It therefore appears that the expression of certain viral functions is dependent upon the physiological state of the cell at the time of infection. That the effect of a tumor virus on its host cell depends upon the operation of certain cellular functions has been previously shown by Temin[11] and Bader[12] for the RNA-containing Rous tumor virus.

Summary.—A combined study by radioautography and immunofluorescence showed that the induction of cellular DNA synthesis in polyoma-infected cultures of mouse kidney cells occurs in productively infected cells.

The competent technical assistance of Mr. Paul Holdy and Mrs. Mildred Barnes is gratefully acknowledged.

* This work was supported by grant no. CA 07592 from the National Cancer Institute, National Institutes of Health.

† Postdoctoral fellow of the U.S. Public Health Service, # 1 F2-AI-30, 286-01 from the National Institute of Allergy and Infectious Diseases.

[1] Dulbecco, R., L. H. Hartwell, and M. Vogt, these PROCEEDINGS, **53**, 403 (1965).
[2] Weil, R., M. R. Michel, and G. K. Ruschmann, these PROCEEDINGS, **53**, 1468 (1965).
[3] Winocour, E., A. M. Kaye, and V. Stollar, *Virology*, **27**, 156 (1965).
[4] Hartwell, L. H., M. Vogt, and R. Dulbecco, *Virology*, **27**, 262 (1965).
[5] Sheinin, R., *Virology*, **28**, 47 (1966).
[6] Hatanaka, M., and R. Dulbecco, unpublished.
[7] Sheinin, R., and P. A. Quinn, *Virology*, **26**, 73 (1965).
[8] Weisberg, R. A., *Virology*, **23**, 553 (1964).
[9] Gershon, D., P. Hausen, L. Sachs, and E. Winocour, these PROCEEDINGS, **54**, 1584 (1965).
[10] Sauer, G., H. Fischer, and K. Munk, in preparation.
[11] Temin, H., *Virology*, **20**, 577 (1963).
[12] Bader, J. P., *Virology*, **22**, 462 (1964).

TEMPLATE ACTIVITY OF URIDYLIC ACID-DIHYDROURIDYLIC ACID COPOLYMERS

BY FRITZ ROTTMAN* AND PETER CERUTTI†

NATIONAL HEART INSTITUTE AND NATIONAL INSTITUTE OF ARTHRITIS AND METABOLIC DISEASES, NATIONAL INSTITUTES OF HEALTH, BETHESDA, MARYLAND

Communicated by Seymour S. Kety, February 21, 1966

Dihydrouridylic acid (H_2Up) has been described as a minor constituent of alanine-sRNA[1] and serine-sRNA[2] isolated from yeast. An RNA fraction obtained from pea bud nucleohistones also contains dihydrouridylic acid, in this instance, representing 27 per cent of the total nucleotide composition.[3] Earlier work has demonstrated the enzymatic incorporation of dihydrouridine-5′-monophosphate into RNA[4] and more recently the DNA-dependent incorporation of dihydrouridine-5′-triphosphate into RNA.[5]

Due to its shortened π-electron system, H_2Up occupies a unique position within the minor nucleotides of natural RNA. Since this may well be reflected in its functional role, studies of the coding properties of polymers containing H_2Up are of special interest. The discovery of a convenient photochemical method of preparing partially reduced poly U (poly U/H_2U) of high molecular weight[6] has made these

studies possible. (Such polymers cannot be synthesized with polynucleotide phosphorylase, since dihydrouridine-5'-diphosphate is not a substrate for this enzyme.[7, 8])

Polymers containing various ratios of uridylic acid and dihydrouridylic acid have been tested for template activity in both the amino acid incorporation system and the binding of aminoacyl-sRNA to ribosomes. Our results show that dihydrouridine cannot substitute for A, G, C, or U in U-containing codons, and apparently is not recognized in these assays for template activity.

Materials and Methods.—Preparation and characterization of Poly U/H_2U: Poly U (Miles Chemical Co.) was photoreduced ($NaBH_4/h\nu$) at pH 9.5 and 50° for varying intervals of time in a nitrogen atmosphere. A mercury low-pressure lamp (Hanovia, type 87A-45) was used as the light source. The light was filtered with a 1-cm layer of 0.1 *N* acetic acid. Immediately after the irradiation was terminated, the solutions were adjusted to pH 4 with dilute hydrochloric acid resulting in complete destruction of excessive $NaBH_4$. Desalting was achieved by passage through Sephadex G-25 (elution with 10^{-3} *M* tris-acetate, pH 7.15), and the solutions were concentrated by lyophilization. The preparations were further purified by precipitation with 0.3 vol 95% ethanol (−20°) in the presence of 2 *M* KCl and washed three times with 80% ethanol in the cold. The precipitates were dissolved in deionized water and dialyzed for 3 hr against water (0°). The preparations were characterized by measuring the residual absorbance at 262 mμ (pH 2, ϵ = 9800), the amount of hydrolyzable ribose with the Orcinol assay (which is positive only for the reduced nucleotides), the ureido groups present (Archibald assay) after acidic cleavage of the glycosidic bond followed by alkaline opening of the dihydrouracil ring, and by determination of the total polymer phosphate (Table 1). In all experiments a poly U preparation which was exposed to $NaBH_4$ under exclusion of light and carried through the same purification steps was used as a standard. Correction factors for the Orcinol and Archibald assays were determined from such preparations and from untreated poly U. Whereas photochemical water addition and dimerization of the Up residues seem to be negligible under the outlined photoreductive conditions, minor contamination with a product of further reduction is found, especially in preparations containing more than 30% H_2Up. The average chain length of the polymers was estimated by measuring the ratio of total organic phosphate to phosphate released by *E. coli* alkaline phosphomonoesterase.[9, 10] An average chain length of 120–150 was found for untreated as well as for poly U/H_2U (23:1) and poly U/H_2U (10:1) (cf. ref. 11).

Samples of poly U/H_2U were digested with RNase and the products separated by paper chromatography. Examination of the chromatograms with UV-light and the Fink reaction revealed only two products corresponding to uridylic and dihydrouridylic acid.

Components of reactions: E. coli B sRNA, obtained from General Biochemicals Co., was acylated with C^{14}-phenylalanine (282 μc/μmole, Nuclear-Chicago) in the presence of 19 C^{12}-amino acids as described previously.[12] C^{14}-phenylalanine-sRNA contained 22.1 μμmoles of C^{14}-phenylalanine per A_{260} unit. The remaining AA-sRNA's have been described previously.[13–15] Crude supernatant fraction (S-30) and ribosomes were prepared from *E. coli* W3100 by modifications of published methods.[16] Polymers and oligonucleotides used as controls are described elsewhere.[13, 14, 17]

Incorporation of C^{14}-amino acids into protein[16] and binding of C^{14}- or H^3-aminoacyl-sRNA to ribosomes[18] was assayed by described methods with the components and conditions of each reaction given in the legends to figures and tables.

TABLE 1

CHARACTERIZATION OF POLY U/H_2U PREPARATIONS

Base ratio U:H_2U	Total P, μmoles/ml	Residual Up,* μmoles/ml	Hydrolyzable ribose,† μmoles/ml	Ureido groups,† μmoles/ml
Poly U	9.35	9.30	—	—
23:1	15.81	14.97	0.74	0.66
10:1	10.98	9.87	1.05	0.99
5.9:1	11.78	9.80	1.75	1.67
2.8:1	7.50	5.30	1.95	1.87

* Calculated from the absorbance at 262 mμ (pH 2, ϵ = 9800).
† A correction is made for the Orcinol and Archibald reaction exhibited by unreduced poly U.

TABLE 2

EFFECT OF PARTIALLY REDUCED POLY U ON C^{14}-PHENYLALANINE INCORPORATION

Polymer	$\mu\mu$Moles C^{14}-phenylalanine incorporated into protein
None	1.5
Poly U	37.2
Poly U/H_2U (23:1)	15.7
Poly U/H_2U (10:1)	6.7
Poly U/H_2U (5.9:1)	2.0
Poly U/H_2U (2.8:1)	1.0

Each reaction mixture contained the following components in a final volume of 50 μl: 0.08 *M* tris-acetate, pH 7.8; 0.01 *M* magnesium acetate; 0.04 *M* potassium acetate; 6×10^{-3} *M* mercaptoethanol; 5×10^{-3} *M* ATP; 1.5×10^{-2} *M* potassium phosphoenolpyruvate; 2 μg of crystalline phosphoenolpyruvate kinase; 1×10^{-4} *M* C^{14}-phenylalanine, 9.8 μc/μmole; 2×10^{-4} *M* each of 19 C^{12}-amino acids minus phenylalanine; 8 μg of polynucleotide when specified; and 0.35 mg *E. coli* W3100 preincubated S-30 protein. Reaction mixtures were incubated at 37° for 20 min followed by the addition of 2 ml of 10% TCA and heating at 90–92° for 20 min. Protein precipitation, washing, and counting of nitrocellulose filters were performed by previously described methods.[16]

Results.—Amino acid incorporation into protein: The activity of poly U/H_2U as a template for directing amino acid incorporation into protein was tested with C^{14}-phenylalanine. Poly U/H_2U (23:1) stimulated C^{14}-phenylalanine incorporation into protein but its template activity was approximately 40 per cent that of poly U (Table 2). Copolymers containing a larger percentage of H_2U were even less effective as templates, and poly U/H_2U (2.8:1) was completely inactive. The poly U included in these experiments was exposed to $NaBH_4$ in the absence of light and carried through the same purification procedure as described in *Methods*.

The possibility that poly U/H_2U could direct the incorporation of amino acids other than phenylalanine into protein was examined with seven different amino acids. Only the incorporation of amino acids with two Up residues in their respective codons[15] was investigated. Such a selection will include all combinations

TABLE 3

EFFECT OF POLY U/H_2U UPON AMINO ACID INCORPORATION

C^{14}-amino acid	Polymer	$\mu\mu$Moles C^{14}-amino acid incorporated into protein
Phe	None	4.3
	Poly U/H_2U (23:1)	33.0
	Poly U/H_2U (2.8:1)	4.9
	Poly U	62.5
Val	None	0.4
	Poly U/H_2U (23:1)	0.0
	Poly U/H_2U (2.8:1)	−0.7
	Poly U	0.7
	Poly UG (4:1)	111.0
Cys	None	0.5
	Poly U/H_2U (23:1)	1.6
	Poly U/H_2U (2.8:1)	0.9
	Poly U	3.2
	Poly UG (4:1)	31.8
Tyr	None	6.4
	Poly U/H_2U (23:1)	8.0
	Poly U/H_2U (2.8:1)	8.0
	Poly U	10.6
	Poly UCAG (4:1:1:1)	53.5
Ser	None	27.8
	Poly U/H_2U (23:1)	27.4
	Poly U/H_2U (2.8:1)	22.5
	Poly U	28.3
	Poly UC (2:1)	73.5
Leu	None	15.2
	Poly U/H_2U (23:1)	15.6
	Poly U/H_2U (2.8:1)	11.3
	Poly U	17.9
	Poly UG (4:1)	98.5
Ileu	None	6.2
	Poly U/H_2U (23:1)	1.1
	Poly U/H_2U (2.8:1)	−0.9
	Poly U	5.3
	Poly UCAG (4:1:1:1)	42.1

Reaction mixture components in a final volume of 50 μl were the same as those described in the legend of Table 2 except for the substitution of C^{14}-amino acid (1×10^{-4} *M*, approx. 25 μc/μmole), 19 C^{12}-amino acids (2×10^{-4} *M*), and polynucleotide (8 μg) as shown above. Assays were performed as described in Table 2.

of two U's and a third nucleoside and thus provide a test for the possible recognition of H_2Up by AA-sRNA as either A, G, C, or U.

Table 3 summarizes the results obtained with Phe, Ser, Leu, Ileu, Tyr, Cys, and Val using two different poly U/H_2U preparations. In each case, poly U which had been exposed to $NaBH_4$ in the absence of light is included since small ambiguities are observed with poly U preparations. Except for phenylalanine, we find no stimulation of amino acid incorporation by poly U/H_2U above background levels which cannot be attributed to residual sequences of U. In fact, the incorporation of C^{14}- or H^3-amino acids into protein is generally lower in the presence of poly U/H_2U than without it. Polynucleotides containing appropriate codons for each amino acid were included as controls to demonstrate the activity of the system for every amino acid tested. Poly U had little effect upon the incorporation of amino acids other than phenylalanine; however, slight ambiguous responses of Cys, Tyr, and Leu were observed.

Binding of aminoacyl-sRNA to ribosomes: The binding of specific aminoacyl-sRNA to ribosomes in response to added polynucleotide provides a sensitive method for determining the specificity of codon recognition prior to peptide bond synthesis.

The effect of H_2U concentration in poly U/H_2U polymers on phenylalanine-sRNA binding is shown in Figure 1. In contrast to amino acid incorporation, poly U/H_2U (23:1) is as active as poly U, even at limiting polynucleotide concentrations. Only when the proportion of H_2U in the polymer is increased does one begin to observe an inhibition of phenylalanine-sRNA binding. Poly U/H_2U (2.8:1), which was completely inactive as a template in phenylalanine incorporation into protein (Table 2), still directs the binding of significant amounts of phenylalanine-sRNA to ribosomes.

Poly U/H_2U was tested for its ability to direct the binding of various AA-sRNA's to ribosomes (Table 4). As in the case of amino acid corporation, no stimulation of AA-sRNA binding other than phenylalanine was observed with poly U/H_2U, and generally one sees a marked depression of background binding. The ambiguous recognition of poly U by Ileu- and Ser-sRNA is even decreased with the introduction of H_2U, presumably due to a loss of uridine residues available for sRNA recognition.

The theoretical frequencies of the four possible trinucleotide codewords in poly U/H_2U are presented in Table 5 for three different polynucleotide copolymers. The frequency calculations assume a random distribution of U and H_2U in the copolymers which has not yet been proved. The relative incorporation of C^{14}-Phe into protein and the relative binding of C^{14}-Phe-sRNA to ribosomes are both compared to poly U. The stimulation of

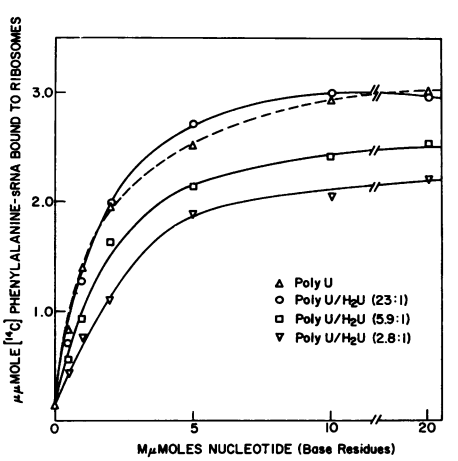

FIG. 1.—Each 50-μl reaction mixture contained: 0.05 *M* tris-acetate, pH 7.2; 0.05 *M* potassium acetate; 0.015 *M* magnesium acetate; 1.1 A_{260} units of ribosomes; 0.155 A_{260} units of AA-sRNA containing 3.90 μμmoles of C^{14}-phenylalanine; and polynucleotide as shown. Reaction mixtures were incubated for 15 min at 24°. The assay for ribosomal-bound C^{14}-AA-sRNA has been described.[18]

C^{14}-Phe-sRNA binding is given for both limiting and saturating concentrations of polynucleotide. In all cases, the poly U/H_2U directed incorporation of C^{14}-Phe is much more sensitive to the amount of H_2U in the copolymer than is the ribosomal binding of C^{14}-Phe-sRNA.

Discussion.—Grossman *et al.*[19] have demonstrated that the UV-induced water addition product of uracil is recognized as cytosine, and copolymers of U and the water addition product direct the incorporation of serine into protein. Our results imply

TABLE 4

BINDING OF C^{14}- OR H^3-AA-sRNA TO RIBOSOMES WITH PARTIALLY REDUCED POLY U

AA-sRNA	Oligonucleotide or polynucleotide control	μμMoles aminoacyl-sRNA bound to ribosomes	AA-sRNA	Oligonucleotide or polynucleotide control	μμMoles aminoacyl-sRNA bound to ribosomes
C^{14}Arg	None	0.39	C^{14}Leu	None	0.70
	Poly U	0.34		Poly U	0.66
	Poly U/H_2U (2.8:1)	0.28		Poly U/H_2U (2.8:1)	0.68
	Poly CG (8:1)	0.84		Poly UC (2:1)	1.01
C^{14}Asp	None	0.13	C^{14}-Lys	None	0.52
	Poly U	0.06		Poly U	0.44
	Poly U/H_2U (2.8:1)	0.04		Poly U/H_2U (2.8:1)	0.47
	GpApC	1.24		pApApA	3.75
H^3Cys	None	0.40	C^{14}Ser	None	0.77
	Poly U	0.31		Poly U	1.09
	Poly U/H_2U (2.8:1)	0.25		Poly U/H_2U (2.8:1)	0.94
	Poly UG (4:1)	1.44		Poly UC (2:1)	4.17
C^{14}Glu	None	0.09	C^{14}Thr	None	0.31
	Poly U	0.06		Poly U	0.18
	Poly U/H_2U (2.8:1)	0.05		Poly U/H_2U (2.8:1)	0.14
	Poly AG (4:1)	0.52		Poly AC (1:1)	3.61
C^{14}His	None	0.72	C^{14}Tyr	None	0.19
	Poly U	0.45		Poly U	0.09
	Poly U/H_2U (2.8:1)	0.42		Poly U/H_2U (2.8:1)	0.05
	Poly AC (1:1)	2.78		UpApU	0.41
C^{14}Ileu	None	0.10	C^{14}Val	None	0.20
	Poly U	0.97		Poly U	0.19
	Poly U/H_2U (2.8:1)	0.67		Poly U/H_2U (2.8:1)	0.13
	ApUpU	0.50		Poly UG (4:1)	3.63

Reactions contained the components described in the legend of Fig. 1. Each 50-μl reaction mixture contained 8 μg of polynucleotide or 5 μg of oligonucleotide when specified. Details concerning the C^{14}- and H^3-AA-sRNA preparations and the amount used in each assay have been previously described.[13-15]

TABLE 5

COMPARISON BETWEEN CODEWORD FREQUENCIES AND TEMPLATE ACTIVITY OF POLY U/H_2U

	Theoretical Code Word Frequencies		
	Poly U/H_2U (23:1)	Poly U/H_2U (5.9:1)	Poly U/H_2U (2.8:1)
U U U	88.5	63.5	40.5
U U H_2U	3.7	10.4	14.2
U H_2U H_2U	0.15	1.7	5.0
H_2U H_2U H_2U	0.06	0.27	1.75

C^{14}-Phenylalanine Incorporated into Protein (Relative to Poly U)		C^{14}-Phenylalanine-sRNA Bound to Ribosomes (Relative to Poly U)	1.5 mμMoles polynucleotide	10 mμMoles polynucleotide
Poly U	100%	Poly U	100%	100%
Poly U/H_2U (23:1)	40%	Poly U/H_2U (23:1)	100%	100%
Poly U/H_2U (5.9:1)	1.4%	Poly U/H_2U (5.9:1)	75%	82%
Poly U/H_2U (2.8:1)	0%	Poly U/H_2U (2.8:1)	53%	71%

The frequency calculations are based upon the assumption of a random distribution of U and H_2U within the copolymer.[17] The incorporation of C^{14}-Phe in the presence of poly U/H_2U copolymers is expressed relative to poly U and obtained from the data in Table 2. The binding of C^{14}-Phe-AA-sRNA in the presence of poly U/H_2U is also expressed relative to poly U. The data in Fig. 1 was used to calculate values at limiting (1.5 mμmoles) and saturating (10 mμmoles) polynucleotide concentrations.

that this pyrimidine transition is not merely a consequence of the saturation of the 5,6-double bond, but presumably involves other effects related to the introduction of a hydroxyl group at position 6 of the pyrimidine ring.

The ability of H_2U to function as either A, G, C, or U in any position of a codon, i.e., 5′-terminal, internal, or 3′-terminal, has been tested in these studies and has not been detected. Furthermore, if H_2U could not actively pair with a complementary base but could act as a "spacer," thus permitting a partial recognition of two out of three bases in a codon, one would expect to see incorporation or binding of some amino acid in addition to phenylalanine. It should be pointed out, however, that the latter possibility might still exist with other copolymers, e.g., poly A/H_2U, and these polymers are being examined for such activity.

The results are consistent with recent studies measuring the interaction of poly U/H_2U with poly A.[20] Small amounts of H_2U in poly U are sufficient to cause a significant decrease in the observed *Tm*. It has been postulated that H_2U is unable to form a base pair with A and may additionally weaken the interaction normally occurring with adjacent bases.

The sharp decrease in C^{14}-phenylalanine incorporation into protein in the presence of copolymers containing H_2U is not accompanied by a corresponding decrease in C^{14}-Phe-sRNA binding to ribosomes directed by the same polymers. Furthermore, the amount of C^{14}-Phe-sRNA bound to ribosomes in the presence of poly U/H_2U exceeds the calculated frequency of UUU occurring in these polymers. These results suggest that Phe-sRNA can recognize additional nucleotide sequences in poly U/H_2U. H_2U may be permitted in specific positions within the trinucleotide codon, e.g., the 3′-terminal nucleotide, and still function as a phenylalanine codeword. It is also possible that under certain conditions a dinucleotide may be sufficient for recognition, and a sequence of two adjacent U residues may permit Phe-sRNA binding.

The noticeable decrease of amino acid incorporation into protein in the presence of poly U/H_2U could be due to unrecognized nucleotide sequences within the polymer, such as a trinucleotide containing H_2U in the wrong position or a sequence of H_2U residues, both resulting in interrupted peptide bond formation. Michelson and Grunberg-Manago[21] have examined the template properties of copolymers containing either N^1-methyluridylic or N^6-methyladenylic acid and observed an inhibition of amino acid incorporation. They suggested that the reading mechanism is unable to "jump" nonsense sequences.

The distribution of H_2U in naturally occurring RNA is perhaps more widespread than anticipated, and only when pure RNA fractions are subjected to analytical examination beyond conventional UV absorption can H_2U be detected. Our results indicate that the presence of only small amounts of H_2U in RNA can modify its properties as a template for protein synthesis. Thus H_2U could conceivably eliminate certain RNA fractions as potential templates for protein synthesis or possess some unique function related to its lack of recognition as a conventional nucleotide.

The authors wish to thank Dr. Marshall Nirenberg, in whose laboratory this work was performed, for many valuable discussions throughout the course of this investigation, and Mr. William Landis for excellent technical assistance.

Abbreviations: H_2Up, dihydrouridylic acid; poly U/H_2U, copolymers of uridylic and dihydrouridylic acid; A, adenosine; G, guanosine; C, cytidine; U, uridine; AA-sRNA, aminoacyl-

sRNA; Phe, phenylalanine; Ser, serine; Leu, leucine; Ileu, isoleucine; Tyr, tyrosine; Cys, cysteine; Val, valine; Arg, arginine; Asp, aspartic acid; Glu, glutamic acid; His, histidine; Lys, lysine; Thr, threonine.

* One of the authors (F.R.) was supported by grant PF-244 from the American Cancer Society. Present address: Department of Biochemistry, Michigan State University, East Lansing, Michigan.

† Present address: Department of Chemistry, Princeton University, Princeton, New Jersey.

[1] Madison, J. T., and R. W. Holley, *Biochem. Biophys. Res. Commun.*, **18**, 153 (1965).

[2] Zachau, G. F., D. Dütting, and H. Feldmann, *Angew. Chem.*, **22**, 1043 (1965).

[3] Huang, R. C., and J. Bonner, these PROCEEDINGS, **54**, 960 (1965).

[4] Carr, D. O., and S. Grisolia, *J. Biol. Chem.*, **239**, 160 (1964).

[5] Roy-Burman, P., S. Roy-Burman, and D. W. Visser, *Biochem. Biophys. Res. Commun.*, **20**, 291 (1965).

[6] Cerutti, P., K. Ikeda, and B. Witkop, *J. Am. Chem. Soc.*, **87**, 2505 (1965).

[7] Ochoa, S., and L. Heppel, in *The Chemical Basis of Heredity*, ed. W. McElroy and B. Glass (Baltimore: John Hopkins Press, 1957), p. 631.

[8] Szer, W., and D. Shugar, *Acta Biochim. Polon.*, **8**, 235 (1961).

[9] Horiuchi, S., *Japan J. Med. Sci. Biol.*, **12**, 429 (1959).

[10] Torriani, A., *Biochim. Biophys. Acta*, **38**, 460 (1960).

[11] Jones, O. W., E. E. Townsend, H. A. Sober, and L. A. Heppel, *Biochemistry*, **3**, 238 (1964).

[12] Nirenberg, M. W., J. H. Matthaei, and O. W. Jones, these PROCEEDINGS, **48**, 104 (1962).

[13] Trupin, J., F. Rottman, R. Brimacombe, P. Leder, M. Bernfield, and M. Nirenberg, these PROCEEDINGS, **53**, 807 (1965).

[14] Nirenberg, M., P. Leder, M. Bernfield, R. Brimacombe, J. Trupin, F. Rottman, and C. O'Neal, these PROCEEDINGS, **53**, 1161 (1965).

[15] Brimacombe, R., J. Trupin, M. Nirenberg, P. Leder, M. Bernfield, and T. Jaouni, these PROCEEDINGS, **54**, 954 (1965).

[16] Nirenberg, M. W., in *Methods in Enzymology*, ed. S. P. Colowick and N. O. Kaplan (New York: Academic Press, 1964), vol. 6, p. 17.

[17] Nirenberg, M. W., O. W. Jones, P. Leder, B. F. C. Clark, W. S. Sly, and S. Pestka, in *Synthesis and Structure of Macromolecules*, Cold Spring Harbor Symposia on Quantitative Biology, vol. 28 (1963), p. 549.

[18] Nirenberg, M., and P. Leder, *Science*, **145**, 1399 (1964).

[19] Grossman, L., J. Ono, and R. G. Wilson, *Federation Proc.*, **24**, S-80 (1965).

[20] Cerutti, P., H. T. Miles, and J. Frazier, *Biochem. Biophys. Res. Commun.*, in press.

[21] Michelson, A. M., and M. Grunberg-Manago, *Biochim. Biophys. Acta*, **91**, 92 (1964).

---

*THE MOLECULAR BASIS FOR THE GENETIC CODE**

BY C. R. WOESE, D. H. DUGRE, W. C. SAXINGER, AND S. A. DUGRE

DEPARTMENT OF MICROBIOLOGY, AND DEPARTMENT OF CHEMISTRY AND CHEMICAL ENGINEERING, UNIVERSITY OF ILLINOIS, URBANA

Communicated by S. Spiegelman, January 17, 1966

The central problem of the genetic code is to identify the factors giving rise to the observed codon assignments. In particular, the key issue is whether or not amino acid-oligonucleotide steric interactions play or have played a role in determining these assignments, and if so, to what extent.[1] Although in the beginning there were no pertinent facts bearing on this matter, there initially was certainly no dearth of theoretical speculation. Gamow and others assumed amino acid-nucleic acid steric interactions to be the sole factors determining codon assignments.[2]