juniper woodland zone. The shrubs are more closely spaced than is usual for *Larrea,* but average only about 40 cm in height [P. V. Wells, *Ecology* **41**, 553 (1960)].

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4. A fair indication of relative annual precipitation and temperature regimes in the pinyon-juniper, *Coleogyne* and *Larrea* zones in Nevada, Utah, Arizona, and California is afforded by records of meteorological stations situated in the respective zones [*Climate and Man,* U.S. Department of Agriculture Yearbook (Government Printing Office, Washington, D.C., 1941)]. The following is a comparison of mean annual precipitation in the three zones: Pinyon-juniper zone (20 stations): range, 26.4 to 42.4 cm; mean, 33.3 cm. *Coleogyne* zone (9 stations); range, 11.4 to 32.3 cm; mean, 20.6 cm. *Larrea* zone (20 stations): range, 6.4 to 22.1 cm; mean, 12.4 cm. The differences in precipitation among zones are reinforced by differences in temperature, the pinyon-juniper zone (highest in elevation) being coolest, the *Larrea* zone (lowest in elevation) warmest, and the *Coleogyne* zone intermediate.
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Amino Acid Code in Alcaligenes faecalis

Abstract. *Amino acid incorporation into protein, promoted by synthetic polynucleotides, was studied in a cell-free extract of* Alcaligenes faecalis *(combined guanine and cytosine content of DNA, 66 percent). In the limited survey, 18 code triplets were found to specify the amino acids as in* Escherichia coli *(combined guanine and cytosine content of DNA, 52 percent). At least six of the eight possible triplets containing adenine and uracil were meaningful. No exceptions to the universality of the amino acid code were found.*

The base composition of the coding units of messenger RNA (*1*) has been studied by using polyribonucleotides of widely varying base composition as artificial messengers in a cell-free system of *Escherichia coli.* More than 40 code triplets have been assigned to the 20 amino acids (*2*), and the list is probably incomplete. These results suggest extensive degeneracy of the amino acid code. There are indications that the code is universal (*3*). These indications received some support from studies with cell-free systems derived from mammalian cells and organisms other than *E. coli* (*4, 5*). In general, polyA codes for lysine, polyU for phenylalanine, and polyUA, polyUC, and polyUG have coding units for phenylalanine, isoleucine, and tyrosine, for phenylalanine and serine, and for phenylalanine and valine, respectively, as in *E. coli.* In the case of animal tissues and yeast a more extensive survey is precluded by the low activity of the available systems, the incorporation of amino acids into protein and the effect of polynucleotides thereon being in general quite small. It was therefore deemed of interest to examine microorganisms other than *E. coli.*

Alcaligenes faecalis was chosen because its DNA differs significantly in base composition from that of *E. coli.* The polynucleotides used were polyA, polyC, and polyU as homopolymers; polyAC (5 : 1), polyAG (5 : 1), and polyAU (5 : 1) as A-rich copolymers; and polyUA (5 : 1), polyUC (5 : 1), and polyUG (5 : 1) as U-rich copolymers. Eighteen triplets of the base composition indicated could be assigned to the following 13 amino acids: arginine, 2A1C; cysteine, 2U1G; glutamic acid, 2A1G; lysine, AAA; phenylalanine, UUU; proline, CCC, 2C1A, 2C1U; serine, 2U1C; threonine, 2A1C; tyrosine, 2U1A; and valine, 2U1G. Comparison with the data on *E. coli* (*6*) shows identical assignments. Thus, as far as the data go there is agreement between *A. faecalis* and *E. coli.* About as many A- and U-containing triplets specify amino acids in *A. faecalis* (DNA, 66 percent GC) as in *E. coli* (DNA, 52 percent GC). However, the difference in GC content between the DNA's of these two organisms is hardly large enough to expect significant differences in this regard. The present survey, although more extensive than previous ones in animal tissues, was not broad enough to rule out the occurrence of coding units in *A. faecalis* differing in amino acid specificity from

Table 1. Amino acid incorporation in the *A. faecalis* system with various polynucleotides.* The amount of polynucleotide added (+) was 80 μg/ml except for polyC where the amount was 400 μg/ml, and for polyUA, polyUC, and polyUG where the amount was 160 μg/ml. The minus signs indicate no addition (control).

Amino acid	Polynucleotide																	
	A		C		U		AC (5 : 1)		AG (5 : 1)		AU (5 : 1)		UA (5 : 1)		UC (5 : 1)		UG (5 : 1)	
	−	+	−	+	−	+	−	+	−	+	−	+	−	+	−	+	−	+
Alanine													60	50				
Arginine									30	190			10	10				
Asparagine					40	50	20	365			29	153	29	118	40	30	40	30
Aspartic acid					70	80	27	639			30	153	38	168	70	50	70	60
Cysteine													70	110			140	799
Glutamic acid	64	73	36	45	11	15	20	40	20	150			40	30				
Glutamine							14	252	20	20			14	10				
Glycine					20	10							20	30			24	339
Histidine							220	240					30	10				
Isoleucine											21	156	29	639				
Leucine					80	430					74	89	83	509	57	2170	34	1083
Lysine	64	1750			20	10	52	2600	60	770	54	1190	9	49				
Methionine											42	36						
Phenylalanine					182	50,600							53	3120	118	12,630	44	4415
Proline			9	156			10	140					10	10	32	516		
Serine													20	50	49	2120		
Threonine							25	1064			40	30	20	20				
Tryptophan													230	220			139	731
Tyrosine											71	96	38	695				
Valine													28	28	42	35	15	1180

* μμmole/mg ribosomal protein. The values with copolynucleotides are averages of experiments carried out in duplicate.

corresponding coding units in *E. coli*.

Alcaligenes faecalis (*7*) was grown according to Pinchot (*8*). Ribosomes and sRNA were prepared as described for *E. coli* (*9*). The polynucleotides used and their base ratios were as previously stated (*5*). A mixture of supernatant and ribosomes (0.9 ml) containing 0.35 mg of ribosomal protein per milligram of supernatant protein was incubated for 30 minutes at 37°C with 0.1 ml of a solution (solution A) containing, per milliliter, tris-HCl buffer, *p*H 7.9, 280 μmole; $MgCl_2$, 37.4 μmole; KCl (or NH_4Cl), 390 μmole; Lubrol *w*, 12.4; mercaptoethylamine, 78 μmole; ATP, 6.2 μmole; GTP, 1.55 μmole; creatine phosphate, 83 μmole; and creatine kinase, 0.62 mg. The purpose of this preliminary incubation was to reduce the blank amino acid incorporation in the absence of added polynucleotides. For measurement of amino acid incorporation, each 0.1 ml of the above mixture was supplemented with 0.03 ml of solution A, 0.3 mg of *A. faecalis* sRNA, 50 μmole of a single 1-C^{14}-labeled amino acid (specific radioactivity, 1 to 10 μc/μmole), 50 μmole each of the remaining nonlabeled amino acids, and made up to a final volume of 0.25 ml, with or without the addition of polynucleotide as indicated in Table 1.

After incubation for 30 minutes at 37°C, the incorporation of individual amino acids into protein-like products was determined as previously described, with the precipitating reagent being either 5 percent trichloroacetic acid (*9*) in experiments with U-rich polynucleotides, 5 percent trichloroacetic acid with 0.25 percent sodium tungstate (*5*) in experiments with A-rich polymers, or 20 percent trichloroacetic acid (*6*) in experiments with polyC.

Since Lubin and Ennis (*10*) found ammonium ions superior to potassium ions in amino acid incorporation experiments with a cell-free system of rat liver, KCl and NH_4Cl were compared. The total incorporation of phenylalanine, in the presence of polyU, was twice as high with NH_4Cl as with KCl. Moreover, whereas with KCl the incorporation ceased virtually after 11 minutes, with NH_4Cl it continued for at least 30 minutes. Therefore NH_4Cl was used in place of KCl in our experiments.

Code triplet assignments were made, as previously explained (*6*, *9*), by matching the experimentally found ratios of amino acid incorporation with the calculated ratios of nucleotide triplets in the added polynucleotide. These can be calculated from the base composition because the added polynucleotides are of random sequence. It is assumed that the relative incorporation of any two amino acids will be equal to the relative abundance in which the triplets specifying these amino acids occur in the added polynucleotide. This method is based on random copolymers and allows the determination of triplet base composition but not of base sequence within the triplets.

Table 2. Calculated mean relative frequency of triplets occurring in synthetic copolynucleotides.

Relative frequency of each triplet* (%)	Base composition of triplets occurring in polynucleotides					
	AC(5 : 1)	AG(5 : 1)	AU(5 : 1)	UA(5 : 1)	UC(5 : 1)	UG(5 : 1)
100	AAA	AAA	AAA	UUU	UUU	UUU
20	2A1C	2A1G	2A1U	2U1A	2U1C	2U1G
4	2C1A	2G1A	2U1A	2A1U	2C1U	2G1U
0.8	CCC	GGG	UUU	AAA	CCC	GGG

* For a triplet of base composition 2A1C, frequency of each of the triplets AAC, ACA, and CAA.

The actual incorporation of amino acids promoted by the various polymers used in this study is shown in Table 1. The calculated mean relative frequency of the various triplets in these polymers is given in Table 2. The relative incorporation of amino acids promoted by the polymers is listed in Table 3. With the exception of threonine, whose incorporation relative to that of lysine in the presence of polyAC (5 : 1) was twice as high as expected for 20 percent frequency of 2A1C relative to AAA triplets, the relative incorporation of various amino acids agreed reasonably well with the mean relative frequency of the triplets assigned to them. These are listed in the last column of Table 3. The incorporation of glutamine with polyAC (5 : 1) was between the calculated values for 2A1C and 2C1A triplets, and that of tryptophan with polyUG (5 : 1) between those for 2U1G, and 2G1U triplets. No triplet assignments were therefore made in these two cases. Furthermore, no assignments could be made to aspartic acid and asparagine because of their ready interconversion in the *A. faecalis* system.

The extensive degeneracy of the amino acid code makes it possible for organisms with DNA of the high GC type to utilize a triplet high in GC to specify an amino acid that may be specified by a triplet of high AU content in organisms with DNA of the low GC

Table 3. Code triplet assignments.*

Amino acid	Polynucleotide						Base composition of code triplets
	AC (5 : 1)	AG (5 : 1)	AU (5 : 1)	UA (5 : 1)	UC (5 : 1)	UG (5 : 1)	
Alanine				0			
Arginine		22.5		0			2A1G
Cysteine				0		15.0	2U1G
Glutamic acid	0	18.3		0			2A1G
Glutamine	10.0	0		0			Contains A and C
Glycine				0		7.2	2G1U
Isoleucine			12.0	19.8			2A1U, 2U1A
Leucine			1.2	13.9	16.8	24.0	2U1A, 2U1C, 2U1G
Lysine	100	100	100	1.3			(AAA)
Phenylalanine				100	100	100	(UUU)
Proline	5.0			0	4.1		2C1A, 2C1U, (CCC)
Serine				0	16.5		2U1C
Threonine	41.0		0	0			2A1C
Tryptophan				0		13.5	Contains U and G
Tyrosine			2.1	21.4			2U1A
Valine				0	0	26.4	2U1G

* The values given are calculated from the data of Table 1. The net polymer-stimulated incorporation of each amino acid is given as percentage of net phenylalanine incorporation in the case of UA(5 : 1), UC(5 : 1), and UG(5 : 1) copolymers, and of net lysine incorporation in that of AC(5 : 1), AG(5 : 1), and AU(5 : 1) copolymers. For example, in the case of isoleucine with polyUA(5 : 1), from Table 1:

$$\frac{\text{Net isoleucine incorporation}}{\text{Net phenylalanine incorporation}} \times 100 = \frac{(639 - 29)}{(3120 - 53)} \times 100 = 19.8 \text{ percent.}$$

Assignment of AAA, CCC, and UUU (triplets in parentheses) is based on the stimulation of the incorporation of lysine, proline, and phenylalanine by polyA, polyC, and polyU, respectively.

type. We have shown that a bacterial species with DNA of the high GC type does nevertheless possess the means (activating enzymes and sRNA) to read coding units which do not contain G or C.

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References and Notes

1. Abbreviations: RNA, ribonucleic acid; DNA, deoxyribonucleic acid; sRNA, transfer (soluble)RNA; ATP and GTP, the 5'-triphosphate of adenosine and guanosine; the capital letters A, C, G, and U, are used for the nucleotides adenylic, cytidylic, guanylic, and uridylic acids, respectively, or their corresponding residues in polynucleotide chains. Figures in parentheses after (or under) the abbreviated polynucleotide names give the molar ratios of nucleoside diphosphates used in the preparation of the polymers with polynucleotide phosphorylase. Thus "polyUA (5 : 1)" means that the polynucleotide was prepared from a mixture of five parts of uridine diphosphate and one part of adenosine diphosphate. The converse would be true for polyAU (5 : 1). The analytically determined base ratios of the copolymers agreed closely with the input ratios of nucleoside diphosphate precursors.
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Sonic Energy Effects in Bovine Serum Albumin Solutions

Abstract. *Bovine serum solutions exposed to high-frequency sound were examined by ultracentrifugal, electrophoretic, viscometric, conductivity, light scattering, and optical rotatory dispersion procedures. Parameters determined with treated material were the same as those determined with untreated albumin solutions except for slight differences in rotation, the dispersion constant, and in weight-average molecular weight.*

In contrast to DNA and collagen, which are highly anisometric molecules (*1–4*), the globular protein bovine serum albumin is strikingly insensitive to treatment with high-frequency sound. Doty *et al.* (*2*) and Nishihara and Doty (*3*) observed scissions in DNA in 1 minute and in collagen within 10 minutes when these substances were exposed to sound from a 50-watt, 9-kc Raytheon generator. The fragmentation of DNA is a nonrandom process and the strand is split preferentially near the midpoint (*2, 4*). The fragmentation of collagen results in shorter pieces which retain the three-stranded helical structure (*3*). Kanig and Künkel (*5*) reported turbidity, flocculation, and increased viscosity in bovine serum albumin and fibrinogen solutions treated with high-frequency sound. In contrast, Tolgyessy and Kovacs (*6*) found no change in viscosity but noted an increase in the conductivity of ovalbumin solutions exposed to ultrasound waves.

Since sonic energy is used extensively to disrupt microorganisms (*7, 8*), it seemed worthwhile to inquire into the effects of the energy from high-frequency sound on globular proteins. A variety of procedures, such as ultracentrifugation, electrophoresis, viscosity, optical rotation, light scattering, and conductivity, have been used to examine sound-treated solutions of bovine serum albumin. Crystalline bovine serum albumin (Armour Pharmaceutical Co.) was dissolved in 0.1*M* KCl; the solution was placed directly in the steel cup of a 50-watt, 9-kc Raytheon magnetostriction generator and exposed to high-frequency sound without excluding oxygen for 120 minutes. The power output of the sonic oscillator was checked according to the procedure recommended by the manufacturer. Viscosity measurements were carried out at 20.0° ± 0.1°C (*9*). Protein concentrations were determined spectrophotometrically or by the Lowry procedure (*10*). In experiments carried out in a Model E Spinco centrifuge, control and treated samples were run simultaneously with wedge-window and normal cells under essentially identical conditions. The weight-average sedimentation coefficient $\bar{S}$ (*11*) was calculated from the rate of movement of the square root of the second moment of the entire gradient curve according to the method of Goldberg (*11*). Synthetic boundary experiments were carried out in a 12-mm cell with a 2° sector modified according to the method of Kegeles (*12*). Experimental procedures used in light scattering have been considered previously (*13*). A Rudolph automatic recording spectropolarimeter was used for optical-dispersion measurements.

Table 1. Comparison of bovine serum albumin exposed to 9-kc sound waves for 120 minutes with untreated bovine serum albumin. The results are averages of two to six experiments.

Physical parameters	Control	Treated
Intrinsic viscosity $[\eta]$ deciliters/gram	0.039	0.039
$(Kc/R_{90})_0$* $\times 10^5$	1.48	0.91
Weight-average molecular weight, M_w	68,000	110,000
Specific rotation, $[\alpha]_D$	—59.5	—56.0
Dispersion constant λ_c (mμ)	264	266
Mobility, $u \times 10^5$ cm^2 volt^{-1} sec^{-1}	—6.6	—6.6
$S^0_{20,w}$ from the second moment of the gradient curve	4.8	4.9
Refractive increment, $\frac{dn}{dc}$, in H_2O at 436 mμ; c in g/ml	0.1940	0.1940
Extinction coefficient $E^1\%_{208}$ deciliters/gram	6.45	6.45

* The limiting value of the scattering.

The results show (Table 1), despite the extensive exposure of the protein to sonic energy, a similarity in parameters determined from the treated and the nontreated samples. In electrophoretic patterns of sound-treated bovine serum albumin we observed a barely detectable shoulder moving more slowly than the main component. In synthetic-boundary studies, the differences in areas under the gradient curves obtained at 3000 rev/min and at 59,780 rev/min, after correction for radial dilution and the stretch of the rotor, reflect the removal from the boundary