[4] Burton, K., and G. B. Petersen, *Biochem. J.*, **75,** 18 (1960).
[5] Chen, P. S., T. Y. Toribara, and H. Warner, *Anal. Chem.*, **28,** 1756 (1956).
[6] Wasserman, E., and L. Levine, *J. Immunol.*, **87,** 290 (1961).
[7] Lehman, I. R., and E. A. Pratt, *J. Biol. Chem.*, **235,** 3254 (1960).
[8] Stollar, D., and L. Grossman, *J. Molec. Biol.*, **4,** 31 (1962).
[9] Tamm, C., M. E. Hodes, and E. Chargaff, *J. Biol. Chem.*, **195,** 49 (1952).
[10] Spencer, J. A., and E. Chargaff, *Biochim. et Biophys. Acta*, **51,** 209 (1961).
[11] Kabat, E. A., *J. Immunol.*, **84,** 82 (1960).
[12] Townsend, E., W. T. Murakami, and H. Van Vunakis, *Federation Proc.*, **20,** No. 1, 438 (1961).
[13] Burton, K., *Biochem. J.*, **77,** 547 (1960).
[14] Kabat, E. A., in *Chemistry and Biology of Mucopolysaccharides*, Ciba Foundation Symposium, ed. G. E. W. Wolstenholme and M. O'Connor (London: J. A. Churchill, 1958).
[15] Staub, A. M., in *Transactions of the Fifth Conference on Polysaccharides in Biology*, June 1–3, 1959, ed. G. F. Springer (Princeton, N. J.: Josiah Macy, Jr., Foundation, 1960).
[16] McCarty, M., *J. Exptl. Med.*, **104,** 629 (1956).
[17] Cebra, J. J., *J. Immunol.*, **86,** 205 (1961).
[18] Rebers, P. A., E. Hurwitz, and M. Heidelberger, *J. Bacteriol.*, **82,** 920 (1961).

---

*THE BIOSYNTHESIS OF RNA: PRIMING BY POLYRIBONUCLEOTIDES**

BY TOKUMASA NAKAMOTO AND SAMUEL B. WEISS

ARGONNE CANCER RESEARCH HOSPITAL† AND DEPARTMENT OF BIOCHEMISTRY, UNIVERSITY OF CHICAGO

Communicated by Charles Huggins, March 26, 1962

Two distinctly different enzymes have been described as being involved in polyribonucleotide formation, polynucleotide phosphorylase[1] and RNA polymerase.[2–6] The former enzyme catalyzes the synthesis of RNA[7] in the presence of nucleoside diphosphates but in the absence of any known directing primer.[8] A similar reaction is catalyzed by RNA polymerase, but this enzyme requires the presence of all four nucleoside triphosphates as well as a DNA primer which directs the assembly of complementary RNA chains.[9, 10] Reports from a number of different laboratories have suggested that perhaps a third reaction, one which uses RNA as a primer for the synthesis of RNA, may also be present in living cells.[11–15]

In the course of testing various primers with somewhat crude preparations of RNA polymerase isolated from extracts of *Micrococcus lysodeikticus*, we observed that these preparations catalyzed the incorporation of labeled ribonucleotides into RNA if either DNA or plant viral RNA was present. Optimum labeling of RNA required the presence of all four triphosphates with either primer. While incorporation in the presence of viral RNA was relatively low (approximately 20% of the incorporation achieved with DNA) this phenomenon was consistently observed. However, as the *M. lysodeikticus* extracts were purified with respect to the DNA-dependent reaction (over 400 fold), the RNA-dependent incorporation was markedly reduced. This suggested that separate enzymes might be responsible for the priming of polyribonucleotide synthesis by DNA and RNA. Experiments with synthetic polynucleotides of known composition have provided a clear demonstration of the RNA-dependent synthesis of RNA in extracts from *M. lysodeikticus*, and are reported in this communication.

Methods and Materials.—Ribonucleoside triphosphates, labeled with P^{32} in the ester phosphate only, were prepared as previously described[3] and in addition by the method of Tener.[16] Turnip yellow mosaic virus RNA (TYMV) free from protein was supplied by Dr. R. Haselkorn. Calf thymus DNA was purchased from the Sigma Chemical Company, 3500 DeKalb Street, St. Louis 18, Missouri. Polynucleotide phosphorylase, purified from *M. lysodeikticus* by the procedure described by Steiner and Beers,[17] and poly A, poly C, and poly U were generously contributed by Dr. S. Yachnin, to whom we are deeply grateful. Polyribonucleotides prepared with this enzyme were phenol treated,[18] precipitated with ethanol, dissolved in 0.05 *M* NaCl–0.005 *M* sodium citrate, and exhaustively dialyzed first against 0.05 *M* NaCl and then against water. Poly CA was prepared from a mixture of CDP and ADP in molar ratio 4:1, poly CU from a mixture of CDP and UDP in molar ratio of 4:1, poly CAU from a mixture of CDP, ADP, and UDP in molar ratio of 4:1:1 and poly AU from a mixture of ADP and UDP in a molar ratio of 2:1. The base composition of the mixed polyribonucleotides described above was determined by alkaline hydrolysis and paper electrophoresis as reported elsewhere.[19] Nearest neighbor studies were performed as previously described.[20]

The enzyme was prepared from extracts of lysozyme-treated *M. lysodeikticus*. The lysates were exposed to sonic oscillation for 5 min, and centrifuged for 90 min at 105,000 × *g*. After centrifugation the clear supernatant was treated with protamine sulfate, and the precipitate extracted once with 0.05 *M* phosphate of pH 7.5, and then again with 0.20 *M* phosphate of the same pH. The second phosphate extract was adsorbed onto calcium phosphate gel and the gel was extracted once with 0.20 *M* phosphate, pH 7.5. This preparation had an average protein concentration of 4–5 mg per ml and a ratio of absorbency of about 1.35 at 280 to 260 mμ.

Routine assays for labeled nucleotide incorporation into RNA were performed by adding cold 50% TCA to the reaction vessels after incubation to give a final concentration of 10%. The vessels were iced for 3 min, 2 ml of cold 5% TCA was added, and the precipitate was collected by filtration through a millipore filter. The precipitate was washed three times with 5 ml portions of cold 5% TCA. The millipore filters were dried in planchets, and the radioactivity of the acid-insoluble residue determined with a windowless gas flow counter.

TABLE 1

THE NUCLEOTIDE REQUIREMENT FOR RNA LABELING WITH TYMV-RNA AS PRIMER

Experiment number	Labeled substrate	Nucleotides added	Labeled substrate incorporated into RNA, mμmoles
1	CTP^{32}	Complete	1.48
		Omit GTP	0.07
		Omit UTP	0.51
		Omit ATP	0.52
		Omit ATP, UTP, GTP	0.08
2	ATP^{32}	Complete	1.80
		Omit GTP	0.14
		Omit UTP	0.57
		Omit CTP	0.80
		Omit CTP, UTP, GTP	0.15

The complete system contained 2 μmoles of $MnCl_2$, 30 μmoles of Tris-PO_4 buffer (1:1) of pH 7.5, 0.10 μmole each of ATP, UTP, CTP, and GTP, 50 μgrams of TYMV-RNA, and 0.04 ml of the enzyme preparation containing 6 mg of protein per ml. 0.10 μmole of CTP^{32} (1.24×10^6 cpm per μmole) and 0.10 μmole of ATP^{32} (1.40×10^6 cpm per μmole) were substituted for unlabeled CTP and ATP where indicated. The final volume of the system was 0.50 ml and the vessels were incubated for 30 min at 30°.

Experimental.—The enzyme preparation described above, when incubated with all four ribonucleoside triphosphates in the presence of TYMV-RNA, catalyzes the incorporation of labeled ribonucleotides into RNA (Table 1). Optimum incorporation requires the addition of a full complement of the triphosphates.

With TYMV-RNA as primer, omission of any one ribonucleotide resulted in a significant decrease in label incorporation, although the system appeared to be more sensitive to the deletion of GTP than to any of the other three ribonucleotides (Table 1). This phenomenon was consistently observed with TYMV-RNA as

primer but not with DNA. A number of explanations was considered for the apparent "essentiality" of GTP in this system, but none was satisfactory. For this reason, a series of experiments was conducted using synthetic polyribonucleotides and the observations made above were confirmed and extended.

When various homopolymers of ribonucleotides were incubated with the microbial preparations, it was found that poly C caused a rapid and remarkably high incorporation of P^{32}-GMP into the acid-insoluble fraction (Table 2). This incorporation was independent of ATP, UTP, and CTP, and only GTP^{32} resulted in label incorporation. This activity could not be attributed to polynucleotide phosphorylase, since the reaction system contained a relatively large concentration of inorganic phosphate which would have inhibited polymerization by this enzyme. Furthermore, the addition of GDP, 8 times in excess of GTP^{32}, resulted in no decrease of label incorporated. The apparent priming by poly C for poly G synthesis was quite vigorous. Indeed, net quantities of poly G have been recently prepared. In view of these results, we were surprised to find that neither poly U nor poly A alone could prime the incorporation of ATP^{32} or UTP^{32} respectively. This suggested that cytidine residues in RNA primers were important for the polymerization of ribonucleotides by the RNA-dependent enzyme. To test this

TABLE 2

THE PRIMING ACTION OF POLY C FOR GTP^{32} INCORPORATION

Experiment number	Labeled substrate	Primer	Additions	Labeled substrate incorporated into RNA, mμmoles
1*	GTP^{32}	Poly C	None	37.0
			ATP, UTP, CTP	27.0
			None (heated enzyme)	0.2
2†	GTP^{32}	Poly C	None	13.8
			GDP (2.0 μmoles)	15.1
	CTP^{32}		None	<0.1
	ATP^{32}		None	<0.1
		Poly U	None	0.2
	UTP^{32}	Poly A	None	<0.1

* Each vessel contained 1 μmole of $MnCl_2$, 30 μmoles of Tris:PO_4 buffer (1:1) of pH 7.5, 0.20 μmole of GTP^{32} (1.9×10^6 cpm per μmole), 10 μgrams of poly C, and 0.4 mg of the enzyme preparation. Where indicated, 0.4 μmole each of unlabeled ATP, UTP, and CTP were added and enzyme heated at 100° for 5 min was used as the heated enzyme. The final volume of the system was 0.25 ml and the vessels were incubated for 15 min at 37°.

† The reaction mixture and the conditions of incubation were the same as above except that each vessel contained 0.20 mg of enzyme preparation. Where indicated 0.08 μmole of CTP^{32} (1.6×10^6 cpm per μmole), 0.04 μmole of ATP^{32} (4.0×10^6 cpm per μmole), and 0.10 μmole of UTP^{32} (4.7×10^6 cpm per μmole) replaced GTP^{32}, and 10 μgrams of poly U or poly A replaced poly C.

TABLE 3

THE PRIMING BY POLYNUCLEOTIDES OF LABELED NUCLEOTIDE INCORPORATION

Experiment number	Polynucleotide	Labeled substrate	Additions	Labeled nucleotide incorporated into RNA, mμmoles
1	Poly C	GTP^{32}	None	26.60
	Poly CA		UTP	4.50
			None	0.59
	Poly CU		ATP	7.83
			None	0.80
	Poly CAU		ATP, UTP	2.96
			ATP	0.47
			UTP	0.71
			None	0.33

The reaction mixture and conditions of incubation were the same as indicated in Table 2 except that 20 μgrams each of poly C, poly CA, poly CU, and poly CAU were used where indicated.

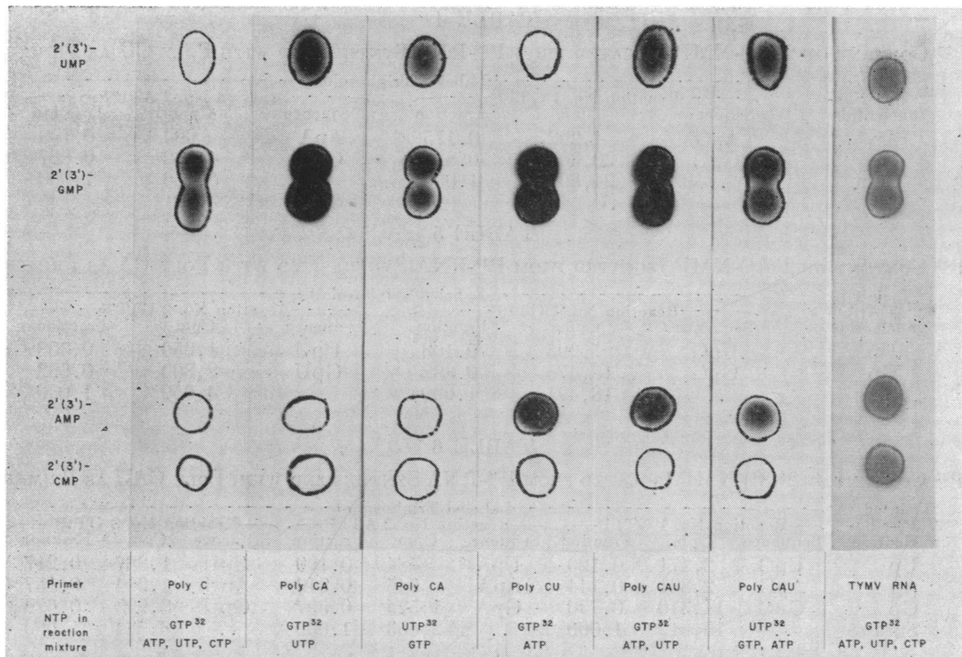

FIG. 1.—Paper electrophoresis of P^{32}-RNA alkaline digest products prepared enzymatically with different polyribonucleotide primers. The primers, labeled substrates and nucleotides added for each reaction are listed below the individual electropherogram. The encircled areas represent ultraviolet absorbing regions. Since carrier yeast RNA was added to the P^{32}-RNA product during isolation, the four 2'(3')-nucleoside monophosphates are found in each experiment. The exposed dark areas indicate the presence of P^{32}.

hypothesis, a number of synthetic polymers containing cytidine and other ribotides were prepared.

Table 3 shows that when various cytidine copolymers were tested as primers for RNA synthesis, each of the copolymers primed the incorporation of labeled GTP. In addition, optimum incorporation was dependent on the presence of a complete complement of the ribonucleoside triphosphates complementary to those bases present in the synthetic copolymers. Similar results were obtained with UTP^{32} and ATP^{32}. Base analysis of the copolymers revealed that the content of cytidine reflected the high ratio of CDP to ADP and/or UDP used during preparation of the synthetic polyribonucleotides (see *Methods*). This would suggest that large regions of the copolymers consisted of poly C sequences. In the face of this, the data shown in Table 3 are remarkable in that the omission of one of the complementary ribonucleotides from the reaction mixture for a given copolymer results in an impressive decrease in label incorporated.

When the RNA's prepared in the presence of the different cytidine-containing polymers were isolated and subjected to alkaline hydrolysis and paper electrophoresis, only the 2',3'-nucleoside monophosphates of the bases complementary to the copolymers employed were labeled. Figure 1 is a composite of various electropherograms of the alkaline digest products of the RNA's prepared with the various polyribonucleotide primers. The results suggest that each synthetic polymer primes the polymerization of a complementary strand of RNA in a fashion similar

TABLE 4

P^{32} CONTENT OF 2′(3′)-NMP ISOLATED FROM P^{32}-RNA SYNTHESIZED WITH POLY CU AS PRIMER

Isolated 2′(3′)-ribonucleotide	Labeled Triphosphate: Reaction No. 1 GTP^{32}			Reaction No. 2 ATP^{32}		
	Sequence	Cpm	Fraction	Sequence	Cpm	Fraction
Ap	ApG	4,292	0.175	ApA	994	0.193
Gp	GpG	20,305	0.825	GpA	4,162	0.807
Sum	..	24,597	1.000	..	5,156	1.000

TABLE 5

P^{32} CONTENT OF 2′(3′)-NMP ISOLATED FROM P^{32}-RNA SYNTHESIZED WITH POLY CA AS PRIMER

Isolated 2′(3′)-ribonucleotide	Labeled Triphosphate: Reaction No. 1 GTP^{32}			Reaction No. 2 UTP^{32}		
	Sequence	Cpm	Fraction	Sequence	Cpm	Fraction
Up	UpG	1,995	0.108	UpU	1,250	0.308
Gp	GpG	16,476	0.892	GpU	2,809	0.692
Sum	..	18,471	1.000	..	4,059	1.000

TABLE 6

P^{32} CONTENT OF 2′(3′)-NMP ISOLATED FROM P^{32}-RNA SYNTHESIZED WITH POLY CAU AS PRIMER

Isolated 2′(3′)-ribonucleotide	Labeled Triphosphate: Reaction No. 1 GTP^{32}			Reaction No. 2 ATP^{32}			Reaction No. 3 UTP^{32}		
	Sequence	Cpm	Fraction	Sequence	Cpm	Fraction	Sequence	Cpm	Fraction
Up	UpG	2,471	0.126	UpA	484	0.190	UpU	1,387	0.217
Ap	ApG	2,831	0.144	ApA	545	0.213	ApU	1,004	0.157
Gp	GpG	14,310	0.730	GpA	1,524	0.597	GpU	3,993	0.626
Sum	..	19,612	1.000	..	2,553	1.000	..	6,384	1.000

to the DNA-dependent reactions described previously.[10] Data which are consistent with and in support of this idea were obtained by determining the base composition of the newly formed polyribonucleotides by the method described earlier by Josse and co-workers.[21]

Tables 4, 5, and 6 present the P^{32} content in the various 2′,3′-nucleotides isolated after alkaline digestion and electrophoresis of the enzymatically synthesized RNA's. From these data, one may calculate the composition of each polyribonucleotide synthesized, independent of the spectrophotometric analysis used for primer base composition determinations. The total amount of any one nucleotide incorporated as a 5′-nucleotide must be equal to the total amount of the same nucleotide isolated as the 2′,3′-isomer after alkaline digestion. For poly CU, the equation for guanine would be: (ApG + GpG) = (GpG + GpA). Substituting the appropriate values given in Table 4, a ratio of G:A is obtained as follows:

$$0.175 \text{ g} + 0.825 \text{ g} = 0.825 \text{ g} + 0.807 \text{ a}$$
$$\text{g/a} = 4.6$$

One can also assume that $g + a = 1$.

Similarly, the base composition may be derived for the RNA's synthesized in the presence of poly CA and poly CAU. In the latter case, the equation involves three unknowns but may be readily solved since four equations are available.

Table 7 shows that the molar proportions of the complementary bases in the primer and product synthesized are nearly identical. Hence, poly C, poly CA, poly CU, and poly CAU prime the synthesis of poly G, poly GU, poly GA, and poly GUA, respectively, where the molar proportions of GMP, UMP, and AMP incorporated are determined by the molar proportions of cytidylate, adenylate, and uridylate in the primer used.

TABLE 7

COMPARISON OF BASE COMPOSITION DETERMINED FOR PRIMER AND RNA PRODUCT

	Mole, per cent, composition							
Base	Primer poly C	Synthesized RNA	Primer poly CA	Synthesized RNA	Primer poly CU	Synthesized RNA	Primer* poly CAU	Synthesized RNA
C	100	..	86.1	..	82.7	..	70.3	..
A	..	..	13.9	..	..	17.8	13.5	13.1
U	..	..	..	13.5	17.3	..	16.2	14.7
G	..	100	..	86.5	..	82.2	..	73.2

* The determination of base composition by alkaline hydrolysis and isolation of the ribonucleoside monophosphates only, as was reported here, may result in significant error if the terminal residue is a base whose molar proportions in the chain are relatively small (e.g., A or U in poly CAU) since this residue will be lost as the nucleoside and the data have not been corrected for this loss.

TABLE 8

THE PRIMING OF NUCLEOTIDE INCORPORATION BY AMP- AND UMP-CONTAINING POLYMERS

Primers	Labeled substrate	Additions	Labeled substrate incorporated into RNA, mμmoles
Poly U	C^{14}-ATP	None	0.15
Poly A	UTP^{32}	None	0.09
Poly AU		ATP	1.35
		None	4.20
Poly A + Poly U		ATP	2.46
		None	2.24
	C^{14}-ATP	UTP	0.74
		None	1.73

The reaction mixture and conditions of incubation was the same as indicated in Table 2 except that 30 μgrams each of Poly U, Poly A, and Poly AU were used as primers and 0.20 μmole of C^{14}-ATP (9.4×10^5 cpm/μmole) was used where indicated.

At present, no clear separation of the DNA- and RNA-dependent reactions has been achieved. In both *M. lysodeikticus* and *E. coli* extracts, all fractions containing RNA polymerase activity (including our 400-fold purified preparations), also catalyze RNA labeling with poly C or TYMV-RNA as primers. Other evidence suggests, however, that separate enzymes are involved since (*a*) the ratio of the two activities alters markedly as the extracts are purified with respect to the DNA primed reaction, (*b*) temperature stability studies indicate that the rate of enzyme inactivation is not the same for the polydeoxy and polyribonucleotide primers, and (*c*) poly C at high concentrations does not interfere with the DNA primed synthesis of RNA. Furth and co-workers[9] have reported that *E. coli* RNA polymerase can be primed by deoxy poly T. One would expect that if one enzyme were involved, poly U should also prime. In our hands, no labeled RNA is formed when *E. coli* extracts are incubated with ATP^{32} and poly U alone. The question of one or several enzymes cannot be further resolved at this time.

The importance of cytidine as a constituent of the polyribonucleotide primer is emphasized by the inability of poly A and poly U alone to prime RNA synthesis by these extracts. However, Table 8 shows that both the AU copolymer and the 1:1 poly A + poly U complex prime the incorporation of labeled ATP or UTP. Analysis of the products formed in both cases (data not presented here) indicates that poly AU primes the synthesis of poly AU when both ATP and UTP are included in the reaction mixture, and that the poly A + poly U complex primes the synthesis of poly A and/or poly U. It is important to note that in both cases the incorporation of labeled substrates is not strictly dependent on a full complement of triphosphates as is the case with the cytidine copolymers. In addition, calculation of the base composition for the poly AU primed product by the isotope method described

earlier shows no relationship to the molar proportions of AMP and UMP in poly AU itself.

Discussion.—We feel that there must be some common denominator which relates the various experiments reported here. If we assume that separate enzymes are involved for the different RNA primers, then we must await purification and a study of the different mechanisms involved. If we assume that the same enzyme which utilizes the poly C-containing primers is also active with poly AU and the poly A + poly U complex, then one possible explanation for the results obtained may be the degree of order which these polymers exhibit.

In solution, at alkaline pH and at temperatures above 8°, poly A and poly U exhibit little or no ordered fine structures:[22] poly A plus poly U, on the other hand, form a highly ordered structure.[23] Evidence exists which shows that AU copolymers have a reasonable degree of order and it has been postulated that there are intramolecular regions within the copolymer chain which are helical.[22] Two types of ordered structure may exist in the AU copolymers: (*a*) the type represented by the poly A + poly U complex and (*b*) complementary chains of complex A-U sequences. Under these circumstances the relationship between primer and product composition might well depend upon the nucleotides present in the reaction mixture, as well as their concentrations. If we assume that only the ordered regions prime, then we can readily understand why the base composition of the poly AU primer bears no relationship to the composition of the product formed.

If ordered structure is important for priming, why then should poly C and the cytidine-containing copolymers function so well in this capacity? In a manner which we do not understand at present, it may be possible for poly C and other cytidine copolymers to establish a degree of order (e.g., perhaps by interaction with the enzyme), which the homopolymers poly A, poly U, and poly I cannot produce alone. These experiments demonstrate not only the synthesis of RNA from polyribonucleotide primers but also that cytidine holds a central position for the type of priming observed. It is obvious that this phenomenon requires further understanding which our present data do not afford. Although the explanation presented here for the data obtained must be regarded as speculative, it provides a basis for further experimentation.

Summary.—An enzyme system, in extracts from *M. lysodeikticus*, has been described which utilizes natural and synthetic polyribonucleotides to prime the synthesis of RNA. The composition of the primer determines the composition of the product formed. The presence or absence of cytidine residues in the RNA primer appears to play a central role in the type of priming observed.

The authors wish to acknowledge the many helpful discussions with Drs. E. P. Geiduschek and R. Haselkorn in preparation of this manuscript.

* This investigation was supported by funds from the Argonne Cancer Research Hospital and from the Helen and Joseph Regenstein Foundation.

† Operated by The University of Chicago for the U.S. Atomic Energy Commission.

[1] Grunberg-Manago, M., and S. Ochoa, *J. Am. Chem. Soc.*, **77**, 3165 (1955).

[2] Weiss, S. B., and L. Gladstone, *J. Am. Chem. Soc.*, **81**, 4118 (1959).

[3] Weiss, S. B., these PROCEEDINGS, **46**, 1020 (1960).

[4] Hurwitz, J., A. Bresler, and R. Diringer, *Biochem. Biophys., Research Commun.*, **3**, 15 (1960).

[5] Stevens, A., *ibid.*, **3**, 92 (1960).

[6] Weiss, S. B., and T. Nakamoto, *J. Biol. Chem.*, **236**, PC18 (1961).

[7] The following abbreviations are used: RNA, ribonucleic acid; DNA, deoxynucleic acid; CTP, ATP, UTP, and GTP for the tri- and CDP, ADP, UDP, and GDP for the di- and CMP, AMP, UMP, and GMP for the monophosphates of cytidine, adenosine, uridine, and guanosine; the capital letters C, A, U, G, T, and I for the above nucleotides including thymine and inosine or their corresponding residues in polynucleotide chains; Tris, tris (hydroxymethyl) aminomethane and TCA, trichloroacetic acid.

[8] Ochoa, S., and L. A. Heppel, in *The Chemical Basis of Heredity*, ed. W. D. McElroy and B. Glass (Baltimore: Johns Hopkins Press, 1957), p. 615.

[9] Furth, J. J., J. Hurwitz, and M. Goldman, *Biochem. Biophys., Research Commun.*, **4**, 362 (1961).

[10] Geiduschek, E. P., T. Nakamoto, and S. B. Weiss, these PROCEEDINGS, **47**, 1405 (1961).

[11] Chung, C. W., H. R. Mahler, and M. Enrione, *J. Biol. Chem.*, **235**, 1448 (1960).

[12] Straus, D. B., and E. Goldwasser, *ibid.*, **236**, 849 (1961).

[13] Burdon, R. H., and R. M. S. Smellie, *Biochim. et Biophys. Acta*, **51**, 153 (1961).

[14] Reddi, K. K., presented at the 98th meeting of the *National Academy of Sciences* (1961).

[15] Hurwitz, J., J. Furth, M. Anders, P. J. Ortiz, and J. T. August, in *Cellular Regulatory Mechanisms*, Cold Spring Harbor Symposia on Quantitative Biology, vol. 26 (1961), p. 91.

[16] Tener, G. M., *J. Am. Chem. Soc.*, **83**, 159 (1961).

[17] Steiner, R. F., and R. F. Beers, in *Polynucleotides* (Amsterdam: Elsevier Publishing Co., 1961), p. 374.

[18] Gierer, A., and G. Schramm, *Nature*, **177**, 702 (1956).

[19] Weiss, S. B., and T. Nakamoto, these PROCEEDINGS, **47**, 694 (1961).

[20] *Ibid.*, 1400 (1961).

[21] Josse, J., A. D. Kaiser, and A. Kornberg, *J. Biol. Chem.*, **236**, 864 (1961).

[22] Steiner, R. F., and R. F. Beers, in *Polynucleotides* (Amsterdam: Elsevier Publishing Co., 1961), p. 189.

[23] Warner, R., *Ann. N. Y. Acad. Sci.*, **69**, 314 (1957).

NONDEGRADATIVE ISOLATION OF DESOXYRIBONUCLEIC ACID IN SUBUNIT FORM FROM CALF THYMUS NUCLEI

BY RICHARD S. WELSH*

UNIVERSITY OF REDLANDS, REDLANDS, CALIFORNIA

Communicated by Bruno H. Zimm, March 12, 1962

A water-soluble form of desoxyribonucleoprotein (DNP) from calf thymus nuclei has been prepared and recently described by the author.[1,2] Some of the extensive evidence against its being an enzymatically degraded product has already been reported.[2] Since the material was prepared rapidly, in good yield, by a mild procedure minimizing any enzymatic reactions, involving first the preparation of purified nuclei, it appeared possible that the nonfibrous product could represent a subunit existing naturally in the nucleus. The desoxyribonucleic acid (DNA) isolated from this DNP has also been characterized, and the preliminary results are given in this report.

Materials and Methods.—Whole nuclei were prepared with the minimum of enzyme action by a method similar to that briefly described, only modified to yield nuclei in a less altered and less clumped condition. A particularly important requirement is that the glands be removed from the calves as quickly as possible (within minutes) after killing and be quick-frozen immediately on dry ice. Any delays at this stage lead to greatly reduced yields of the DNA in smallest subunit form, otherwise obtained. The medium for washing was 0.44 *M* sucrose, 40% glycerol, 0.039 *M*