Kimura, F., Harada, F., and Nishimura, S. (1971), *Biochemistry 10*, 3277.
Litt, M. (1968), *Biochem. Biophys. Res. Commun. 32*, 507.
Litt, M. (1969), *Biochemistry 8*, 3249.
Litt, M. (1971), *Biochemistry 10*, 2223.
Powers, D. M. (1970), Ph.D. Thesis, Cornell University, p 60.
RajBhandary, U. L., and Chang, S. H. (1968), *J. Biol. Chem. 243*, 598.
Randerath, K., and Randerath, E. (1971), *in* Procedures in Nucleic Acid Research, Cantoni 'G. L., and Davies, D. R., Ed., Vol. 2, New York, N. Y., p 796.
Uziel, M. (1967), *Methods Enzymol. 12A*, 407.
von Ehrenstein, G. (1967), *Methods Enzymol. 12A*, 588.
Wimmer, E., Maxwell, I. H., and Tener, G. M. (1968), *Biochemistry 7*, 2623.
Yaniv, M., and Barrell, B. G. (1969), *Nature (London) 222*, 278.
Zachau, H. G. (1968), *Eur. J. Biochem. 5*, 559.
Zachau, H. G., Dutting, D., Feldman, H., Melchers, F., and Karau, W. (1966), *Cold Spring Harbor Symp. Quant. Biol. 31*, 417.

Isolation of Nuclei and Characterization of Ribonucleic Acid Synthesized *in Vitro* from Swine Aortic Tissue†

K. Janakidevi

ABSTRACT: The object of the present study was to devise a method for isolating substantial quantites of purified nuclei from aortic tissue, and to study the nature of the ribonucleic acids synthesized *in vitro* by the nuclei. A new method of homogenization is described using a stepwise disintegration of the tissue with a Virtis 60 homogenizer. This method gives a good yield of highly purified, enzymatically active nuclei. DNA-dependent RNA polymerase was assayed using the isolated nuclei and the product analyzed by nearest-neighbor base frequency analysis. At low ionic strength and in the presence of Mg^{2+}, the nuclei synthesize a ribosomal type of RNA while in the presence of Mn^{2+} and 0.3 M ammonium sulfate, a DNA-like RNA is produced. Some properties of the two reactions are also described.

Weiss (1960) first demonstrated the presence of DNA-dependent RNA polymerase (nucleoside triphosphate:RNA nucleotidyl transferase, EC 2.7.7.6) in an extract of nucleated cells, called the "aggregate enzyme." The existence of more than one polymerase was suggested by Widnell and Tata (1966) in rat liver nuclei, one active at low ionic strength in the presence of Mg^{2+}, and the other requiring high salt concentrations and Mn^{2+}. Solubilization and purification of RNA polymerases has since been reported, and it is generally agreed that there are at least two distinct enzymes in the nuclei of most eukaryote tissues examined, and a third one in some tissues (Roeder and Rutter, 1969, 1970). Intact nuclei provide a good system to study the nature of the RNAs synthesized during cell transformations.

The importance of DNA-dependent RNA synthesis in the developing and proliferating tissue has been well recognized. The formation of new nuclear mRNAs has been demonstrated in the early period of liver regeneration following partial hepatectomy (Church and McCarthy, 1967). It is generally accepted that when a cell passes from interphase to mitosis, a process of derepression is set into operation resulting in the synthesis of some messengers, which will in turn code for enzymes and proteins necessary for DNA synthesis.

During the development of atherosclerosis, some smooth muscle cells (SMC)[1] of the aortic intima media (Scott *et al.*, 1969) undergo proliferation. In experimental models, such as swine fed hypercholesteremic diets, increased DNA synthesis and cell division have been demonstrated (Florentin and Nam, 1968; Thomas *et al.*, 1968). Any investigation of the factors involved in SMC proliferation of the aorta in the atherosclerotic process must take into consideration the role of transcription and other nuclear functions. It is, therefore essential to develop a method for the isolation of substantial amounts of aortic nuclei in an enzymatically pure state. The present paper describes such a method and some of the properties of the RNA polymerases and the products they synthesize *in vitro*.

Materials and Methods

Male Yorkshire swine weighing 7–10 kg were used. They were on an unrestricted diet (commercial pig chow) and water was provided *ad libitum*.

ATP, GTP, CTP, UTP, and actinomycin D were purchased from Sigma Chemical Co., St. Louis, Mo. DNase and RNase are from Worthington Chemical Co. [^{3}H]UTP was obtained from New England Nuclear Corp., Boston, Mass. [α-^{32}P]-Nucleoside triphosphates were purchased from Schwarz BioResearch, New York, N. Y.

† From the Department of Pathology, Albany Medical College, Albany, New York 12208. *Received November 1, 1971.* Supported by NIH Grants HE-7155 and HE-14177, Research Grant from Albany Medical College, and a grant from the Heart Association of Eastern New York, Inc.

[1] Abbreviations used are: SMC, smooth muscle cells; nnf, nearest-neighbor frequency; RNase, ribonuclease; DNase, deoxyribonuclease.

Isolation of Nuclei from Swine Aorta. Nuclei were isolated by a modification of the procedure described by Widnell and Tata (1964). The animals were killed with a gun, and the aorta from 2 or 3 pigs quickly excised and dropped into a mixture of 0.32 M sucrose containing 3 mM $MgCl_2$ (medium A). The intima media was stripped from the aorta, weighed, and thoroughly minced with scissors. Six to 8 volumes of medium A were added and the suspension homogenized for 15–20 sec in a Virtis 60 homogenizer between 15,000 and 25,000 rpm. The homogenizing flask used is of 165-ml capacity (Cat. No. 16-207) in which a minimum of 2 g can be easily homogenized. For smaller weights of tissue, flasks of Cat. Nos. 16-099 and 16-117 can be used. The homogenate was filtered through two layers of Nylon Bolting cloth, 110 mesh (John Staniar and Co., Manchester, England). The residue was then resuspended in medium A, homogenized, and filtered as described above. This process was repeated 3 or 4 times to ensure complete homogenization. The filtered homogenates were pooled and the crude nuclear pellet was obtained by sedimenting at 700*g* for 10 min in an International refrigerated centrifuge. This pellet was evenly suspended in a small volume of medium A and sufficient 2.4 M sucrose containing 1 mM $MgCl_2$ was added to a final molarity of 2.2, and centrifuged for 1 hr at 50,000*g* using a No. 40 rotor in a Beckman L-2 ultracentrifuge. The pellet of pure nuclei was washed twice with 0.25 M sucrose containing 1 mM $MgCl_2$ and suspended in the same medium for use in RNA polymerase assays.

All the above operations were performed in the cold between 0 and 4°.

RNA polymerase was assayed according to Widnell and Tata (1966) in the presence and absence of ammonium sulfate. The assay was carried out at 30° instead of 37°. The enzyme reaction was terminated with ice-cold 0.5 N $HClO_4$. Carrier RNA (1 mg) (yeast RNA, type II from Sigma Chemical Co.) was then added and, after 2 hr in ice, the precipitate was collected by centrifugation for 20 min at 2000 rpm. The pellet was washed three times with ice-cold 0.2 N $HClO_4$, twice with ethanol–ether (3:1), and once with ethanol. Nucleic acids were extracted twice from the precipitate with 10% NaCl at 100°, and to the pooled NaCl extracts was added two volumes of ethanol. Nucleic acids were allowed to precipitate overnight in the freezer and collected by centrifugation in cold for 30 min at 2000 rpm. The precipitate was dissolved in 5 mM NaOH. To one aliquot was added 10 ml of Bray's solution and the radioactivity was counted in a liquid scintillation spectrometer (Nuclear-Chicago Corp.), at an efficiency of 10–20% for tritium and 80–90% for ^{32}P. DNA was measured in a second aliquot.

For determining nnf, the components of the assay mixture were raised fivefold and the ^{32}P-labeled RNA was processed as described above but the perchlorate used contained 0.02 M sodium pyrophosphate. The ethanolic precipitate of nucleic acids was washed twice with ice-cold 0.2 N $HClO_4$, once with cold ethanol, and hydrolyzed for 16 hr at 37° in 0.3 M KOH. The hydrolysate was acidified with 6 N $HClO_4$, and the insoluble $KClO_4$ was removed by centrifugation. The 2′,3′-mononucleotides in the supernatant were separated according to Katz and Comb (1963). The individual nucleotide fractions were pooled separately and evaporated in a rotary evaporator. The residue was dissolved in a small volume of 5 mM NaOH and radioactivity determined as before. The nnfs were calculated according to Josse *et al.* (1961).

Cytochrome oxidase was determined according to Cooperstein and Lazarow (1951) and NADH–NADPH cytochrome *c* reductases by the method of Tata *et al.* (1963). NAD pyrophosphorylase was assayed according to Widnell and Tata (1964).

DNA was estimated by Burton's method (1956) and RNA by the orcinol method (Hurlbert *et al.*, 1954). Protein was measured with Folin–Ciocalteau reagent (Lowry *et al.*, 1951) with bovine serum albumin as standard.

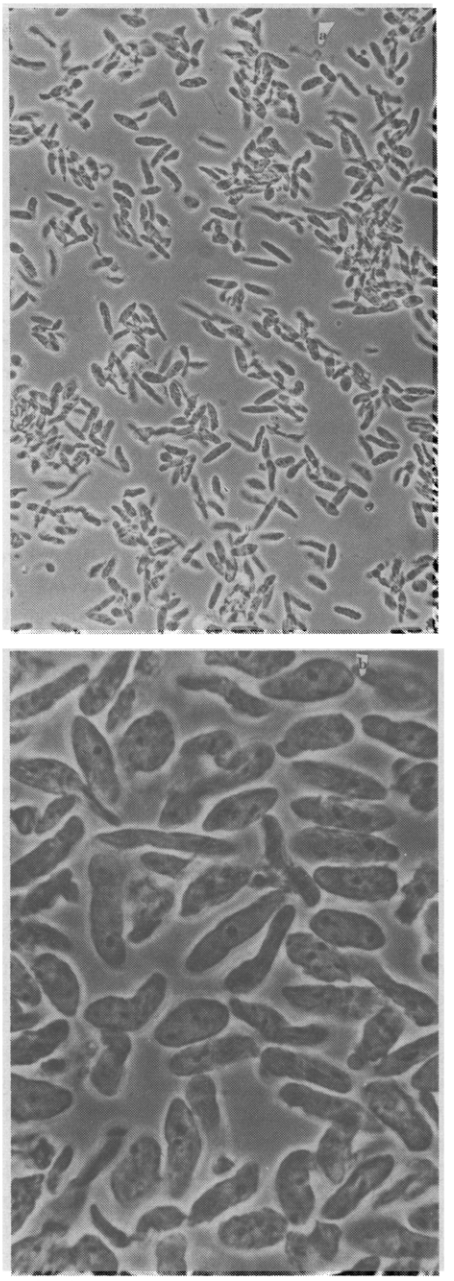

FIGURE 1: Phase-contrast micrographs of the nuclear preparation from intima media of swine aorta: (a) at a magnification of 300×; (b) nuclei at 1125× magnification.

Results

The nuclear preparation obtained from the aorta of pig was viewed under a Zeiss phase microscope. As shown in Figure 1 the nuclei were intact and free from contamination.

Table I gives the ratios of protein to DNA, and RNA to DNA. It is evident from these ratios and from morphological examination that the nuclear preparation is of a highly puri-

TABLE I: Chemical and Enzymatic Constitution of Isolated Nuclei from Swine Aorta.

	Specific Activity[a]				
				RNA Polymerase	
	Protein/DNA	RNA/DNA	NAD Pyrophosphorylase	Mg^{2+} Activated	Mn^{2+} $(NH_4)_2SO_4$ Activated
Homogenate	27.5	1.5			
Nuclei	2.87	0.21	54 ± 9	68 ± 8.8 (10)	1026 ± 117 (10)

[a] NAD pyrophosphorylase—nmoles of NAD synthesized for 20 min per mg of DNA. RNAp olymerase—pmoles of UMP incorporated per mg of DNA. Values are given as means and std dev. The numbers in the parentheses represent the number of determinations.

fied nature and is comparable to preparations from other tissues. The specific activities of NAD pyrophosphorylase and RNA polymerase activities are also given in this table. The activity of NAD pyrophosphorylase, a good marker enzyme for nuclei, is much less in the aorta than in liver but is comparable to the values reported for uterus (Widnell *et al.*, 1967).

Cytochrome oxidase and NADH–NADPH cytochrome *c* reductase, marker enzymes for mitochondria and microsomes, respectively, were assayed in the homogenate and the purified nuclear preparation. As shown in Table II, contamination of nuclear preparation from these sources is minimal.

In Table III are listed the various requirements for the two RNA polymerases from aortic nuclei. Both activities are sensitive to RNase as well as DNase and all of the four nucleoside triphosphates are essential for optimum activity. A cation is essential in both cases, Mn^{2+} in the presence of ammonium sulfate and Mg^{2+} in its absence. The pH optima are similar to those reported for rat liver nuclei by Widnell and Tata (1966). Ammonium sulfate concentrations of 0.3 M are required for maximum stimulation (Figure 2).

Figure 3 shows the rate of incorporation of [^{3}H]UTP into RNA. The Mg^{2+}-dependent reaction is linear for 5 min, reaching a maximum at 15 min, while the Mn^{2+}, ammonium sulfate activated enzyme is linear up to 30 min and continues to rise up to 60 min.

Figure 4 illustrates the effect of varying the concentrations of [^{3}H]UTP on its incorporation into RNA. As shown in the figure, increasing the nucleotide concentrations above 0.04 mM for Mg^{2+}-dependent reaction and 0.2 mM for ammonium sulfate activated reaction did not appreciably increase the incorporation of [^{3}H]UTP into RNA.

Tables IV and V give nnfs of the RNA synthesized *in vitro* by aortic nuclei.

In the Mg^{2+}-dependent reaction, the RNA synthesized by the nuclei is rich in GpG, CpC, CpG, and GpC pairs. In contrast, ApA, UpU, UpA, and ApU dinucleotide sequences are much less frequent. Also of interest is the high UpG content, which is much higher than that reported for rat liver. UpC and CpC pairs occur with less frequency than in rat liver.

When high salt concentrations are used in the assay, GpG, CpC, and GpC frequencies become less frequent. Particularly interesting is the low amount of CpG pair, which is in general agreement with the findings that vertebrate DNA is deficient in this pair (Josse *et al.*, 1961 and Swartz *et al.*, 1962). ApA, UpU, UpA, CpA, CpU, and ApU pairs are significantly increased while UpG is as high as in the absence of ammonium sulfate.

The base composition calculated from nnf analysis are given in Table VI. The overall base composition of the RNA synthesized in the Mg^{2+}-dependent reaction is of the ribosomal type, particularly 28S RNA. Nair *et al.* (1967) have also reported that the RNA synthesized by rat heart nuclei in the absence of high salt resembles more the 28S RNA than the overall rRNA. The addition of salt significantly shifts

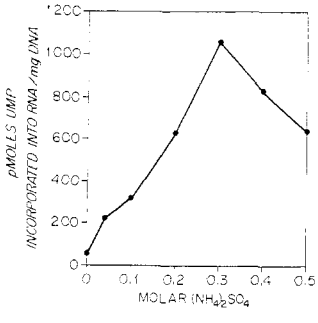

FIGURE 2: The effect of ammonium sulfate on the activity of RNA polymerase of aortic nuclei. The incubation mixture is as given in Table III. Ammonium sulfate 2 M adjusted to pH 7.5 with NH_4OH and aliquots added to the incubation mixture to the required molarity. Incubation for 45 min at 30°.

TABLE II: Specific Activities of Cytoplasmic Marker Enzymes.

	Specific Activity[a]		
	Cytochrome Oxidase	NADH Cytochrome *c* Reductase	NADPH Cytochrome *c* Reductase
Homogenate	24	4.8	1.05
Nuclei	0	0.03	0

[a] Cytochrome oxidase expressed as $\Delta \log A_{550}$/min per g of intima media. One unit of NADH/NADPH cytochrome *c* reductase expressed as ΔA_{550}/min per mg of protein and given in the table as units per g of intima media for homogenate and total units present in the nuclei isolated from 1 g of tissue.

TABLE III: Characteristics of RNA Polymerase Reaction of Isolated Pig Aortic Nuclei Carried Out in the Presence and Absence of $(NH_4)_2SO_4$.

Conditions	Specific Activity[b]	
	Mg^{2+}-Activated Polymerase	Mn^{2+}-$(NH_4)_2SO_4$-Activated Polymerase
Complete system[a]	61	1090
ATP, CTP, GTP omitted	7	12
ATP omitted	8	47
GTP omitted	19	28
CTP omitted	20	52
Mg^{2+} omitted	4	
Mn^{2+} omitted		17
Mn^{2+} instead of Mg^{2+}	3	
Mg^{2+} instead of Mn^{2+}		297
Complete + Actinomycin D, 0.5 μg	7	164
Actinomycin D, 1.0 μg	3.5	82
Actinomycin D, 2.0 μg	1.0	41
2 mM Mg^{2+} and 2 mM Mn^{2+}		364
Complete + ribonuclease, 25 μg at time 0	2	35
Complete + deoxyribonuclease, 25 μg at time 0	3	27

[a] Complete system contains for Mg^{2+}-activated polymerase in a total volume of 0.5 ml, ATP, CTP, GTP, 0.2 mM; [^{3}H]-UTP, 0.04 mM (specific activity, 100 Ci/mole); $MgCl_2$, 5 mM; NaF, 6 mM; Tris-HCl, pH 8.5, 100 mM; and nuclei containing 100–150 μg of DNA. Incubation for 15 min at 30°. In a total of 0.5 ml, $(NH_4)_2SO_4$-activated polymerase contains ATP, CTP, GTP, 0.2 mM; [^{3}H]UTP, 0.2 mM (20 Ci/mole); $MnCl_2$, 4 mM; $(NH_4)_2SO_4$, 300 mM; Tris-HCl, pH 7.5, 100 mM; and nuclei containing 100–150 μg of DNA. Incubation for 45 min at 30°. [b] Specific activity expressed as pmoles per mg of DNA.

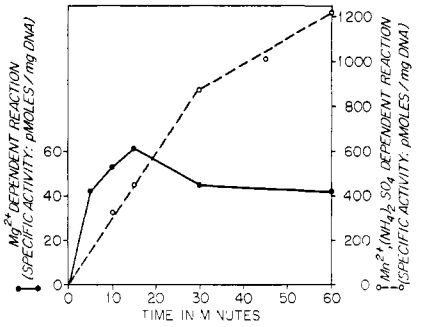

FIGURE 3: Time course of the RNA polymerase activities of the aortic nuclei. Experimental conditions as given in Table III.

the ratio of incorporation of the 4 nucleoside triphosphates and results in the synthesis of a DNA-like RNA with a higher A-U content than obtained for the Mg^{2+}-dependent reaction.

Discussion

As far as can be ascertained, preparation of purified nuclei has not been reported from aortic tissue. The present paper describes a method for obtaining substantial quantities of nuclei from swine aortic intima media preparations. Part of the problem in isolating the nuclei from aorta is the toughness of the tissue that renders it resistant to conventional homogenizing techniques. This is overcome by using a sheering mechanism as provided by the Virtis homogenizers, which, combined with a sequential disintegration for very short periods of time (15 to 20 sec), provides a method that yields adequate quantities of nuclei. As judged by morphological appearance, plus RNA/DNA and protein/DNA ratios, the nuclei appear intact and pure. Based on the recovery of DNA, the yield of nuclei is between 20 and 30%, which is reasonable considering the nature of the tissue. Cytoplasmic contamination is virtually absent as indicated by the study of marker enzymes. Cytochrome oxidase and NADPH–cytochrome *c* reductase, marker enzymes for mitochondria and microsomes, respectively, were not detected in the nuclear preparation. There is only 0.68% of the total tissue NADH–cytochrome *c* reductase in the nuclei.

Nuclei incorporate ribonucleoside triphosphates into an acid-insoluble product which is sensitive to exogenous ribonuclease. The enzyme is DNA dependent as judged by its sensitivity to added deoxyribonuclease. The presence of all the ribonucleoside triphosphates is essential for optimum activity and the elimination of any one of the nucleotide precursors reduces the activity to varying degrees of the optimal levels depending on which of the precursors is excluded. The remaining residual activity could be due to the presence of small pools of nucleotides in the cell nucleus. The kinetics of the two reactions, pH optima, and other requirements are similar to those reported for rat liver nuclei by Widnell and Tata (1966). However, the ammonium sulfate concentration required for optimum stimulation is 0.3 M and is lower than that reported for rat liver nuclei or rat heart nuclei.

The study of RNA polymerases and the nature of the products they synthesize is also reported for the first time from aortic tissue. The specific activities obtained for RNA polymerase of isolated aortic nuclei are generally lower than those reported for liver but much higher when compared to

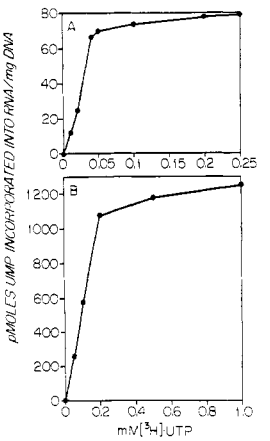

FIGURE 4: Effect of [^{3}H]UTP concentrations on the activity of RNA polymerases: (A) Mg^{2+}-dependent reaction; (B) Mn^{2+}, $(NH_4)_2SO_4$-dependent reaction.

TABLE IV: Nearest-Neighbor Frequency Analysis of the Product of RNA Polymerase in the Presence of Mg^{2+}.

Radioactive Nucleoside Triphosphate	% of Total ^{32}P Recovered in 2′,3′-Nucleoside Monophosphate					Expt	Isolated 2′,3′-Nucleoside Monophosphate			
	UMP	GMP	CMP	AMP			U_p	A_p	C_p	G_p
							Calculated Result: Nearest-Neighbor Frequency			
							U_pA	A_pA	C_pA	G_pA
[α-^{32}P]ATP	18	34	33	15	[α-^{32}P]ATP	a	0.032	0.027	0.059	0.061
	18	31	30	21		b	0.034	0.039	0.056	0.058
							U_pU	A_pU	C_pU	G_pU
[α-^{32}P]UTP	21	33	29.5	16.5	[α-^{32}P]UTP	a	0.042	0.033	0.059	0.066
	19	34	29	18		b	0.037	0.035	0.056	0.066
							U_pG	A_pG	C_pG	G_pG
[α-^{32}P]GTP	30	32.5	18.5	19	[α-^{32}P]GTP	a	0.09	0.059	0.057	0.101
	29	29	24	18		b	0.09	0.056	0.074	0.09
							U_pC	A_pG	C_pC	G_pC
[α-^{32}P]CTP	17.5	35.5	32.5	14.5	[α-^{32}P]CTP	a	0.054	0.045	0.101	0.11
	18.5	33.6	30.4	17.5		b	0.057	0.054	0.094	0.104
						Sum	0.218	0.164	0.276	0.338
							0.218	0.184	0.280	0.318

TABLE V: Nearest-Neighbor Frequency Analysis of the Product of RNA Polymerase in the Presence of Mn^{2+} and $(NH_4)_2SO_4$.

Radioactive Nucleoside Triphosphates	% of Total ^{32}P Recovered in 2′,3′-Nucleoside Monophosphate					Expt	Isolated 2′,3′-Nucleoside Monophosphate			
	UMP	GMP	CMP	AMP			U_p	A_p	C_p	G_p
							Calculated Result: Nearest-Neighbor Frequency			
							U_pA	A_pA	C_pA	G_pA
[α-^{32}P]ATP	22	27	25	26	[α-^{32}P]ATP	a	0.045	0.045	0.052	0.056
	24	25	26	25		b	0.065	0.067	0.07	0.067
							U_pU	A_pU	C_pU	G_pU
[α-^{32}P]UTP	30	24	27	19	[α-^{32}P]UTP	a	0.075	0.047	0.067	0.06
	29	24	29	18		b	0.08	0.05	0.08	0.066
							U_pG	A_pG	C_pG	G_pG
[α-^{32}P]GTP	39	27.5	8.5	25	[α-^{32}P]GTP	a	0.106	0.068	0.023	0.075
	37	31	8	24		b	0.084	0.054	0.018	0.07
							U_pC	A_pC	C_pC	G_pC
[α-^{32}P]CTP	26	25	29	20	[α-^{32}P]CTP	a	0.071	0.054	0.079	0.068
	27	27	28.5	17.5		b	0.061	0.04	0.065	0.06
						Sum	0.297	0.223	0.221	0.259
							0.29	0.211	0.233	0.263

skeletal muscle (Sobel and Kaufman, 1970 and Baieve and Florini, 1970). The lower activity as compared to liver might reflect the lower metabolic activity of this tissue or the ribonuclease activity could be higher in aorta than in liver. The nature of the RNAs synthesized is, however, similar to that reported for rat liver and heart. Although more than one species of RNA may be synthesized in each of these two systems described in this paper, nnf analysis shows that the overall base composition of the RNA resembles either ribosomal or DNA-like depending on the ionic strength of the assay mixture. There are, however, some differences in the dinucleotide sequences of RNA synthesized by the pig aortic nuclei in the Mg^{2+}-dependent reaction as compared to rat liver nuclei (Chambon *et al.*, 1968). These differences might

TABLE VI: Base Composition of RNA from Various Sources.

	Percentage of 2′,3′-Nucleoside Monophosphate				
	UMP	GMP	CMP	AMP	Reference
Mg^{2+}-dependent reaction	21.985	32.765	27.8	17.5	Present study (NNF analysis)
28S RNA, rat heart	23.6	35.7	24.9	15.8	Posner and Fanburg (1966)
18S RNA, rat heart	33.9	28	21.5	16.2	Posner and Fanburg (1966)
Mn^{2+} $(NH_4)_2SO_4$-dependent reaction	29.135	26.18	22.725	21.7	Present study (NNF analysis)
Complementary RNA	28.6	21.6	21.4	28.4	Nair *et al.* (1967)

be inherent to the type of tissue or result partially from the lower temperature (30°) employed in the present study. Chambon *et al.* (1968) have shown that temperature has a marked effect on the activity of ribonuclease, the activity increasing with a rise in temperature. In the absence of ammonium sulfate—a potent inhibitor of ribonuclease—some species of RNA, like DNA-like RNA, which are more sensitive to the action of ribonuclease than ribosomal RNA (Chambon *et al.*, 1968) are degraded to a greater extent at 37° than at 30°. The minor components thus remain stable when the assay of the Mg^{2+}-dependent reaction is carried out at the lower temperature. In this context, it is interesting to mention that Chambon *et al.* (1968) reported slightly different base compositions for RNA synthesized in the absence of ammonium sulfate when the assay was carried out at 17° instead of 37° and concluded that this difference is due to the stability of (A-U)-rich RNA.

Derepression of the genome is considered a vital step in the induction of DNA synthesis and cell division, and would, therefore, constitute an important step in the development of atherosclerosis. Since RNA polymerase is the key enzyme in the process of derepression, the levels or activities of this enzyme would play an important role on the control and synthesis of various RNA species under different metabolic conditions, such as those involved in hormonal stimulation, embryonic development or in various pathological conditions.

References

Baieve, D., and Florini, J. R. (1970), *Arch. Biochem. Biophys. 139*, 393.

Burton, K. (1956), *Biochem. J. 62*, 315.

Chambon, P., Ramuz, M., Mandel, P., and Doly, J. (1968), *Biochim. Biophys. Acta 157*, 504.

Church, R., and McCarthy, B. J. (1967), *Proc. Nat. Acad. Sci. U. S. 58*, 1548.

Cooperstein, S. J., and Lazarow, A. (1951), *J. Biol. Chem. 189*, 665.

Florentin, R. A., and Nam, S. C. (1968), *Exp. Mol. Pathol. 8*, 263.

Hurlbert, R. B., Schmitz, H., Brumm, A. F., and Porter, V. R. (1954), *J. Biol. Chem. 209*, 23.

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

Katz, S., and Comb, D. G. (1963), *J. Biol. Chem. 238*, 3065.

Lowry, O. H., Rosebrough, N. J., Farr, A. L., and Randall, R. J. (1951), *J. Biol. Chem. 193*, 265.

Nair, K. G., Rabinowitz, M., and Tu, M.-H. C. (1967), *Biochemistry 6*, 1898.

Posner, B. I., and Fanberg, B. L. (1966), *Circ. Res. 19*, 805.

Roeder, R. G., and Rutter, W. J. (1969), *Nature (London) 224*, 234.

Roeder, R. G., and Rutter, W. J. (1970), *Proc. Nat. Acad. Sci. U. S. 65*, 675.

Scott, R. F., Morrison, E. S., and Kroms, M. (1969), *J. Atheroscler. Res. 9*, 5.

Sobel, B. E., and Kaufman, S. (1970), *Arch. Biochem. Biophys. 137*, 169.

Swartz, M. A., Trautner, J. A., and Kornberg, A. (1962), *J. Biol. Chem. 237*, 1961.

Tata, J. R., Ernster, L., Lindberg, O., Arrhenius, E., Pedersen, S., and Hedman, R. (1963), *Biochem. J. 86*, 408.

Thomas, W. A., Florentin, R. A., Nam, S. C., Kim, D. N., Jones, R. M., and Lee, K. T. (1968), *Arch. Pathol. 86*, 621.

Weiss, S. B. (1960), *Proc. Nat. Acad. Sci. U. S. 46*, 1020.

Widnell, C. C., Hamilton, T. H., and Tata, J. R. (1967), *J. Cell Biol. 32*, 766.

Widnell, C. C., and Tata, J. R. (1964), *Biochem. J. 92*, 313.

Widnell, C. C., and Tata, J. R. (1966), *Biochim. Biophys. Acta 123*, 478.