uridine is an equally efficient precursor of both the uridylic and cytidylic acid constituents of the ribonucleic acid, but that very little of the radioactivity appears in the purine nucleotides, indicating the insignificant participation of transribosidases (Expts. 1 and 2); (b) that adenosine participates to an equal extent in the formation of both adenylic and guanylic acids (Expt. 3), whereas guanosine gives rise mainly to guanylic acid (Expt. 4), both purine nucleosides

References p. 554.

being virtually ineffective as precursors of the pyrimidine nucleotides; (c) that the uptake of labeled uridine is not affected by the presence of a large supply of unlabeled guanosine, *i.e.*, of the other 6-keto compound[14] found in pentose nucleic acids (Expt. 2); (d) that the nucleosides are remarkably efficient as precursors of ribonucleic acid, as much as 50% of the newly incorporated constituent being derived from the precursor; and, finally, (e) that the suppression of protein synthesis is without effect on the incorporation, both in its qualitative and quantitative aspects, of the nucleoside employed as nucleic acid precursor. One could speak of the anabolic invariability of the ribonucleic acid of *E. coli*.

Invariable composition of ribonucleic acid

In the preceding section it was shown that nucleosides are utilized very efficiently as precursors of ribonucleic acid under the experimental conditions observed here; in the present one we discuss the nucleotide composition of the ribonucleic acid synthesized by *E. coli* in the presence or absence of a single nucleoside and under conditions permitting or preventing the simultaneous synthesis of protein. The experimental conditions were similar to those described before. The incubation at 37° of the bacterial cells transferred to fresh medium containing the additions listed in Table II was permitted to proceed for 30 (Expt. 7), 60 (Expts. 13a and b), or 120 minutes (Expts. 5, 6, 8 to 12). In most experiments the quantity of ribonucleic acid formed during the treatment equaled or surpassed that initially present in the cells.

The nucleotide composition of the ribonucleic acid found in the various experiments is listed in Table II. The following conclusions emerge. (a) The ribonucleic acid of *E. coli* grown under normal conditions (Expt. 5) appears identical in composition with that found in cells exposed to chloramphenicol for 2 hours (Expt. 6), though nearly one half of the nucleic acid (44% in the particular experiment) had been synthesized after the addition of the drug. A similar observation has been reported recently[15]. We have, however, been unable to confirm the nucleotide proportions given in that publication for what would appear to be the same strain of *E. coli*. (b) The addition of adenosine, guanosine, cytidine or uridine, which, as has been shown in the preceding section, are utilized to a large extent as precursors of the nucleotides comprising the ribonucleic acid, is without perceptible effect on the composition of the ribonucleic acid thus synthesized, regardless of the conditions—simultaneous synthesis of protein or its suppression—under which the formation of new nucleic acid takes place. If a very slight shift in the nucleotide proportions is enforced by the unbalancing presence of a single nucleoside, it could be revealed only by a statistical treatment for which the available data do not suffice. (c) Assuming that the ribonucleic acid is indeed invariable, we present in Table III the average composition of the ribonucleic acid of *E. coli*, strain B, derived from all experiments summarized in Table II. The figures do not differ appreciably from those found in a previous analysis of the ribonucleic acid of another *E. coli* strain[14]. In accordance with prior observations[14], the ratio of 6-amino to 6-keto nucleotides was, in most analyses, found near unity.

General remarks

In conclusion, we shall briefly discuss the invariability, with respect both to the composition and to the utilization of precursors, of the total ribonucleic acid of the *E. coli*

References p. 554.

TABLE II

COMPOSITION OF *E. coli* RNA FORMED IN THE PRESENCE AND ABSENCE OF SIMULTANEOUS PROTEIN SYNTHESIS*

Experiment No.	Nucleoside as precursor in experiment μmole/ml				Chloramphenicol γ/ml	Moles per 100 moles nucleotide in RNA				Molar ratios**	
	Adenosine	Guanosine	Cytidine	Uridine		Adenylic acid	Guanylic acid	Cytidylic acid	Uridylic acid	Purines / Pyrimidines	6-Am / 6-K
5		—				26.8	28.9	24.6	19.7	1.26	1.06
6		—			30	25.6	28.9	25.3	20.2	1.20	1.04
7	0.15		—	—	30	26.9	29.3	24.8	19.0	1.28	1.07
8a	0.37		—	—		26.9	29.3	25.7	18.1	1.28	1.11
8b	0.37		—	—	30	26.6	28.7	26.3	18.5	1.23	1.12
9a		0.28				26.9	29.7	24.9	18.5	1.30	1.07
9b		0.28	—	—	30	26.4	31.9	24.1	17.6	1.40	1.02
10a	—	0.28	—			26.1	30.7	24.7	18.5	1.31	1.03
10b	—	0.28	—		30	25.8	30.7	25.3	18.1	1.30	1.05
11a	—	0.28	—			25.9	28.9	25.8	19.5	1.21	1.07
11b	—	0.28	—	—	30	26.2	28.5	25.2	20.1	1.21	1.06
12a	—	—	0.33	—		25.4	27.6	26.1	20.9	1.13	1.06
12b	—	—	0.33	—	30	25.4	28.4	26.2	20.0	1.16	1.07
13a		—		0.16	—	26.7	29.4	24.5	19.4	1.28	1.05
13b		—		0.16	30	25.7	29.0	24.9	19.5	1.25	1.02

* All experiments were performed with *E. coli*, Strain B. See the text for general experimental conditions. In Experiments 8 to 13, portions of the same culture were treated with the nucleoside alone (a) or with the nucleoside and chloramphenicol (b).

** The first column reports the ratio of the molar sum of purine nucleotides (adenylic and guanylic acids) to that of the pyrimidine nucleotides (cytidylic and uridylic acids); the second column indicates the ratio of the molar sum of 6-amino nucleotides (adenylic and cytidylic acids), abbreviated 6-Am, to that of the 6-keto nucleotides (guanylic and uridylic acids), abbreviated 6-K.

TABLE III

E. coli RIBONUCLEIC ACID: MEAN NUCLEOTIDE PROPORTIONS AND MOLAR RELATIONSHIPS*

Moles per 100 moles nucleotide		Molar ratio	
Adenylic acid	26.3 (2)	Pu/Py	1.27 (5)
Guanylic acid	29.6 (4)	6-Am/6-K	1.06 (2)
Cytidylic acid	25.1 (3)	(A + U)/G + C)	0.83 (4)
Uridylic acid	19.0 (5)		

* The values are based on 21 analyses performed on 15 preparations listed in Table II as Experiments 5 to 13. The coefficients of variation are given in parentheses. Abbreviations: A, G, C, U stand for adenylic, guanylic, cytidylic, uridylic acids. Pu = A + G; Py = C + U. 6-Am = A + C; 6-K = G + U.

cell observed under our experimental conditions; an invariability that appears to apply also to many other cellular systems[2,3,14,16]. It will be understood that the criterion applied by us here, *viz.*, the absence of observable changes in the pattern of the distribution of polymer-bound ribonucleotides, is crude and obtuse: it will fail to reveal very small changes in the entire polymer or important modifications of a small fraction; it will not demonstrate alterations of the nucleotide sequence that are not accompanied by changes in the nucleotide proportions; nor will it permit the recognition of changes in the physical properties of the ribonucleic acids or in their conjugation with proteins. We hope, in this connection, to discuss some aspects of stability in a later publication.

References p. 554.

Nevertheless, it can be said that the total polymer of ribonucleic acid made by *E. coli* in the course of its normal growth does not appear to differ in composition or in anabolic history, as regards the utilization of precursors, from that produced in the absence of protein synthesis, even when large amounts of a single nucleoside that can be utilized efficiently are supplied. If the formation of normal ribonucleic acid were a corollary of protein synthesis, it could have been conceivable that, when the latter is prevented, phosphorylases or similar enzymes, though normally acting only as scavengers in the direction of the degradation of the polymer, might come into play; so that through an oversupply of one precursor it would have been possible to produce unusual polymers and thus to drive the nucleotide ratios in one direction or another. This does not seem to be the case.

One gains the impression that the prevention of the formation of new protein does not remove the newly forming ribonucleic acid of the cell from the controls that ensure its inviolate reproduction. The search for the nature of these controls, presumably indispensable if the cell is to maintain itself in a state of specificity, has not been attended by much success; it has become a field criss-crossed with short cuts that lead nowhere. We are faced with a paradox: on the one hand, there must exist a level of strict integration of all macromolecular cell constituents that together define the specificity of the cell; on the other hand, there appear to exist levels of autonomy permitting the independent replication of these molecules. How these contradictions can be reconciled, we do not know.

SUMMARY

This paper discusses the invariability of the total ribonucleic acid of *E. coli* (Strain B and a lysine auxotroph), both with respect to the proportions of constituent nucleotides and to the anabolic history of the polymer, synthesized in the presence or absence of a single nucleoside acting as precursor and with or without simultaneous protein synthesis. Experiments on the identification of the sugar component are included.

REFERENCES

[1] M. DEMEREC AND E. CAHN, *J. Bacteriol.*, 65 (1953) 27.
[2] D. ELSON, T. GUSTAFSON AND E. CHARGAFF, *J. Biol. Chem.*, 209 (1954) 285.
[3] D. ELSON, L W. TRENT AND E. CHARGAFF, *Biochim. Biophys. Acta*, 17 (1955) 362.
[4] B. MAGASANIK, E. VISCHER, R. DONIGER, D. ELSON AND E. CHARGAFF, *J. Biol. Chem.*, 186 (1950) 37.
[5] G. R. WYATT, *Biochem. J.*, 48 (1951) 584.
[6] R. MARKHAM AND J. D. SMITH, *Biochem. J.*, 52 (1952) 552.
[7] E. CHARGAFF, E. VISCHER, R. DONIGER, C. GREEN AND F. MISANI, *J. Biol. Chem.*, 177 (1949) 405.
[8] S. ZAMENHOF, G. LEIDY, P. L. FITZGERALD, H. E. ALEXANDER AND E. CHARGAFF, *J. Biol. Chem.* 203 (1953) 695.
[9] M. A. JERMYN AND F. A. ISHERWOOD, *Biochem. J.*, 44 (1949) 402.
[10] E. F. GALE AND J. P. FOLKES, *Biochem. J.*, 53 (1953) 493.
[11] C. L. WISSEMAN, J. E. SMADEL, F. E. HAHN AND H. E. HOPPS, *J. Bacteriol.*, 67 (1954) 662.
[12] I. A. ROSE AND B. S. SCHWEIGERT, *J. Biol. Chem.*, 202 (1953) 635.
[13] E. T. BOLTON AND A. M. REYNARD, *Biochim. Biophys. Acta*, 13 (1954) 381.
[14] D. ELSON AND E. CHARGAFF, *Biochim. Biophys. Acta*, 17 (1955) 367.
[15] A. B. PARDEE AND L. S. PRESTIDGE, *J. Bacteriol.*, 71 (1956) 677.
[16] A. LOMBARD AND E. CHARGAFF, *Biochim. Biophys. Acta*, 20 (1956) 585.

Received April 26th, 1957