Table 1. Effect of temperature on the ostracod *Potamocypris* at test exposure times. Values for the initiation of heat coma are the lowest temperatures at which heat coma was observed. The thermal death range is from the lowest temperature at which death was recorded to the temperature above which no specimens survived. The LD_{50} values were calculated from slopes determined by the method of least squares (*10*).

Exposure time (min)	Initiation of heat coma (°C)	Thermal death range (°C)	Calculated LD_{50} (°C)
60	49.50	49.50–50.75	50.44
40	50.25	50.50–51.25	50.96
20	50.50	51.00–51.75	51.43
10	51.75	51.75–52.50	52.03
5	52.00	52.00–53.00	52.77
1	53.75	54.50–55.75	55.12

genetic or physiological population differences can provide tolerance to such high temperatures.

Mason (*5*) and Phelps (*11*) found ostracods in the field at 51.5°C. Mason found that *Heterocypris balnearia* did not survive longer than 4 hours at 49°C, and the *Cypridopsis* species examined by Phelps did not live more than a few hours at this temperature. The Oregon *Potamocypris* was exposed to 49°C (± 0.5°C in a heated aquarium) for 12 hours and a three-tube series was removed every hour. Under these conditions no deaths occurred until 5 hours, and there was 33 percent survival even after 12 hours. Food was not considered a source of error during this longer incubation as adults have survived at 35°C with no food for up to 10 days in the laboratory.

As a result of these findings we believe that this ostracod may have the highest temperature tolerance of any aquatic metazoan. Since the ostracod is easily cultured (reproducing populations at 40° to 45°C have been maintained in glass vessels with algae for over 2 years), it may prove to be a valuable laboratory animal for study of biochemical and physiological systems and their genetic control at the upper temperature limits to which aquatic metazoan life has evolved. In view of the production of thermal waters in the United States and other countries through the use of surface waters as coolants for power plants, the study of temperature stresses in this organism may provide valuable guidelines to the study of more economically important animal species.

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9. Least squares calculations of the slopes were performed according to C. Bliss [*The Statistics of Bioassay* (Academic Press, New York, 1952), p. 452] and M. Moroney [*Facts from Figures* (Penguin, Baltimore, ed. 3, 1956), p. 277]. The calculations were done with actual values, not percentages, and do not include any zero mortalities unless there was some mortality at equal or lower temperatures, nor do they include any 100 percent mortalities unless there is less than 100 percent at equal or higher temperatures.
10. Methods described by R. Sokal and F. Rohlf [*Biometry* (Freeman, San Francisco, 1969), pp. 424, 446] were used to determine the significance of the regressions and the LD_{50} values and corresponding 95 percent confidence limits. The LD_{50} values were found by solving for X with Y set at 15. The 95 percent confidence intervals were: 49.14° to 52.80°C for 60 minutes; 50.16° to 51.91°C for 40 minutes; 50.48° to 52.65°C for 20 minutes; 51.40° to 52.58°C for 10 minutes; 51.45° to 55.46°C for 5 minutes; and 54.76° to 55.47°C for 1 minute.
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23 February 1973; revised 24 May 1973

Synthesis of RNA–Polyadenylic Acid by Isolated Brain Nuclei

Abstract. *Nuclei, isolated from mouse brain tissue at various stages of postnatal development and incubated under cell-free conditions, synthesized RNA molecules that were associated with polyadenylic acid [poly(A)]. The RNA synthesized by these nuclei was similar to the poly(A)–associated products described for intact eukaryotic cells. The brain nuclei synthesized a similar proportion of RNA-poly(A) in the presence either of Mg^{2+} or of Mn^{2+} with $(NH_4)_2SO_4$. The RNA from neonatal brain nuclei appeared to have a greater proportion of poly(A)-containing RNA than nuclear products obtained from more mature neural tissue.*

The rate of RNA synthesis in intact mouse brain nuclei has been observed to be altered dramatically during postnatal development (*1*). Several investigators have demonstrated the presence of polyadenylic acid [poly(A)] sequences in nuclear and cytoplasmic messenger RNA (mRNA) of eukaryotic cells (*2, 3*). The presence of these poly(A) sequences in the RNA molecules has enabled the selective isolation and measurement of these molecules by a variety of techniques. In our study we have used cellulose columns, which have been shown to separate RNA-poly(A) from the bulk of cellular RNA (*4, 5*), to ascertain the presence of poly(A) sequences in the RNA synthesized by isolated mouse brain nuclei during various stages of postnatal maturation. We have sought to determine whether the RNA products synthesized by brain nuclei under cell-free conditions resembled the RNA products produced in intact eukaryotic cells (*3*).

Since previous investigators have

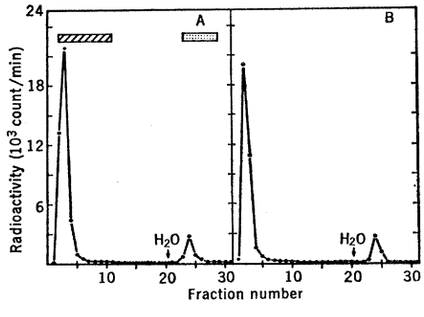

Fig. 1. Elution profiles of nuclear RNA from cellulose columns. RNA was isolated from adult mouse brain nuclei after their incubation in the presence either of Mg^{2+} (A) or of MN^{2+} with $(NH_4)_2SO_4$ (B), as described in Table 1. Elution with water is indicated by arrows. Fractions (2 ml) were collected during elution with buffer, and 1-ml fractions were collected during elution with water. In independent experiments, the elution patterns of free [^{3}H]GTP (hatched bar) and polyadenylic acid (stippled bar) were determined.

demonstrated that ionic conditions, especially ionic strength, can influence the RNA polymerase activities of mammalian cells (*6*), RNA synthesis was measured in brain nuclei incubated in the presence of either Mg^{2+} or of Mn^{2+} with $(NH_4)_2SO_4$. Initially, intact nuclei isolated from adult neural tissue were incubated with ^{3}H-labeled guanosine triphosphate ([^{3}H]GTP) (*1*), and the RNA was chromatographed on Sigmacell, type 38, cellulose columns essentially as described by Schutz *et al.* (*4*). Although we have shown that the labeled nuclear products synthesized under these conditions (Table 1) are resistant to prolonged exposure to deoxyribonuclease (*1*), each preparation was treated with deoxyribonuclease prior to chromatography to minimize the possible interference of DNA, which has been shown to have some affinity to cellulose (*5*). After the addition of the samples, the column was washed with a buffer solution (10 m*M* tris-HCl, *p*H 7.6; 500 m*M* KCl; 0.2 m*M* $MgCl_2$) at room temperature until no further elution of radioactive RNA could be detected. The adsorbed RNA was then eluted from the cellulose with distilled water (*4*). The amount of radioactivity in each fraction was determined as described (*7*). A portion of the RNA product from adult brain nuclei, synthesized under either ionic condition, was retained by the cellulose. Approximately 10 percent of the RNA made in the presence of Mg^{2+} and 12 percent of that synthesized in the presence of Mn^{2+} with $(NH_4)_2SO_4$ remained bound to the cellulose column, and was freed only when the eluent was changed from buffer to distilled water (Fig. 1). In order to be assured that homopolymers [poly(A)] remained quantitatively bound to the cellulose columns during the initial buffer elution, 1.0 absorbancy ($A_{260\ nm}$) unit of poly(A) was added to the cellulose column and chromatographed as described. The poly(A) was retained by the cellulose during extensive washing with the buffer solution. However, like the bound fraction of radioactive nuclear RNA, the poly(A) was easily eluted from the column with water (Fig. 1A, stippled bar). Since our RNA preparations might have been contaminated with free radioactive GTP, a sample of [^{3}H]GTP was also run on a cellulose column to determine its elution pattern. The results showed that the radioactive precursor was eluted from the column during the initial wash with the buffer solution and would not have contaminated the bound material (Fig. 1A, hatched bar).

Table 1. Effect of postnatal brain development on the synthesis of poly(A)-containing RNA by isolated nuclei. Nuclei, isolated from brain tissue of mice of various ages by differential centrifugation through sucrose (*1*), were incubated in reaction mixtures (1.0 ml) containing 40 μmole of tris-HCl, *p*H 8.6; 70 μmole of KCl; either 8 μmole of $MgCl_2$ or 0.5 μmole of $MnCl_2$ (*9*); 1.6 μmole of phosphoenolpyruvate; 1 to 2 enzyme units of pyruvate kinase; 0.1 μmole each of adenosine, cytidine, and uridine triphosphates; 25 μg of polyvinyl sulfate; 2.0 μc of [^{3}H]guanosine triphosphate (5.66 c/mmole) and nuclei (100 to 250 μg DNA). When incubations were carried out at high ionic strength conditions, 150 μmole of $(NH_4)_2SO_4$ was added. Nuclei were incubated for 20 minutes at 22°C. Prior to chromatography, 50 μg of electrophoretically purified deoxyribonuclease were added to each reaction mixture, the tubes were gently mixed and reincubated at 37°C as described (*1*). Labeled RNA was extracted with phenol, in the presence of 16 m*M* EDTA (*p*H 7.8) and 0.5 percent sodium dodecyl sulfate, and repeatedly precipitated with ethanol (*9*).

Age (days)	Mg^{2+}			$Mn^{2+}/(NH_4)_2SO_4$		
	Total RNA (count/min)	Poly(A)-containing RNA		Total RNA (count/min)	Poly(A)-containing RNA	
		Count/min	Percent of total		Count/min	Percent of total
1–2	15,030	2,730	18	42,790	7,060	16
3–5	35,280	5,830	16			
12	63,090	8,380	13	48,640	4,470	9
30	47,100	3,400	7	30,900	2,520	8
Adult	46,760	4,860	10	38,750	4,570	12

These results are interesting in view of the observations of Schutz *et al.* (*4*) who showed that RNA molecules for both globin and ovalbumin were retained on cellulose columns until their elution with water. So far as we know, our study is the first to demonstrate that isolated nuclei, incubated under cell-free conditions, can synthesize RNA that presumably is associated with poly(A) sequences. This would suggest that the enzyme responsible for the addition of adenylic acid residues to the terminus of eukaryotic mRNA's is localized in the nucleus and is retained during nuclear isolation and purification.

In order to determine whether there were maturation-dependent alterations in the proportion of poly(A)-containing RNA to the total RNA products, nuclei were isolated and purified from mouse brain tissue at various stages of postnatal development. In each case the nuclei were incubated in cell-free reaction mixtures which contained either Mg^{2+} or the Mn^{2+} with $(NH_4)_2SO_4$. Regardless of the age of mouse, a portion of the [^{3}H]GTP incorporated into its nuclear RNA was always associated with poly(A) sequences and bound to the cellulose column (Table 1). However, under both ionic conditions, the RNA synthesized by nuclei isolated from neonatal brain tissue, as compared to more mature neural tissue, appeared to have a greater proportion that remained bound to the cellulose column during the initial wash with the buffer solution. This result is consistent with the observation of DeLarco and Guroff (*8*), who found that RNA synthesized in brain slices from young rats and in vivo appeared to contain a greater percentage of molecules that bound to oligo(deoxythymidylate)-cellulose columns.

The relative proportion of brain RNA that remained bound to the cellulose columns (7 to 18 percent) was very similar to that reported with intact mammalian cells (*3*). These techniques could be applied to examine the RNA products synthesized by the specific RNA polymerases that have been identified in cell nuclei (*6*), as well as to study the control of their subsequent translation (*4*).

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30 April 1973; revised 18 June 1973