Laboratory, U.S. National Museum, Washington, D.C. The fish are now stored in 70 percent ethyl alcohol; the initial fixative is unknown. R. L. Haedrich informs us that *Aldrovandia macrochir* (Günther) 1878 has been reassigned to the genus *Halosauropsis*.
19. *Baseline Studies of Pollutants in the Marine Environment and Research Recommendations*, report of the IDOE Baseline Conference, 24–26 May 1972, New York.
20. G. E. Miller, P. M. Grant, R. Kishore, F. J. Steinkruger, F. S. Rowland, V. P. Guinn, *Science* **175**, 1121 (1972).
21. We thank F. Schwartz for identifying the benthopelagic fish and H. Windom for help in the Hg analysis. We acknowledge the technical assistance of L. H. Hardy and N. Y. Jones of the Atlantic Estuarine Fisheries Center, and the Oceanographic Program of Duke University Marine Laboratory for the use of R.V. *Eastward* on cruises E-19-71 and E-12-72. The Oceanographic Program at DUML is supported by NSF grants GA-27725 and GD-28333; other DUML support was from NSF grant GA-28742. The research conduced at AEFC was supported jointly by the National Marine Fisheries Service and the AEC, agreement No. AT(49-7)-5.

8 September 1972

Mitochondrial Polyriboadenylate Polymerase: Relative Lack of Activity in Hepatomas

Abstract. *An enzyme that polymerizes adenylate residues from adenosine triphosphate was prepared from rat liver mitochondria and compared to similar preparations from the mitochondria of three hepatomas. Enzyme activity in the hepatomas was only 1 to 2 percent of that in normal liver.*

An enzyme in eucaryotes that can incorporate adenylate residues into nonterminal internucleotide linkages has been characterized in the nuclei (*1*) and in the supernatant from microsomal centrifugation (*2*). During our studies on DNA-dependent RNA polymerase of rat liver mitochondria, we solubilized and partially purified an enzyme that can synthesize polyadenylate[poly(A)] chains from adenosine triphosphate (ATP). The activity of this enzyme was considerably reduced in preparations from Morris hepatomas 7777 (well differentiated), 3924A (poorly differentiated), and 3683F (undifferentiated) (*3*).

Mitochondria were isolated by a procedure similar to that of Sordhal and his colleagues (*4*). The mitochondrial pellet was suspended in 50 m*M* tris(hydroxymethyl)aminomethane (tris) buffer (*p*H 9.0) containing 5 m*M* $MgCl_2$, 0.1 m*M* ethylenediaminetetraacetic acid (EDTA), and 1 m*M* dithiothreitol (TMED buffer), and sonicated for 1 minute (four 15-second periods) in a Branson Sonifier (model W 140) at full output. The suspension was mixed with glycerol (final concentration, 20 percent, by volume), incubated at 37°C for 30 minutes, and centrifuged at 105,000*g* for 1 hour. The supernatant, which contained enzyme activity, was dialyzed against several volumes of TMED buffer (*p*H 7.5) containing glycerol (20 percent, by volume) to deplete the nucleotides. In some experiments, the enzyme was further purified by differential precipitation with ammonium sulfate. The fraction precipitated by increasing the ammonium sulfate saturation from 40 percent to 50 percent had most of the activity, although this procedure resulted in a considerable loss of activity.

The enzyme from liver had a specific activity of 13,000 units (1 unit = 1 pmole of nucleotide incorporated per milligram of protein per 30 minutes) (Table 1). The reaction was linear for 30 minutes. The specific activity of the liver enzyme was comparable to the highest values reported for the corresponding enzymes from *Escherichia coli* (*5*) or from calf thymus nuclei (*6*). The capacity of the enzyme preparation to incorporate labeled cytosine triphosphate (CTP), guanosine triphosphate (GTP), or uridine triphosphate (UTP) was relatively insignificant. The enzyme activity was 380 units for [^{3}H]UTP, 300 units for [^{3}H]CTP, and 40 units for [^{3}H]GTP. The enzyme preparations obtained from the same amount of tumor tissue had no significant poly(A)-forming activity. The material that was not solubilized by our procedure did not contain significant poly(A) polymerase activity ($<$ 1000 units for liver and $<$ 50 units for hepatomas).

The product was characterized as poly(A) on the basis of the following properties (Table 1). (i) Pancreatic ribonuclease had no inhibitory effect if it was incubated with the enzyme preparation before the polymerization reaction or if it was incubated with the reaction product. (ii) The product was retained on a Millipore filter in the presence of 0.5*M* KCl, which dissolves the bulk of cellular RNA (*7*). (iii) Deletion of the remaining three nucleotides did not inhibit enzyme activity. The internucleotide or nonterminal incorporation of adenylate was tested by estimating the radioactivity in the nucleoside mono-

Table 1. Comparison of poly(A) polymerase activities in the mitochondrial preparations of normal rat liver, host liver, and hepatomas. The enzyme was prepared from 30 g each of liver and hepatoma. The standard reaction mixture (0.35 ml) contained 10 μmole of tris-HCl (*p*H 8.0), 2 μmole of $MgCl_2$, 1 μmole of $MnCl_2$, 13 μmole of KCl, 3 μmole of phosphoenolpyruvate, 20 μg of pyruvate kinase, 0.1 μmole of unlabeled ATP, 0.00075 μmole of [^{3}H]ATP [generally labeled, 9.53 to 15 c/mmole (New England Nuclear)] and 0.8 to 1.0 mg of enzyme protein. After incubation at 30°C for 30 minutes, the reaction was stopped by the addition of 0.1 μmole of unlabeled ATP and a cold solution of 10 percent trichloroacetic acid and 0.04*M* sodium pyrophosphate. The precipitate was collected either on Whatman GF/C filters or on Millipore filters (1.2-μm pore size), washed three times with a cold solution of 5 percent trichloroacetic acid and 0.02*M* sodium pyrophosphate, and finally with cold glass-distilled water. In some experiments, the product was filtered in the presence of 0.5*M* KCl to test for poly(A) (*7*). The radioactivity in the filters was determined by counting in 10 ml of a toluene-based scintillation solution containing 4 g of Omnifluor (New England Nuclear) and 0.1 g of 1,4-bis-[2-(4-methyl-5-phenyloxazolyl)]-benzene (POPOP) per liter of solution. The enzymes from liver and tumor were used in saturating amounts and were assayed in triplicate (1 unit is the amount of enzyme that incorporates 1 pmole of nucleotide into trichloroacetic acid–insoluble product per milligram of protein per 30 minutes; *N* is the number of experiments).

Enzyme source	Additional treatment	Enzyme activity (10^3 units)	*N*
Host liver, rats with 7777		13.80 ± 0.98	4
Host liver, rats with 3924A		13.10 ± 0.51	3
Host liver, rats with 3683F		10.00 ± 0.64	2
Hepatoma 7777		0.25 ± 0.05	4
Hepatoma 3924A		0.15 ± 0.09	3
Hepatoma 3683F		0.10 ± 0.03	2
Hepatoma 3683F	Unlabeled ATP + (0.1 μmole)	0.11 ± 0.03	2
Normal liver		13.50 ± 1.70	10
Normal liver	Prior 30-minute incubation with ribonuclease (100 μg/ml)	12.90 ± 0.94	2
Normal liver	Product incubated 30 minutes with ribonuclease (100 μg/ml)	13.40 ± 0.90	3
Normal liver	+ GTP, CTP, UTP	13.10 ± 0.61	2
Normal liver	+ 0.5*M* KCl during filtration	12.90 ± 1.08	3
Normal liver	+ Deoxyribonuclease (100 μg/ml)	13.10 ± 0.71	2
Normal liver	+ Mitochondrial RNA (50 μg/ml)	13.70 ± 0.75	2

phosphates obtained after alkaline hydrolysis of the product. Almost all the radioactivity (88 percent) was recovered in adenosine monophosphate; whereas terminal nucleosides would be hydrolyzed to adenosine.

The lack of inhibition of the enzyme activity by treatment with ribonuclease or by addition of exogenous RNA (Table 1) indicates that the bulk of the mitochondrial RNA does not act as a primer. Initial experiments, however, suggest that poly(A) itself can act as a primer, as has been reported for the corresponding nuclear enzyme (*6*).

Because tumors contain fewer mitochondria per cell (*8*) and consequently less mitochondrial protein per gram of wet tissue than do control tissues (*9*), they can be expected to show relatively low poly(A)-synthesizing activity. However, the low enzyme activity of hepatomas (only 1 to 2 percent of that in normal liver) cannot be explained on this basis. Furthermore, enzyme activity has been expressed per milligram of protein. The loss of enzyme activity in tumors cannot be due to adenosine triphosphatase that may be present in the enzyme preparation, because (i) Ca^{2+}, which is required for the nucleotidase activity (*10*), was completely removed by exhaustive dialysis, and (ii) additional ATP included in the reaction mixture did not increase the specific activity of the hepatoma enzyme (Table 1).

The tumor enzymes could have been associated with an inhibitor or inhibitors of poly(A) polymerase. To test this possibility, we conducted two sets of experiments. First, the liver enzyme was incubated together with each tumor enzyme under optimal assay conditions. Inhibition of liver enzyme activity was not significant (about 20 percent), which demonstrates the absence of any potent inhibitors in the tumor enzyme. In the second experiment, the liver enzyme was incubated for 30 minutes, and the product formed was further incubated for 20 minutes with tumor enzyme. Again, there was no significant change in the liver enzyme activity (less than 20 percent). In these experiments, 0.3 to 0.6 mg of tumor enzyme protein was used.

The experiments reported here show that transformation of parenchymal cells into hepatoma cells results in a considerable loss of mitochondrial poly(A) polymerase. Further experiments with other normal tissues and with growing tissue such as regenerating liver are required to prove unequivocally whether the relative lack of this enzyme in hepatomas is unique to malignant cells.

Unlike nuclear poly(A), which is involved in the transport of messenger RNA (mRNA) from the nucleus to the cytoplasm (*11*), the function of mitochondrial poly(A) is not known. Hence, the significance of the lowered activity of this enzyme in hepatomas cannot be assessed. Since mRNA containing poly(A) segments can be retained on Millipore filters in the presence of 0.5*M* KCl (*7, 12*), it is possible that liver mitochondrial mRNA, as compared to mRNA of hepatomas, contains larger segments of adenylate residues at the 3′ terminal end.

SAMSON T. JACOB*
DANIEL G. SCHINDLER†
Physiological Chemistry Laboratories, Massachusetts Institute of Technology, Cambridge 02139

HAROLD P. MORRIS
Department of Biochemistry, Howard University, Washington, D.C. 20001

References and Notes

1. M. Edmonds and R. Abrahms, *J. Biol. Chem.* **235**, 1142 (1960); M. Edmonds and M. G. Caramela, *ibid.* **244**, 1314 (1969); R. H. Burdon, *Biochem. Biophys. Res. Commun.* **11**, 472 (1963); E. A. Hvatt, *Biochim. Biophys. Acta* **142**, 246 (196→ P. Chambon, J. D. Weill, P. Mandel, *Biochem. Biophys. Res. Commun.* **11**, 39 (1963).
2. C. W. Chung, H. R. Mahler, M. Envione, *J. Biol. Chem.* **235**, 1448 (1960); H. G. Klemperer, *Biochim. Biophys. Acta* **72**, 416 (1963).
3. H. P. Morris and B. P. Wagner, *Methods Cancer Res.* **4**, 125 (1968). The hepatomas are transplanted subcutaneously or intramuscularly. The highly differentiated tumors deviate the least from normal liver. The degree of differentiation is based on histological observations.
4. L. A. Sordhal and A. Schwartz, *ibid.* **6**, 159 (1970); L. A. Sordhal, Z. R. Blailock, A. G. Liebilt, G. H. Kraft, A. Schwartz, *Cancer Res.* **29**, 2002 (1969). The animals were killed by decapitation, and the livers were rapidly removed, minced well, and homogenized with 10 volumes of a cold solution of 0.25*M* sucrose and 1 m*M* EDTA (*p*H 7.4, adjusted with 1*M* tris). The homogenate was centrifuged at 800*g* for 10 minutes, and the supernatant was again centrifuged at 800*g* for 10 minutes to remove the nuclei completely. The portion of the supernatant that was at least 1 cm above the pellet was removed carefully with a Pasteur pipette and centrifuged at 8000*g* for 10 minutes. The pellet was suspended in the starting buffer and centrifuged again at 8000*g* for 10 minutes to obtain purified mitochondria. All the operations were carried out at 4°C. Hepatoma mitochondria were prepared by the same procedure except that 1 percent bovine serum albumin was included in the buffers to stabilize the structure (and hence biochemical functions) of mitochondria during isolation.
5. P. J. Ortiz, J. T. August, M. Watanabe, A. M. Kaye, J. Hurwitz, *J. Biol. Chem.* **240**, 423 (1965).
6. M. Edmonds and R. Abrahms, *ibid.* **237**, 2636 (1962).
7. S. Y. Lee, J. Medecki, G. Brawerman, *Proc. Nat. Acad. Sci. U.S.A.* **68**, 1331 (1971).
8. C. Allard, R. Mathieu, G. DeLanurande, A. Cantero, *Cancer Res.* **12**, 407 (1952).
9. H. A. Mintz, D. H. Yawn, B. Safer, E. Bresnick, A. G. Liebelt, Z. R. Blailock, E. R. Rabin, A. Schwartz, *J. Cell Biol.* **34**, 513 (1967).
10. B. M. Braganca and I. Aravindakshan, *Biochem. Biophys. Res. Commun.* **3**, 484 (1960).
11. J. E. Darnell, L. Philipson, R. Wall, M. Adesnick, *Science* **174**, 507 (197→ M. Edmonds, M. H. Vaughan, H. Nakazato, *Proc. Nat. Acad. Sci. U.S.A.* **68**, 1336 (1971).
12. A. R. Means, J. P. Comstock, G. C. Rosenfeld, B. W. O'Malley, *Proc. Nat. Acad. Sci. U.S.A.* **69**, 1146 (1972).
13. Supported by PHS grant GM 18534 to S.T.J. We thank M. C. Linder for some rats carrying hepatoma 3683F.

* Present address: Department of Pharmacology, Hershey Medical Center, College of Medicine, Pennsylvania State University, Hershey 17033.
† Present address: Department of Biochemistry, University of Wisconsin, Madison 53706.

12 June 1972; revised 28 August 1972

Hypothalamic Norepinephrine: Circadian Rhythms and the Control of Feeding Behavior

Abstract. *The time of day is a decisive determinant of the effects of* l-*norepinephrine on feeding behavior. During the dark, direct application of* l-*norepinephrine to the hypothalamus of rats suppressed feeding behavior. During the light, treatment with the same dose of* l-*norepinephrine facilitated feeding behavior. Thus,* l-*norepinephrine has dual and opposite effects on feeding behavior. A hypothalamic substrate that fluctuates in a circadian rhythm could account for both actions of* l-*norepinephrine.*

The addition of exogenous *l*-norepinephrine (*l*-NE) to the lateral hypothalamus affects feeding behavior. Both stimulant (*1*) and suppressant (*2*) effects have been reported, but the conditions that determine when each of these opposite actions will occur are unknown. This has led to the development of a controversy between proponents of the noradrenergic-feeding and the noradrenergic-satiety theories. The implications of each theory have been reviewed by Hoebel (*3*). We now report data that appear to resolve the controversy. The effects of the addition of exogenous *l*-NE to the hypothalamus appear to be dependent on differences in the internal state of the hypothalamus associated with the environmental cycle of darkness and light. In the dark, this treatment suppressed feeding behavior. In the light, the same dose of