say that the quantity $AL/\Delta t$ is proportional to C_r. This quantity is given by

$$A_{\mathrm{I}}L/\Delta t = (KRT/6t_f)(\Delta t/T)^2 \quad (\text{for } \Delta t < LT), \tag{10a}$$

$$A_{\mathrm{II}}L/\Delta t = (KRT/6t_f)[3L(\Delta t/T) - 3L^2 + L^3(T/\Delta t)] \quad (\text{for } \Delta t > LT). \tag{10b}$$

These equations are plotted in Figure 2, with L as the abscissa. It is evident that for Case I, C_r is independent of L and varies as the square of Δt, while for the extreme Case II, or large $\Delta t/T$, C_r becomes nearly proportional to L. These relationships are the same as those proposed by Commoner *et al.* from a more qualitative analysis of the model.

In the previous paper, Commoner *et al.* demonstrate that the relationship among the values of A, L, and Δt determined by experiments with TMV is in reasonably good agreement with equations (8) and (10) and leads to the calculation of the value of T for the growth of the TMV rod, 15 min. It is to be expected that better data will require refinements such as changes in the description of the increase of the pool activity P and perhaps the introduction of a growth rate which is a function of rod length. However, it is difficult to see how these possible changes could invalidate the main conclusion, i.e., TMV particles grow linearly with one process taking about 15–20 min per 3,000 Å rod, and all other processes taking much less time.

The model under discussion is unusual in that it relates isotope data descriptive of a chemical process, biosynthesis, to the geometry of the particle being synthesized and in particular to its length. If it proves possible to obtain corresponding geometrical data for other chemical and biochemical processes, the foregoing mathematical treatment may be applicable to them.

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[2] Lucas, Z. J., unpublished experiments.

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*SYNTHETIC POLYNUCLEOTIDES AND THE AMINO ACID CODE, VII**

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Previous papers of this series[1] have dealt with the effect of uracil-containing synthetic polyribonucleotides on amino acid incorporation in a cell-free system of *Escherichia coli*. The present paper deals mainly with the effect of polynucleotides which do not contain uracil. It was found that poly A stimulates the incorporation of lysine, and of no other amino acid, by the *E. coli* system. Poly-L-lysine appears to be the product of this reaction. This observation made it possible to use

adenine-rich copolymers in studies with cell-free systems and led to the assignment of additional, non-U code triplets to several amino acids.

While studying the effect of synthetic polynucleotides on amino acid incorporation by a cell-free rat liver system, it was observed that poly A consistently produced a small stimulation of the incorporation of lysine into products insoluble in trichloroacetic acid. As no such effect had previously been noted with *E. coli* preparations, this matter was reinvestigated. Polylysine is soluble in trichloroacetic acid[2] and it was possible that poly A-promoted synthesis of this polypeptide might have escaped detection in earlier experiments with use of trichloroacetic acid as the protein precipitating agent. Since polylysine is insoluble in tungstic acid,[2] the effect of poly A on the incorporation of lysine in the *E. coli* system was therefore studied with use of a mixture of trichloroacetic and tungstic acids as the precipitating reagent. Under these conditions poly A consistently promoted a marked incorporation of C^{14}-lysine into trichloroacetic-tungstic acid insoluble material.

With the tungstic acid technique non-U copolymers, such as poly AG and poly AC, stimulated the incorporation of several amino acids by the *E. coli* system. On the basis of these experiments, additional (non-U) code triplets have been assigned to the amino acids arginine (2A1G), asparagine (2A1C), glutamic acid (2A1G), glutamine (1A2G, 2A1C), glycine (1A2G), histidine (1A2C), lysine (AAA), proline (1A2C), and threonine (2A1C). These results suggest extensive degeneracy of the amino acid code. Recently Bretscher and Grunberg-Manago,[3] using the trichloroacetic acid procedure, reported stimulation of the incorporation of glutamine, histidine, proline, and threonine, by A and C containing copolymers. Stimulation of the incorporation of several amino acids by non-U copolymers, with use of conventional techniques, was also recently reported by Nirenberg.[4]

Preparations and Methods.—Polynucleotides: Prior to the preparation of the polynucleotides used in this work, the ribonucleoside 5′-diphosphates were purified by ion-exchange chromatography.[5] The polyribonucleotides were prepared with *Azotobacter* polynucleotide phosphorylase as previously described.[6] Their sedimentation coefficients are listed in Table 1. We are indebted to J. Y. Chen for these determinations. Base composition of the copolymers used in this and in previous work was determined as described by Smith and Markham.[7] As seen from Table 2 the

TABLE 1

SEDIMENTATION COEFFICIENTS OF SYNTHETIC POLYNUCLEOTIDES

Polynucleotide	$S_{20,w}$
Poly A	9.2
Poly U	7.6
Poly AC (5:1)	0.9
Poly AG (5:1)	4.2
Poly AU (5:1)	2.8

TABLE 2

BASE RATIOS OF SYNTHETIC POLYNUCLEOTIDES

Polynucleotide	Ratio of nucleoside diphosphates in reaction mixture	Base ratios of isolated polynucleotides
Poly UA	5:1	4.8:1
Poly UC	5:1	4.7:1
Poly UG	5:1	5.2:1
Poly AC	5:1	4.9:1
Poly AG	5:1	4.9:1
Poly AU	5:1	4.7:1
Poly UAC	6:1:1	6:1.25:0.9
Poly UAG	6:1:1	6:1.3:1
Poly UCG	6:1:1	6:0.6:1

actual molar base ratios agreed closely with the molar ratios of the ribonucleoside diphosphates used in the preparation of the polymers.

S-30 fraction from rat liver: Livers from 24-hr fasted, three-month-old Wistar rats were homogenized with 3 volumes of cold 0.05 *M* Tris-HCl buffer, pH 7.6, containing 0.25 *M* sucrose. The homogenate was centrifuged at 0° for 15 min at 15,000 *g*, and the supernatant fluid centrifuged at 30,000 *g* for 30 min at 0°. The supernatant (S-30) was lyophilized and the dry powder stored at −20°. The S-30 fraction is similar to the preparations used by Sachs[8] and by Weinstein and Schechter.[9] Microsomal protein was determined with use of the procedure of Lowry *et al.*,[10] after isolating the microsomes by centrifugation for 2 hr at 100,000 *g*. The lyophilized fraction was suspended in water, before use, in the proportion of 0.1 gm of powder/ml. To decrease "blank" incorporation in the absence of added polynucleotides, the samples were preincubated at 37° as previously described for the *E. coli* system.[1, 6] Preincubation for 8 min was optimal for maximal stimulation of phenylalanine incorporation by poly U. Composition of the samples and incubation conditions were essentially the same as in previous experiments with the *E. coli* system[1, 6] except that 4, rather than 50, mμmoles of each amino acid at specific activities 40–80 mc/mM were used in 0.30 ml of reaction mixture. The amount of polynucleotide used per sample was 100 μg in the case of poly U and 200 μg in that of all other polymers. Addition of transfer RNA increased amino acid incorporation. 100 μg of rat liver transfer RNA were added to each sample in all cases.

Tungstic acid procedure: In all experiments with the *E. coli* system described in this paper, 0.2 mg of carrier polylysine was added to each sample after incubation. This was followed by the addition of 10 ml of a solution containing 0.25% sodium tungstate and 5% trichloroacetic acid; final pH, 2.0. The mixture was centrifuged for 10 min at 10,000 *g* and the supernatant discarded. The precipitate was washed twice, each time for 15 min at 90°, with the trichloroacetic-tungstic reagent, plated on Whatman No. 1 filter paper disks, washed with absolute acetone, dried, and its radioactivity measured in a windowless gas-flow counter.

Other materials: Twice-crystallized salt-free trypsin and three-times crystallized α-chymotrypsin were obtained from the Worthington Biochemical Corporation, Freehold, New Jersey, and C^{14}-labeled amino acids of high specific radioactivity (1mc/mg) from Schwarz Bioresearch, Orangeburg, New York. Other preparations were as noted in previous papers of this series.

Results.—Polynucleotide-dependent amino acid incorporation in rat liver system: Typical results are illustrated in Table 3. They confirm the recent observations of Weinstein and Schechter[9] and Maxwell[11] in showing that the coding triplets found with this system had previously been found with the *E. coli* system. This adds further support to the view[1] that the genetic code is universal. However, a new code triplet was suggested by the rat liver experiments, namely AAA for lysine. As mentioned in the introduction AAA is a lysine code triplet also in *E. coli*.

Effect of poly A on lysine incorporation in E. coli system: Table 4 shows that, with use of the tungstic acid procedure, a poly A-promoted incorporation of lysine

TABLE 3

AMINO ACID INCORPORATION IN RAT LIVER SYSTEM WITH VARIOUS POLYNUCLEOTIDES

		Polynucleotide*							
Amino acid	None	A	U	UA (5:1)	UC (5:1)	UG (5:1)	Found†	Calculated‡	Code triplet
Phenylalanine	27	33	778	245	370	346	100	...	UUU
Isoleucine	26	...	...	56	...	...	14	20	2U1A
Lysine	15	55	...	15	...	...	...	...	AAA
Serine	13	...	...	...	59	17	14	20	2U1C
Tyrosine	55	...	...	101	...	...	21	20	2U1A
Valine	26	...	...	...	28	134	34	20	2U1G

* Amino acid incorporation given in μμmoles/mg ribosomal protein.

† Amino acid incorporation given as per cent of the phenylalanine incorporation (blanks without polynucleotide subtracted).

‡ Per cent frequency relative to UUU of triplet most closely matching per cent amino acid incorporation relative to phenylalanine.

TABLE 4

LYSINE INCORPORATION IN *E. coli* SYSTEM AS A FUNCTION OF THE CONCENTRATION OF POLY A

Poly A (μg/ml)	Lysine incorporation*
0	0.04
8	0.43
16	0.97
24	1.03
40	1.12
80	1.06
160	1.12
320	0.75
600	0.64
1,200	0.45

* mμmoles/mg ribosomal protein. Incubation, 60 min at 37°.

was readily detected. Maximal incorporation was obtained with 40 μg of poly A/ml. Incorporation declined at concentrations of poly A above 160 μg/ml. As shown in a subsequent table (Table 8), of 20 amino acids tested individually with poly A, only the incorporation of lysine was stimulated by this polymer.

Table 5 shows that the poly A-dependent lysine incorporation was further dependent on the presence of transfer RNA and the ATP-generating system but did not require the presence of the other amino acids. This suggests that a lysine homopolypeptide is formed under these conditions. The table also shows that the incorporation of lysine was inhibited by puromycin and by chloramphenicol. As in the case of the poly U-dependent incorporation of phenylalanine, which is inhibited by poly A[6], the poly A-dependent incorporation of lysine was inhibited by poly U (Table 5, experiment 2). This is undoubtedly due to loss of the coding ability of the homopolymers through formation of the poly A + U helical complex.

The time course of phenylalanine and lysine incorporation, in the presence of poly U and poly A respectively, is shown in Table 6. While phenylalanine incorporation stopped after 30 min, the incorporation of lysine was still in progress between 45 and 60 min. This difference may be mainly due to the greater susceptibility of poly U to ribosomal ribonuclease and nucleases in the supernatant. It may be noted that the incorporation of lysine and that of phenylalanine are of the same order of magnitude. Unfortunately, the extent of amino acid incorporation in the present experiments was much less than in previous work, for the poly U-promoted stimulation of phenylalanine incorporation, in the presence of

TABLE 5

CONDITIONS FOR LYSINE INCORPORATION IN *E. coli* SYSTEM

Experiment No.	System	Lysine incorporation*
1	Complete†	1.21
	No poly A	0.02
	No transfer RNA	0.21
	No GTP, no ATP, no ATP generating system	0.03
	Complete + 19 cold amino acids	1.03
	Complete + puromycin (720 μg/ml)	0.29
	Complete + chloramphenicol (160 μg/ml)	0.18
2	Complete‡	1.13
	Complete + poly U (320 μg/ml)	0.08
	No poly A, poly U (80 μg/ml)	0.03

* mμmoles/mg ribosomal protein. Incubation, 60 min at 37°.
† 40 μg of poly A/ml.
‡ 160 μg of poly A/ml.

TABLE 6

TIME COURSE OF POLY U-DEPENDENT PHENYLALANINE INCORPORATION AND POLY A-DEPENDENT LYSINE INCORPORATION IN *E. coli* SYSTEM*

Time (min)	Phenylalanine incorporation†	Lysine incorporation‡
0	0.01	0.04
5	1.48	0.45
10	2.57	0.92
15	2.72	1.10
30	3.50	1.57
45	3.48	2.00
60	3.22	2.21

* mμmoles/mg ribosomal protein.
† Poly U, 80 μg/ml.
‡ Poly A, 40 μg/ml.

TABLE 7

EFFECT OF PROTEOLYTIC ENZYMES ON PRODUCT OF C^{14}-LYSINE INCORPORATION*

Conditions	C^{14}-lysine in insoluble form†
120 min incubation	1.62
120 min incubation, no poly A	0.03
60 min incubation followed by 60 min incubation with trypsin (200 μg/ml)	0.05
60 min incubation followed by 60 min incubation with chymotrypsin (200 μg/ml)	1.49

* Incubation at 37°. After incubation, samples worked up by addition of trichloroacetic-tungstic acid reagent with carrier protein (*E. coli* supernatant) and polylysine.
† mμmoles/mg ribosomal protein.

saturating amounts of transfer RNA, may reach values of 30 mμmoles/mg ribosomal protein in 60 min at 37°. This low activity appears to have been due to the presence of inhibitory substances in the batch of *E. coli* transfer RNA available at the time the experiments reported in this paper were carried out.

Nature of lysine incorporation product: Since the product formed from lysine in the presence of poly A is soluble in water and aqueous solvents, it was readily identified as a polypeptide with use of proteolytic enzymes. As shown in Table 7, incubation with trypsin resulted in the almost complete disappearance of the tungstic acid-insoluble radioactive material formed by the *E. coli* system on incubation with C^{14}-lysine in the presence of poly A. This was not the case following incubation with chymotrypsin. Since polylysine is susceptible to trypsin but very resistant to chymotrypsin,[12] these results suggest that poly A directs the synthesis of poly-L-lysine in cell-free systems of protein synthesis.

Additional code triplet assignments: Table 8 lists the effects of poly A, poly AU (5:1), poly AG (5:1), and poly AC (5:1) on the incorporation of each of 20 amino acids with use of the tungstic acid procedure. Stimulation is noted by underlined amino acid incorporation values.

The basis for the code triplet assignments listed in Table 9 is the same as in previous work,[1] except that with the A-rich copolymers used in the present experiments the stimulation by a polymer of the incorporation of a given amino acid relative to that of lysine was matched to the calculated frequency of a given triplet relative to that of the AAA triplet in this polymer. Some of the matched values, e.g., those on which the assignment of additional code triplets to asparagine (2A1C), glutamic acid (2A1G), glutamine (1A2G), glycine (1A2G), proline (1A2C), and threonine (2A1C) is based, agree rather closely, others, e.g., arginine (2A1G), glutamine (2A1C), histidine (1A2C), do not agree as well. The additional code

TABLE 8

AMINO ACID INCORPORATION IN *E. coli* SYSTEM WITH VARIOUS POLYNUCLEOTIDES*

		Polynucleotide			
Amino acid†	None	A	AU (5:1)	AG (5:1)	AC (5:1)
Alanine (7.5)	29	28	22	23	27
Arginine (5.0)	20	22	19	70	21
Asparagine (1.32)	11	10	142	11	306
Aspartic acid (5.0)	9	9	13	9	14
Cysteine (7.0)	33	28	37	37	40
Glutamic acid (7.25)	19	18	16	130	22
Glutamine (2.05)	25	24	24	46	460
Glycine (7.8)	5	4	3	25	6
Histidine (5.0)	100	106	97	91	190
Isoleucine (5.5)	3	4	100	3	3
Leucine (6.0)	4	5	20	6	7
Lysine (2.0)	21	1,200	495	390	1,014
Methionine (4.75)	49	52	57	41	46
Phenylalanine (6.8)	28	12	14	13	11
Proline (3.2)	76	74	73	72	130
Serine (4.0)	17	18	14	19	16
Threonine (5.0)	10	12	13	13	240
Tryptophan (3.74)	148	128	149	125	149
Tyrosine (6.5)	11	10	26	10	9
Valine (5.0)	6	5	5	7	7

* $\mu\mu$moles/mg ribosomal protein.
† Values in parentheses give specific radioactivity in μc/μmole.

TABLE 9

ADDITIONAL CODE TRIPLET ASSIGNMENTS*

	Polynucleotides						
	AU (5:1)		AG (5:1)		AC (5:1)		
Amino acid	Found	Calculated§	Found	Calculated	Found	Calculated	Code triplets†
Arginine	...	...	13.5	20	...	...	2A1G
Asparagine	28	20	...	...	30	20	2A1U, 2A1C
Glutamic acid	...	...	30	20	...	...	2A1G
Glutamine	...	...	5.9	4	44	20	1A2G, 2A1C
Glycine	...	...	5.4	4	...	...	1A2G
Histidine	...	...	...	...	9.1	4	1A2C
Isoleucine	20	20	...	...	...	...	2A1U
Leucine	3.4	4	...	...	...	...	1A2U
Lysine‡	100	...	100	...	100	...	AAA
Proline	...	...	...	...	5.4	4	1A2C
Threonine	...	...	...	...	23	20	2A1C
Tyrosine	3.2	4	...	...	...	...	1A2U

* The polynucleotide-stimulated incorporation of each amino acid is given as percentage of the stimulation of lysine incorporation by a given copolymer (found columns) and is compared with the percentage frequency of certain triplets to AAA triplets in that polymer (calculated columns). Agreement or proximity of the two values is the basis for the code triplet assignments in the last column of the table.
† New code triplets are underlined.
‡ Incorporation taken as 100%.
§ For the sake of simplicity, values in this column are based only on the AAA triplet for lysine and the 2A1U triplet for isoleucine. Lysine has an additional 2A1U and isoleucine an additional 1A2U triplet. If these triplets were taken into consideration the values in this column would be asparagine, 16.6; isoleucine, 20; leucine, 3.3; tyrosine, 3.3.

triplet for histidine (previously assigned triplet, 1U1A1C) could be either 2A1C (AAC, ACA, or CAA) or 1A2C (ACC, CAC, or CCA) since the percentage histidine incorporation relative to lysine (9.1%) with poly AC (5:1) lies midway between the percentage of each of the 2A1C triplets (20%) and that of each of the 1A2C triplets (4%) relative to AAA triplets in this polymer. However, since two of the three

2A1C triplets can definitely be assigned to asparagine and threonine, respectively, and the remaining one probably corresponds to glutamine (despite the high incorporation of this amino acid), a 1A2C triplet may be assigned to histidine.

It should be noted from Table 9 that the assignments of code triplets to asparagine (2A1U), leucine (1A2U), and tyrosine (1A2U) from the results of the present experiments with poly AU (5:1) coincide with those previously made[1] from experiments with poly UA (5:1) despite the inversion of base ratios. This proves the correctness of the experimental approach and of the assumptions made in the determination of code triplets. It may also be noted that an additional U-containing code triplet (2A1U) can now be assigned to isoleucine from the results with poly AU (5:1) given in Table 9. A 2U1A code triplet had already been assigned to this amino acid[1] from the results with poly UA (5:1).

Discussion and Conclusions.—The results presented in this paper add one U-containing and 10 non-U-containing triplets to the previous list of 22 or 23 U-containing code triplets[1] giving a total of 33 or 34. The results suggest extensive degeneracy of the amino acid code. This would be in agreement with the wide range of DNA composition and with genetic studies.[13] The copolymers used by us thus far contain 58 of the 64 possible triplets in RNA but it is possible that stimulation of the incorporation of some amino acids by the polymers used in the present work may have been missed because of the low frequency of occurrence of certain triplets in these polymers (cf. also reference given as footnote 1). Moreover, CG and ACG copolymers have as yet not been tested. Thus, further degeneracy of the code is not excluded. In any case, the finding of a number of non-U code triplets brings the base composition of the code closer to that of RNA and suggests that criticisms of the code based on its apparent high U content may have been premature.

It is of interest to note that all of the eight triplets occurring in AU copolymers have now been assigned as follows: AAA, lysine; 2A1U, asparagine, isoleucine, lysine; 1U2A, isoleucine, leucine, tyrosine; and UUU, phenylalanine. Thus, AU copolymers must consist of unbroken sequences of meaningful triplets and should direct the synthesis of long polypeptide chains.

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BASIS OF ACTINOMYCIN ACTION, I. DNA BINDING AND INHIBITION OF RNA-POLYMERASE SYNTHETIC REACTIONS BY ACTINOMYCIN

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Actinomycin inhibits DNA-dependent RNA synthesis of intact cells[1] and enzymes from mammalian[2] and bacterial[3] sources but not the growth of RNA-viruses in animal cells.[1, 4] The inhibition of RNA synthesis appears to account completely for the antibiotic and cytotoxic action of actinomycin and is attributable to the fact that the antibiotic is tightly bound[5, 6] to DNA.

Structural changes in the actinomycin molecule which affect its biological activity also influence, in a parallel way, its binding to DNA and its inhibition of the RNA polymerase reaction.[3] The experiments described below suggest a basis for the effect of actinomycin in that the base composition of DNA primers may influence the extent of actinomycin binding and thereby the degree of inhibition of the different reactions catalyzed by RNA-polymerase. The binding of actinomycin appears to be absolutely dependent on the presence of guanine residues in the DNA primers. Kersten[7] showed that high concentrations of deoxyguanosine and several other guanine and adenine compounds could alter the spectral properties of actinomycin solutions. Our data in this and a subsequent paper[8] show that only guanine residues in DNA are indispensable for actinomycin action.

Recently, Hurwitz *et al.* have reported on the effect of actinomycin on some aspects of bacterial RNA-polymerase activity.[9] Several of the findings of these authors appear to differ from those recorded below.

Materials and Methods.—The standard assay conditions used in the experiments and the isolation of RNA-polymerase from *E. coli* B were as described by Chamberlin and Berg,[10] and the incubations were carried out for 15′ at 37° unless otherwise noted.

Incorporation of precursors into RNA under standard conditions in the absence of DNA was less than 0.02 mμmoles/15′ for 4 NTP,† and smaller for single NTP; these control values were always subtracted from the results recorded below. All reactions contained 4.1 μg enzyme protein/0.25 ml. Synthesis of acid-insoluble polyribonucleotides was measured by the incorporation of one of the following precursors: C^{14}-ATP, obtained from Schwarz Bioresearch; UTP^{32}, ψUTP^{32}, CTP^{32}, GTP^{32}, synthesized as previously described,[11] in which the proximal phosphate was radioactive. Nucleic acid concentrations were based on the phosphorus content determined according to Fiske and Subba-Row.[12] Protein was measured according to Lowry *et al.*[13] Actinomycin concentrations were based on the absorption of a standard solution of 25 μg/ml, giving an O.D. (440 mμ) of 0.358 in 0.01 *M* Tris pH 7.4 with 1 cm light path. Absorption of pure actinomycin is appreciably greater than this value, which was established several years ago for a preparation containing inert material.