PURIFICATION AND PROPERTIES OF A NUCLEAR EXORIBONUCLEASE FROM EHRLICH ASCITES TUMOR CELLS

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Recently there have been many investigations on the role of RNA in inducing or maintaining the differentiated state of cells.[1] In dealing with this problem, the process of destruction of RNA, as well as its synthesis and translation, must be considered. There have been numerous reports[2-7] of the destruction, *in situ*, of the rapidly labeled, newly synthesized nuclear RNA of mammalian cells, although the enzymes responsible for this destruction were not isolated in these investigations.

There has been relatively little work on the purification and properties of enzymes of the tumor cell nucleus which destroy RNA. We wish to report here on the isolation from the nucleus of the Ehrlich ascites tumor cell of an exoribonuclease which produces 5′-mononucleotides as its sole product. This enzyme shows a definite preference for degrading single-stranded, random-coil RNA's, in contrast to polynucleotides with a high degree of secondary structure. The exoribonuclease appears to account for the bulk of the Ehrlich nuclear enzyme activity capable of degrading random-coil polyribonucleotides such as polyadenylic acid (Poly A). The properties of the enzyme are similar to those of a leukemia cell exonuclease[8] which shows marked capacity to degrade polyribonucleotides to 5′-mononucleotides, but does not hydrolyze synthetic *p*-nitrophenyl esters of nucleoside-5′-phosphates. The properties of the Ehrlich exoribonuclease are also similar to those of the *Escherichia coli* exoribonuclease which Spahr[9] and Singer and Tolbert[10] have suggested may function as a selective destroyer of messenger RNA. We have also noted the presence of this type of exoribonuclease in normal mouse liver nuclei, and have separated the exoribonuclease from the endoribonuclease I[11] (which produces oligonucleotides bearing 5′-phosphate end groups) which is also present in both types of nuclei. The presence of a ribonuclease which can degrade Poly A to adenosine 5′-phosphate (AMP) has previously been noted in crude extracts of whole Ehrlich ascites tumor cells.[12]

Experimental Procedure.—Materials: Sources of reagents were as follows: Triton N-101, Rohm and Haas, Philadelphia, Pa.; *O*(diethylaminoethyl) (DEAE) Microgranular anion exchange cellulose, H. Reeve Angel, Clifton, N.J.; crystalline bovine serum albumin (BSA), Pentex, Kankakee, Ill.; lyophilized snake venom (*Crotalus adamanteus*), Ross Allen Reptile Institute, Silver Springs, Fla., and Sigma Chemical, St. Louis, Mo.; venom (*Crotalus adamanteus*) phosphodiesterase and calf thymus DNA, Worthington Biochemical, Freehold, N.J.; oligonucleotides and synthetic polynucleotides, Miles Laboratories, Elkhart, Ind.; dinucleoside monophosphates, Gallard-Schlesinger Chemical, Carle Place, L.I., N.Y.; *p*-nitrophenyl thymidine-5′-phosphate (NT5P), Calbiochem, Los Angeles, Calif.; uridine-H^3 and cytidine-H^3, generally labeled, specific activity 3 c/mM and orotic acid-6-C^{14}, specific activity 4 mc/mM, New England Nuclear, Boston, Mass.; CTP-H^3, Schwarz BioResearch, Orangeburg, N.Y. P^1-adenosine-5′-, p^2-phenyl pyrophosphate (ADP-phenol) was synthesized by the method of Moffatt and Khorana.[13] RNA was prepared essentially as described previously.[14,15] For preparation of nuclear RNA labeled with both C^{14} and H^3, mice were inoculated i.p. with both orotic acid-C^{14} (25 μc per mouse, given 18 hr before sacrifice) and either cytidine-H^3 or uridine-H^3 (100 μc per mouse, given 20 min before

sacrifice). A crude nuclear pellet was prepared from the livers[14] and the RNA extracted. Soluble RNA (sRNA), labeled at the 3'-OH(pCpCpA) end with CTP-H^3, was prepared as described by Weiss.[16] Polynucleotides were dialyzed against 0.05 *M* NaCl-0.001 *M* EDTA, and BSA was dialyzed against 0.01 *M* EDTA and water before use. DNA was denatured at 100° for 10 min, followed by quick cooling in ice. The dinucleotide, pApA, was prepared by degradation of Poly A.[11, 17]

Analytical methods: (a) *Enzyme assays:* The standard assay mixture (0.5 or 1.0 ml) for nuclear exoribonuclease and endoribonuclease was as follows: Tris-HCl, pH 7.4, 0.1 *M;* $MgCl_2$, 0.004 *M;* potassium phosphate, pH 7.4, 0.025 *M;* dithiothreitol (DTT), 0.0003 *M;* poly A, either non-radioactive or radioactive, 0.003 *M* (adenine equivalent); BSA, 100 μg/ml; and enzyme (0.1–1.0 unit). After 30 min at 37°, the reaction was stopped by the addition of ice-cold perchloric acid (PCA) (final conc 0.4 *M*), and the tubes were centrifuged for 30 min at 1900 × *g*. The clear supernatants were then assayed either for absorbancy at 257 mμ or for radioactivity in a liquid scintillation counter. A unit of enzyme is defined as that amount which forms 1 micromole of AMP residues per hr; the reaction is essentially linear up to 30% hydrolysis of Poly A. Hydrolysis of NT5P was measured by the method of Razzell,[18] and total protein estimated by the method of Lowry *et al.*[19]

(b) *Thin-layer chromatography:* Plastic sheets, coated with cellulose-thin layers containing fluorescent indicator (Brinkmann Instruments, Westbury, N.Y.) were developed in Eastman thin-layer sandwich chambers (Distillation Products Industries, Rochester, N.Y.). Two solvent systems were used:[20, 21] solvent 1, isopropyl alcohol-concentrated ammonia-water, 7:1:2 (v/v); and solvent 2, *n*-propyl alcohol-concentrated ammonia-water, 55:10:35 (v/v). The following *Rf*'s are observed in solvents 1 and 2, respectively: pA, 0.07, 0.43; Ap, 0.07, 0.42; adenosine, 0.58, 0.68; ApA, 0.32, 0.58; pApA, 0.04, 0.47; ApApA, 0.17, 0.55; $A(pA)_5$, 0.01, 0.42; pC, 0.05, 0.48; cytidine, 0.46, 0.68; pU, 0.04, 0.38; uridine, 0.37, 0.55; pI, 0.04, 0.45; inosine, 0.41, 0.64.

(c) *Gel filtration analysis:* The procedure of Birnboim[22] was used to measure the molecular weight distribution of polynucleotides during their enzymatic degradation. Enzymatic activity during the course of degradation of Poly A or polycytidylic acid (Poly C) was stopped by addition of equal volumes of 1 *M* NaCl-0.01 *M* Na_3EDTA to aliquots of reaction mixtures; the samples were then applied to Sephadex columns for analysis.[22]

Preparation of nuclear enzymes: (a) *Solutions for cell fractionation and enzyme purification:* Solution 1: sucrose, 0.32 *M;* $MgCl_2$, 0.002 *M;* potassium phosphate, pH 6.8, 0.001 *M*. Solution 2: sucrose, 0.32 *M;* potassium phosphate, pH 6.8, 0.001 *M*. Solution 3: sucrose, 0.32 *M;* Triton N-101, 0.3%; $MgCl_2$, 0.001 *M;* adjusted with 0.001 *M* potassium phosphate to pH 6.2–6.4. Solution 4: NaCl, 0.1 *M;* potassium phosphate, pH 6.2, 0.005 *M;* $MgCl_2$, 0.002 *M*, DTT, 0.001 *M*. Solution 5: NaCl, 0.08 *M;* Na_3EDTA, 0.02 *M;* Tris-HCl, pH 8.0, 0.02 *M;* DTT, 0.001 *M*. Solution 6: Tris-HCl, pH 8.0, 0.05 *M;* DTT, 0.0003 *M*. Solution 7: NaCl, 0.6 *M;* Na_3EDTA, 0.02 *M;* Tris-HCl, pH 8.0, 0.01 *M;* DTT, 0.0003 *M*. Solution 8: same as solution 7, without EDTA.

(b) *Preparation of tumor cell nuclei:* The cell line used is known[23] as the hyperdiploid Ehrlich-Lettré tumor or Landschütz sarcoma I and is routinely carried in our laboratory in C57BL6 mice, which are inoculated i.p. every 14 days with 0.1 ml of undiluted ascites. Nuclei were prepared from these cells by a modification of previous detergent procedures,[24, 25] as follows: 25 ml of ascites (approx 10^8 cells/ml) were obtained by aspiration from mice on the 12th or 13th day after inoculation. The rest of the procedure was performed at 0–4°. Ten ml of solution 1 was added to the ascites; the cells were spun down and the supernatant was discarded. The cells were then evenly suspended in 35–40 ml of solution 2 and spun for 10 min at 1300 × *g* (max). The supernatant was discarded and the treatment with solution 2 repeated once. The washed pellet of cells was gently homogenized in 35–40 ml of solution 3 in a Dounce homogenizer. The lysed cells were spun for 10 min at 1300 × *g* and the nuclear pellet was saved and treated twice more with solution 3. The final nuclear pellet was washed once with solution 1 before storage at −80° or below. The entire procedure was monitored with a phase microscope, and the final nuclear pellet was essentially free of cytoplasmic contamination.

(c) *Preparation of mouse liver nuclei:* Five gm of mouse liver were minced, homogenized in 35 ml of solution 1 in a Dounce homogenizer, and then filtered through 230-mesh (75-μ openings) and 465-mesh (25-μ openings) nylon monofilament screening cloth. The homogenate was spun

for 10 min at 1800 × *g*. The crude nuclear pellet was saved, treated two or three times with solution 3 (as described above for Ehrlich nuclei), washed once with solution 1, and then frozen.

(d) *Purification of Ehrlich exoribonuclease:* A typical preparation of enzyme from the nuclei obtained from 1000 ml of ascites is summarized in Table 1. Inasmuch as nuclear protein accounts for only 10% of the total protein of the Ehrlich ascites tumor cell,[26] the nuclear enzyme has been purified over 200 times its original concentration in the whole cell. The entire procedure was performed at 0–4°.

(1) *pH 6.2 extraction:* The nuclear pellets were suspended in a total of 200 ml of solution 4 in several Dounce homogenizers, then spun for 15 min at 13,000 × *g* and re-extracted with a total of 100 ml of solution 4. The combined pH 6.2 extracts were discarded.

(2) *pH 8.0 extraction:* The pellets were extracted twice (as described above) with a total of 260 ml of solution 5. The extracts were saved and the nuclear residue was discarded.

(3) *Ammonium sulfate fractionation:* Dry ammonium sulfate was added to the pH 8.0 extract to bring it to 30% saturation, and the precipitate was discarded after removal by centrifugation. The supernatant was then brought to 50% saturation, and the precipitate was saved after centrifugation.

(4) *$CdCl_2$ fractionation:* The above precipitate was dissolved in 60 ml of solution 6, dialyzed for 2–3 hr against solution 6, and then made 0.002 *M* in $CdCl_2$. The precipitate was collected by centrifugation, suspended in 6 ml of solution 7, and solubilized by dialysis against solution 7.

(5) *Sephadex G-100 fractionation:* Fraction 4 was applied to a Sephadex G-100 column, 2.5 × 40 cm, which had been equilibrated with solution 8. Void volume of the column, as determined with blue dextran, was 62 ml. Solution 8 was run through the column at a flow rate of 15 ml/hr; the enzyme was recovered quantitatively in the eluate. Peak tubes, collected between the 60th and 80th ml of eluate, were saved. The enzyme was precipitated by addition of two volumes of 0.003 *M* zinc acetate in 0.05 *M* Tris, pH 7.4.

(6) *DEAE fractionation:* The enzyme precipitate (fraction 5) was dissolved in 21 ml of solution 6 containing 0.05 *M* Na_3EDTA and dialyzed against solution 6. It was then applied to a 2.5- × 5-cm column of DEAE, buffered with solution 6 (DEAE-1). The column was eluted with 30 ml of 0.1 *M* Tris-HCl, pH 7.4, 0.001 *M* DTT and then with 100 ml of the same buffer containing 0.1 *M* NaCl. The peak of exoribonuclease activity was eluted with the 15th to 35th ml of the latter buffer. It was then dialyzed against solution 6 and applied to a second DEAE column, 1 × 18 cm, buffered with solution 6 (DEAE-2). The column was washed with 30 ml of solution 6 and then eluted with a linear salt gradient, with the mixing chamber containing 50 ml of solution 6 and the reservoir 50 ml of solution 6 plus 0.4 *M* NaCl. Six-ml fractions were collected from the point when enzyme was first applied to the column. Final enzyme fractions were stored at −80° or below.

(7) *Comments on the purification procedure; Preparation of nuclear exoribonuclease and endoribonuclease from mouse liver:* Nuclear exoribonuclease loses activity upon prolonged dialysis or excessive dilution. Quantitative recovery from DEAE columns may be achieved by the use of relatively steep gradients. If it is desired only to separate the nuclear exoribonuclease activity from the endoribonuclease I activity[11] (which produces oligonucleotides bearing 5′-phosphate end groups), this may be done by applying material purified as far as fraction 3 (and then dialyzed briefly against solution 6) directly to a DEAE column of the type used in the second DEAE purification described above. The endoribonuclease I activity is eluted very readily, while the exoribonuclease requires higher salt concentration for elution.

TABLE 1

PURIFICATION OF EHRLICH EXORIBONUCLEASE

Fraction	Total activity (units)	Specific activity (units/mg protein)
1. Whole nuclei	22,600	8.1
1*a*. pH 6.2 extract (discarded)	3,600	8.4
2. pH 8.0 extract	12,600	34
3. Ammonium sulfate 30–50% ppt.	8,900	43
4. $CdCl_2$ ppt.	5,400	67
5. Sephadex	4,600	71
6. DEAE-1	2,100	112
7. DEAE-2, fractions 19–23	1,680	116
7*a*. DEAE-2, fraction 20	540	186

This brief procedure has been used to separate and compare the endoribonuclease I and the exoribonuclease activities of both Ehrlich and mouse liver nuclei, as shown in Figure 1. Nuclei from both Ehrlich tumor (from 70 ml of ascites) and mouse liver (from 20 gm of liver) were extracted twice with solution 4, and the extracts, which contained less than 25% of the total nuclear Poly A-degrading activity, were discarded. The nuclei were then extracted twice with solution 5; and the extracts, which contained the majority of the Poly A-degrading activity, were then fractionated with 30–50% ammonium sulfate, briefly dialyzed against solution 6, and applied to DEAE columns, which were eluted as shown in Figure 1. Recoveries of Poly A-degrading activity from these columns were essentially quantitative. The liver endoribonuclease I and exoribonuclease peak tubes were frozen at −80°.

Results.—Relative activities of exoribonuclease and endoribuclease I in liver and tumor: In liver and Ehrlich ascites tumor nuclear extracts there are two major enzymes which degrade Poly A, namely, endoribonuclease I[11] and the exoribonuclease whose purification is presently described. The relative activities of these two enzymes are strikingly different in extracts of the two tissues (see Fig. 1); in extracts of Ehrlich tumor nuclei, the ratio of the number of units of exonuclease to endonuclease is 50:1, while the corresponding ratio in extracts of liver nuclei is 1:3.

Specificity of exoribonuclease: The exoribonucleases from Ehrlich tumor and mouse liver nuclei produce, as the sole product of Poly A hydrolysis, AMP, which was identified by its chromatographic behavior in solvents 1 and 2 and by cleavage

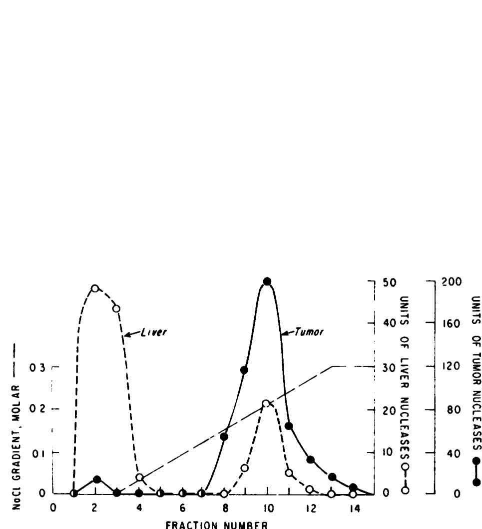

FIG. 1.—Separation of exoribonucleases and endoribonucleases from liver and tumor nuclear extracts, prepared as described in *Methods*. A total of 125 Poly A-degrading units from liver and 500 units from tumor were applied to 1- × 12-cm DEAE columns, buffered with solution 6. Columns were eluted with 30 ml of solution 6 and then with solution 6 plus an NaCl gradient, as shown. Fractions of 10 ml were collected and enzyme was assayed. The first peak of activity is endoribonuclease; the second, exonuclease, as verified by thin layer chromatography of products.

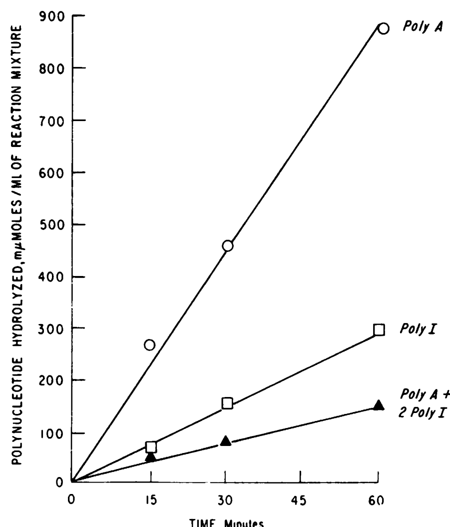

FIG. 2.—Digestion of Poly A, Poly I, and Poly (A + 2I) by Ehrlich exoribonuclease. Polymers, at a total nucleotide concentration of 0.003 *M*, were incubated under standard assay conditions with 0.9 unit of enzyme/ml in a volume of 3.5 ml. One-ml samples were withdrawn at the times indicated, precipitated with ice-cold PCA, and centrifuged. Absorbancy was measured on the supernatants at either 257 or 250 mμ. Digestion was also allowed to continue for 6 hr, and 5-μl aliquots of the digests were spotted on thin layer chromatograms for identification of products.

to adenosine by venom containing 5′-nucleotidase.[27] No 3′-AMP, oligonucleotides, or adenosine are produced. The Ehrlich exoribonuclease also degrades polyinosinic acid (Poly I), polyuridylic acid (Poly U), and Poly C to 5′-mononucleotides at rates that are, respectively, 33, 12, and 4 per cent of the rate for Poly A.

The triple-stranded, helical polymer formed by mixing Poly A and Poly I[28] in nucleotide molar ratios of 1:2 was relatively resistant to hydrolysis, as shown in Figure 2. The hydrolysis that did occur yielded only inosine 5′-phosphate (IMP), as identified by absorption spectrum, chromatography, and hydrolysis to inosine by venom. Similarly, the digestibility of Poly A (0.001 or 0.003 *M*) by enzyme is decreased by 90 per cent when it is complexed in a Poly A (0.001 *M*) + Poly U (0.002 *M*) helix.[29] The relative resistance of Poly C to hydrolysis may be a function of its high degree of secondary structure in solution[28, 30] or of a preference of the enzyme for specific bases. Microsomal RNA was hydrolyzed only 29 per cent, and soluble RNA only 5 per cent as readily as Poly A.

Ehrlich exoribonuclease also shows a marked preference to degrade rapidly labeled, newly synthesized RNA, in contrast to ribosomal RNA. Doubly labeled RNA was isolated from a crude nuclear fraction (see *Methods*) and subjected to attack by the Ehrlich exoribonuclease and mouse liver endoribonuclease I. Figure 3 shows that the exoribonuclease hydrolyzes the rapidly labeled H^3-RNA much more readily than the largely ribosomal C^{14}-RNA, while the endonuclease hydrolyzes the H^3-RNA only slightly more readily than C^{14}-RNA.

Ehrlich nuclear exoribonuclease does not hydrolyze NT5P, or native or heat-denatured DNA; activity toward these substrates was less than 0.4 per cent of the activity toward Poly A. Anderson and Heppel likewise reported[8] that leukemia cell exonuclease does not split NT5P, which is the preferred substrate for microsomal phosphodiesterase I.[18]

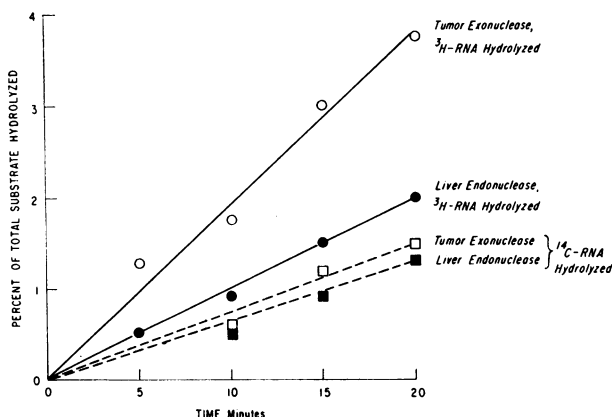

FIG. 3.—Doubly labeled RNA, containing 160,000 cpm of H^3 and 90,000 cpm of C^{14} per mg, was digested under standard assay conditions, in a final volume of 0.5 ml. Separate assays were run for each time point, using 480 μg of RNA and 0.5 unit of either Ehrlich exoribonuclease or liver endonuclease for each assay.

The reaction was stopped with ice-cold PCA, and acid-soluble H^3- and C^{14}-radioactivity measured in a Packard model 4312 liquid scintillation spectrometer, calibrated with H^3- and C^{14}-toluene. Counting conditions were such that less than 0.1% of the counts in the H^3-channel appeared in the C^{14}-channel.

Mechanism of degradation of polynucleotides by Ehrlich exoribonuclease: The phosphodiester bond is broken by hydrolysis, not by phosphorolysis or pyrophosphorolysis, as there is no requirement for P_i or PP_i, and no ADP or ATP can be detected during degradation of Poly A. Neither ADP nor ATP are hydrolyzed under standard assay conditions.

The hydrolysis proceeds stepwise with successive removal of mononucleotides from an end of a polynucleotide; oligonucleotides are never detected by thin-layer chromatography. Moreover, gel filtration analysis[22] of samples of early and late digests of Poly A and Poly C detected no endonuclease activity. When oligonucleotides are attacked by the enzyme, the hydrolysis begins at the 3'-OH end and proceeds to the 5'-end. This was shown by examining the hydrolysis products of ApApA and $A(pA)_5$ by thin layer chromatography; a similar technique was used by Razzell and Khorana[31] to elucidate the mechanism of attack by venom and spleen exonucleases. In our experiments, no adenosine or pApA was found at a time when ApApA was totally degraded to pA and ApA. When $A(pA)_5$ is degraded, pA, not adenosine, is liberated early in digestion; furthermore, ApA does not appear until later stages of digestion, and, in turn, is hydrolyzed extremely slowly to adenosine and pA. In contrast, the dinucleotide, pApA, is readily degraded to pA.

The mechanism of attack on polynucleotides was further investigated by gel filtration analysis[22] of the molecular-weight distribution of Poly A during the course of degradation, as follows: Poly A was incubated under standard assay conditions with 0.9-unit of enzyme per ml. At 0, 40, 80, and 160 minutes of incubation, aliquots of digest were applied to analytical Sephadex columns. By 160 minutes, 66 per cent of the Poly A had been hydrolyzed to AMP. However, at none of the above time points was there any detectable shift in the molecular-weight distribution of the undigested Poly A, both in terms of the elution volume and the band width of the Poly A on the Sephadex column. The mechanism of attack on polynucleotides was also studied with the relatively resistant substrate, sRNA, labeled at the 3'-OH (-pCpCpA) end with H^3-CTP. When this material was degraded with snake venom, we found that 90 per cent of the radioactivity was liberated in acid-soluble form at a time when only 10 per cent of the total polynucleotide was acid-soluble, as reported by Weiss.[16] However, when labeled sRNA was degraded by Ehrlich exoribonuclease, we found equal percentages of radioactivity and total polynucleotide were liberated in acid-soluble form throughout the course of digestion. In contrast, microsomal phosphodiesterase I[18] acts in a fashion similar to snake venom during degradation of end-labeled sRNA.[32] The results of the above experiments with Poly A and sRNA suggest that Ehrlich exoribonuclease degrades polynucleotides by a mechanism similar to the one proposed by Nossal *et al.*[33] (a full discussion of this mechanism will be published shortly by M. F. Singer *et al.*[34]) for the *E. coli* exoribonuclease, namely that the enzyme remains complexed with an individual polynucleotide molecule until it is almost completely reduced to mononucleotides, rather than following the classical Michaelis-Menten model of an enzyme releasing its products (remaining polymer and mononucleotide) after each hydrolytic step.

Inhibitors: Various nucleosides and nucleotides were examined as inhibitors of degradation of Poly A; several cytidine nucleotides were the most potent inhibitors

found. At 1×10^{-3} M, per cent inhibition was as follows: CMP, 20; CDP, 47; CTP, 27; 2′ (3′)-CMP, 37; and dCDP, 30. Cytidine, 3′-CMP, C-cyclic-p, deoxycytidine, dCMP, and dCTP did not inhibit. ADP-phenol, at 1×10^{-3} M, inhibited 50 per cent. The inhibitions observed cannot be accounted for on the basis of binding of Mg^{++}, as the enzyme has essentially identical activity over a range of [Mg^{++}] from 1 to 4×10^{-3} M.

Factors affecting activity and stability of exoribonuclease: The enzyme is active over a broad pH range between 7.4 (Tris buffer) and 9.2 (glycine buffer). It is routinely assayed in the presence of the sulfhydryl reagent, DTT; p-chloromercuribenzoate (1×10^{-3} M) reduced activity by 92 per cent. No hydrolysis of Poly A is observed without Mg^{++}. Mn^{++} can replace Mg^{++} to the extent of 30 per cent, while Ca^{++} and Zn^{++} are totally ineffective. P_i (0.025 M) stimulates activity 15 per cent, K^+ or Na^+ (0.025 M) have no effect; PP_i (0.01 M) inhibits 85 per cent; NaF (0.04 M) inhibits completely.

Purified enzyme is stable at −80° or less for at least two months. Heating for ten minutes at 50 and 60° causes loss of 17 and 100 per cent of activity, respectively. Prolonged dialysis, especially at low ionic strength (0.02 M Tris-HCl, pH 8.0) or at low pH (below 7.0) results in loss of activity.

Discussion.—The preference of the Ehrlich nuclear exoribonuclease for degrading nonhelical, single-stranded polynucleotides and rapidly labeled, newly synthesized nuclear RNA *in vitro* suggests that it may be a principal agent responsible for the *in situ* destruction of rapidly labeled nuclear RNA.[2–7] The properties of Ehrlich exoribonuclease are very similar to those of one of the principal enzymes that may destroy messenger RNA in bacteria.[9, 10, 35] It has been suggested[35, 36] that the 5′-ribonucleotides produced by degradation of rapidly labeled RNA may serve either as precursors for synthesis of stable ribosomal RNA or for incorporation into the DNA precursor pool.

In his classic monograph on the *Biochemistry of Cancer*, Greenstein[37] emphasized that "when a normal tissue becomes neoplastic, many of [its] specific functional activities markedly decrease or are lost altogether. Many enzymes of high activity in the normal tissues are markedly reduced in the neoplasms derived from them." At present, there is no definitive explanation for this deficiency of proteins in cancer cells. Cohen[36, 38] has suggested that the deficiency of proteins in a cancer cell may be partly a function of the destruction of critical RNA molecules which are necessary for synthesis of proteins of the differentiated state. The role of exoribonucleases, such as the one previously described in mouse leukemia cells[8] and the present one from Ehrlich tumor nuclei, in contributing to the deficiency of proteins in the tumor cell remains a matter of conjecture and current investigation.

Summary.—An exoribonuclease and an endoribonuclease have been separated from both Ehrlich ascites tumor and normal liver cell nuclear extracts. There is a marked preponderance of the exoribonuclease in the tumor nuclei and the endoribonuclease in the liver nuclei. The tumor exoribonuclease has been extensively purified. It attacks nonhelical polyribonucleotides to produce 5′-mononucleotides; the enzyme appears to remain complexed with an individual polynucleotide molecule until it is almost totally degraded. Oligonucleotides are attacked from the 3′-OH end. It is suggested that the exoribonuclease may contribute to the relative deficiency of proteins in the tumor cell.

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The abbreviations used are: BSA, bovine serum albumin; NT5P, *p*-nitrophenyl thymidine-5′-phosphate; DTT, dithiothreitol; ADP-phenol, P^1-adenosine-5′-, P^2-phenyl pyrophosphate; PCA, perchloric acid; sRNA, soluble or transfer RNA. Other abbreviations and designations for nucleotides are those used in *J. Biol. Chem.* Column dimensions are given as diam. × length.

[1] For a review, see: Bell, E. P., ed., *Molecular and Cellular Aspects of Development* (New York: Harper and Row, 1965).

[2] Harris, H., *Proc. Roy. Soc. (London), Ser. B*, **158**, 79 (1963).

[3] Hymer, W. C., and E. L. Kuff, *Biochem. Biophys. Res. Commun.*, **15**, 506 (1964).

[4] Warner, J. R., R. Soerio, H. C. Birnboim, M. Girard, and J. E. Darnell, *J. Mol. Biol.*, **19**, 349 (1966).

[5] Soerio, R., H. C. Birnboim, and J. E. Darnell, *J. Mol. Biol.*, **19**, 362 (1966).

[6] Roberts, W. K., and J. F. Newman, *J. Mol. Biol.*, **20**, 63 (1966).

[7] Shearer, R. W., and B. J. McCarthy, *Biochemistry*, **6**, 283 (1967).

[8] Anderson, E. P., and L. A. Heppel, *Biochim. Biophys. Acta*, **43**, 79 (1960).

[9] Spahr, P. F., *J. Biol. Chem.*, **239**, 3716 (1964).

[10] Singer, M. F., and G. Tolbert, *Biochemistry*, **4**, 1319 (1965).

[11] Heppel, L. A., in *Procedures in Nucleic Acid Research*, ed. G. L. Cantoni and D. R. Davies (New York: Harper and Row, 1966), p. 31.

[12] Martin, M. A., unpublished thesis submitted to Yale Univ. School of Medicine, 1962; M. F. Singer, personal communication.

[13] Moffatt, J. G., and H. G. Khorana, *J. Am. Chem. Soc.*, **80**, 3756 (1958). This compound was synthesized by Ash Stevens, Inc., Detroit, Mich., under NIH contract PH43-66-929.

[14] Dingman, W., and M. B. Sporn, *Biochim. Biophys. Acta*, **61**, 164 (1962).

[15] Sporn, M. B., and Dingman, W., *Biochim. Biophys. Acta*, **68**, 389 (1963).

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

[17] Stevens, A., and R. J. Hilmoe, *J. Biol. Chem.*, **235**, 3016 (1960).

[18] Razzell, W. E., in *Methods in Enzymology*, ed. S. P. Colowick and N. O. Kaplan (New York: Academic Press, 1963), vol. 6, p. 236.

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

[20] Heppel, L. A., P. J. Ortiz, and S. Ochoa, *Science*, **123**, 415 (1956).

[21] Lapidot, Y., and H. G. Khorana, *J. Am. Chem. Soc.*, **85**, 3857 (1963).

[22] Birnboim, H., *Biochim. Biophys. Acta*, **119**, 198 (1966).

[23] Hauschka, T. S., S. T. Grinnell, L. Révész, and G. Klein, *J. Natl. Cancer Inst.*, **19**, 13 (1957). We thank Dr. Hauschka and Dr. J. DiPaolo for their help in providing this tumor.

[24] Zalta, J.-P., R. Rozencwajg, N. Carasso, and P. Favard, *Compt. Rend.*, **255**, 412 (1962).

[25] Hymer, W. C., and E. L. Kuff, *J. Histochem. Cytochem.*, **12**, 359 (1964).

[26] Sporn, M. B., unpublished results.

[27] Heppel, L. A., in *The Enzymes*, ed. P. D. Boyer, H. Lardy, and K. Myrback (New York: Academic Press, 1961), 2nd ed., vol. 5, p. 49.

[28] Sarkar, P. K., and J. T. Yang, *Biochemistry*, **4**, 1238 (1965).

[29] Felsenfeld, G., and A. Rich, *Biochim. Biophys. Acta*, **26**, 457 (1957).

[30] Fasman, G. D., C. Lindblow, and L. Grossman, *Biochemistry*, **3**, 1015 (1964).

[31] Razzell, W. E., and H. G. Khorana, *J. Am. Chem. Soc.*, **80**, 1770 (1958).

[32] Razzell, W. E., personal communication.

[33] Nossal, N. G., G. Tolbert, and M. F. Singer, *Federation Proc.*, **26**, 612 (1967).

[34] Singer, M. F. *et al.*, in preparation. We thank Dr. Singer for her discussion of her results prior to publication and for her advice on this problem.

[35] Sekiguchi, M., and S. S. Cohen, *J. Biol. Chem.*, **238**, 349 (1963).

[36] Cohen, S. S., H. D. Barner, and J. Lichtenstein, *J. Biol. Chem.*, **236**, 1448 (1961).

[37] Greenstein, J. P., *Biochemistry of Cancer* (New York: Academic Press, 1954), 2nd ed., p. 362.

[38] Cohen, S. S., *Cancer Res.*, **20**, 698 (1960).