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RNA synthesis in isolated nuclei: the use of mercurated nucleotides

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ABSTRACT

We have used uridine 5' triphosphate-5-mercury (Hg-UTP) in place of UTP to study RNA synthesis in a previously described isolated nuclei system (1). Employing isopycnic density gradient centrifugation to separate RNAs based upon their relative content of Hg-U, several conclusions can be drawn. *In vitro* RNA synthesis consists of end addition onto pre-initiated HnRNA molecules as well as apparent initiation of new HnRNA molecules *de novo*. Synthesis in our system continues linearly for greater than two hours. The chain elongation rate has been measured to be about 500 nucleotides per minute. The methods used to make these measurements are generally applicable to other *in vitro* systems.

INTRODUCTION

The use of mercury-substituted nucleotide triphosphates in the study of transcription *in vitro* was first suggested by Dale *et al*. (2,3,4) and it has been applied to the analysis of transcription in isolated nuclei and chromatin (5-10). In all these studies, RNA molecules synthesized in the presence of mercury-substituted ribonucleoside triphosphates were separated from pre-existing RNA molecules by affinity chromatography on thiol-agarose columns. This separation method does not distinguish RNA molecules according to their content of Hg-nucleotide since mercuration of as little as 0.5 - 1.0% of the totoal nucleotides is sufficient to obtain quantitative retention on the resin (4). Thus pre-existing molecules that have been extended *in vitro* with Hg-nucleotide cannot be separated from molecules initiated *de novo* and contain only Hg-nucleotide.

Dale and Ward (3) showed that mercurated polynucleotides could be separated from unmercurated polynucleotides by isopycnic density gradient sedimentation. In this report we present an application of this principle to the study of RNA synthesis in the isolated nuclei system perviously described (1). However the method has general applicability. Previous studies using the nuclei system suggested that HnRNA and mRNA-like molecules were being produced *in vitro* and preliminary analysis of this system using the

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method described here is consistent with that suggestion.

MATERIALS AND METHODS

Materials -- [^{3}H]GTP (10 Ci/mmole), α-[^{32}P]GTP (30 Ci/mmole) and [^{14}C]GTP (330 mCi/mmole) were purchased from New England Nuclear Corp; ATP, CTP, GTP, UTP, amino acids, yeast tRNA and S-adenosylmethionine from Sigma; creatine phosphate and creatine phosphokinase from Calbiochem; uridine-5'-triphosphate-5-mercury was either prepared according to the procedure of Dale et al. (2,3) or purchased from Calbiochem. Angio Conray (Na-iothalamate) was from Mallinckrodt.

Cell culture and isolation of nuclei -- Myleoma MOPC 315 cells were grown in suspension and nuclei were isolated as previously described (1).

Preparation of cytoplasmic extracts -- Cytoplasmic extracts were prepared from myeloma cells as previously reported (1) except that the preincubation and the Sephadex G25 steps were omitted.

Conditions for RNA synthesis -- RNA synthesis in isolated nuclei was carried out as described (1) except that 2-mercaptoethanol was added to the reaction mixture at a final concentration of 10 mM, $MnCl_2$ to 0.5 mM and EGTA to 50 μM. In the experiments where mercurated RNA was synthesized, UTP was replaced by Hg-UTP at the indicated concentrations.

RNA extraction -- After incubation, the nuclei were separated by centrifugation (1) and resuspended into an equal volume of 25% glycerol containing 5 mM Mg acetate, 0.1 mM EDTA, 5 mM 2-mercaptoethanol and 50 mM Hepes NaOH pH 7.5. Each sample was then made up to 1% SDS, 200 mM NaCl and 50 mM Tris HCl pH 7.5 and 50 mM EDTA, and diluted ten fold with the same buffer. The mixture was incubated at 37°C for 1-2 hours with occasional stirring. After addition of 2-mercaptoethanol to 10 mM,RNA was extracted by the phenol/chloroform procedure described by Penman (11) except that the extraction was carried out at room temperature and not at 60°C as described. The RNA was precipitated with ethanol, dried under reduced pressure, and resuspended into 10 mM Tris HCl pH 7.5, 10 mM NaCl, 10 mM EDTA and 0.5% SDS. The RNA was heated for 3 minutes at 65°C prior to sedimentation analysis.

Velocity sedimentation -- The RNA synthesized in vitro was separated according to molecular weight by sedimentation through 5 to 20% sucrose gradients. The RNA samples (0.2 ml) were layered over 4.4 ml of linear sucrose gradients (5 to 20%) formed over a 0.6 ml cushion of 40% sucrose. The gradients contained 0.5% SDS, 10 mM Tris HCl pH 7.5, 10 mM NaCl, 10 mM EDTA and were centrifuged for 150 minutes at 43,000 rpm and at 23°C in the

SW 50.1 Beckman rotor. After centrifugation fractions were collected from the bottom of the centrifuge tube and aliquots of each fraction were spotted on paper filters. The filters were washed with 5% TCA and ethanol, dried and radioactivity was measured in toluene scintillation liquid. All centrifugations were carried out in siliconized polyalamer centrifuge tubes which had been prewashed with a solution of mercurated nucleic acid prepared as described by Dale et al. (2).

Isopynic density sedimentation -- The density of the RNA synthesized in vitro in the presence of Hg-UTP was measured by sedimenting it to equilibrium, in sodium iothalamate (12). The RNA sample (0.1 ml) was mixed with 4.4 ml of the gradient solution that contained 48% (w/v) of the sodium salt of 5 acetamide-2,4,6-triiodo-N-methylisophthalamic acid (sodium iothalamate), 6 mM Tris HCl pH 7.6, 1 mM EDTA, 0.3% Sarkosyl, 0.6 mM Na_2HPO_4, and centrifuged in the SW 50.1 Beckman rotor for 72 hours at 36,000 rpm at 25°C. Aliquots from each fraction were spotted on paper filters, the filters were washed with cold ethanol, 5% TCA, ethanol, dried and then the radioactivity was measured in a toluene scintillation liquid. Aliquots from every fourth fraction were used to determine the refractive index. The latter parameter is proportional to the iothalamate concentration.

DNA extraction -- DNA was extracted from mouse liver by the procedure described by Berns and Thanes (13).

RESULTS

General properties of Hg-containing RNA -- Initial experiments were performed in order to examine the characteristics of RNA in which UMP is replaced by Hg-UMP. As seen in Figure 1A, RNA synthesized by E. coli RNA polymerase employing murine liver DNA as template and Hg-UTP in place of UTP, has a higher bouyant density in iothalamate. In fact, the density difference between "heavy" and "light" RNA (0.19 g/cc) allows for quantitative separation. As seen in Figure 1B, RNA may be de-mercurated essentially to completion by incubation of the RNA with 2-mercaptoethanol (0.5 M for 18 hr at 37°C).

To determine the affect of increased density, due to Hg content, on the sedimentation behavior, the following experiment was performed. The in vitro synthesized RNA was mixed with ^{14}C labeled 18S and 28S ribosomal markers and sedimented through a 5 to 20% linear sucrose gradient. The fraction corresponding to 18S was collected and split into two equal samples. One sample was demercurated and the second aliquot was incubated at 37°C for 18 hr

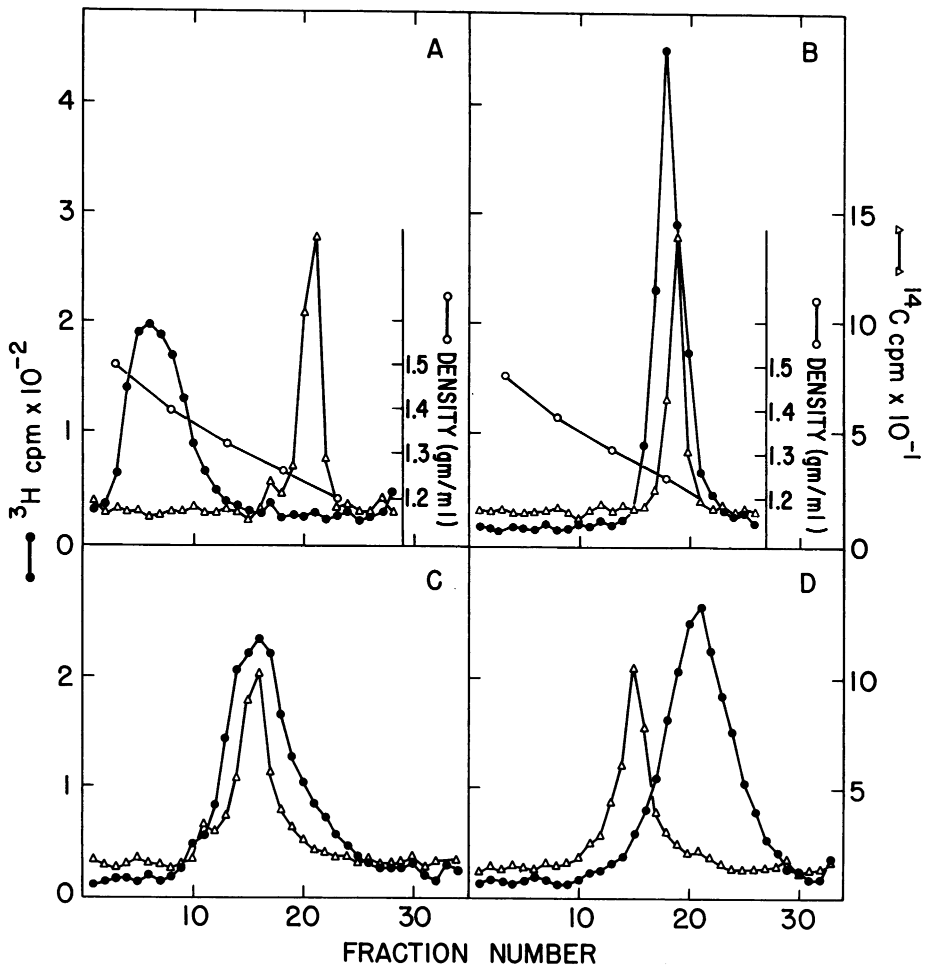

Figure 1. Sedimentation analysis of mercurated and unmercurated RNA. Fully mercurated RNA was synthesized by incubating mouse liver DNA with E. coli RNA polymerase in a reaction mixture that contained in 0.25 ml, 10% glycerol, 40 mM Tris HCl pH 7.9, 10 mM $MgCl_2$, 0.1 mM EDTA, 10 mM 2-mercaptoethanol, 50 mM KCl, 150 μM ATP, 150 μM CTP, 150 μM Hg-UTP, 150 μM GTP, 120 μCi [^{3}H]GTP, 140 μg/ml mouse liver DNA and 40 U./ml E. coli RNA polymerase. The RNA was mixed with ^{14}C labeled ribosomal RNA and sedimented through a 5 to 20% sucrose gradient. The RNA sedimenting at 18S was isolated and resuspended into 0.5% SDS, 10 mM Tris HCl pH 7.5, 10 mM NaCl and 10 mM EDTA and split into two equal samples. One sample was demercurated in 0.5 M 2-mercaptoethanol for 18 hours without the addition of 2-mercaptoethanol. An aliquot of each sample was centrifuged to equilibrium in iothalamate gradients and a second aliquot was resedimented in a 5 to 20% sucrose gradient. A) Equilibrium sedimentation of mercurated RNA; B) equilibrium sedimentation of demercurated RNA; C) velocity sedimentation of mercurated RNA; D) velocity sedimentation of demercurated RNA. ●—● [^{3}H]RNA synthesized in vitro in the presence of Hg-UTP; ▲—▲ [^{14}C]RNA ribosomal marker; o—o density.

without the addition of 2-mercaptoethanol. An aliquot of each sample was then re-sedimented in a second sucrose gradient. As shown in Figure 1, panels C and D, after demercuration the sedimentation rate of 18S RNA is decreased by 25%. The difference in sedimentation rate between Hg-containing RNA (density = 1.44 g/cc) and de-mercurated RNA (density = 1.25 g/cc) is expected. Knowing the density of any RNA sample partially substituted by Hg-nucleotide, its molecular weight may be calculated from its sedimentation coefficient after the latter is corrected. The following formula applies:

$$Sc = S_o - S_o \, 0.25 \, \frac{d - 1.25}{0.19}$$

where Sc is the corrected sedimentation coefficient, S_o is the observed sedimentation coefficient, d is the density of the RNA sample, 1.25 is the value of the density of unmercurated RNA and 0.19 is the difference between the density of a fully mercurated RNA and the density of an unmercurated RNA.

We attempted to utilize the density difference between "heavy" and "light" RNAs to analyze RNA produced in vitro in nuclei isolated from murine plasmacytoma cells. This system had been developed in order to analyze mRNA synthesis in vitro (1). Under conditions optimal for RNA synthesis, the substitution of Hg-UTP for UTP leads to a reduction in the rate of RNA synthesis. This effect has been noted by others (2-10). As seen in Figure 2, the effect on the rate reaches a plateau at about 50-55% inhibition at a concentration Hg-UTP of 1 mM.

Preliminary experiments designed to assess the quality of the RNA produced revealed that the density of the RNA synthesized in the presence of 0.22 mM Hg-UTP (40% inhibition) was not fully "heavy" as expected. This could be due to either of two possibilities. RNA chains cannot initiate in the system, thus all molecules have pre-existing, "light" 5' ends and "heavy" 3' ends or that there exists an endogenous pool of UTP of such proportion that all molecules, regardless of their origin, are not synthesized with only Hg-UTP. The results presented below suggest the latter. The density of the RNA produced is a function of the concentration of Hg-UTP employed thus indicating that the influence of the endogenous "light" pool on the density of the resultant RNA is diminished as the amount of Hg-UTP employed is increased. These results are summarized in Figure 3. It can be seen that although there are other density species evident (presumably mixed heavy-light molecules), RNA of fully heavy density can be achieved provided that Hg-UTP is added at a concentration of 1 mM (panel D). By extrapolation from the concentration of Hg-UTP required to achieve half heavy RNA (between panel A and

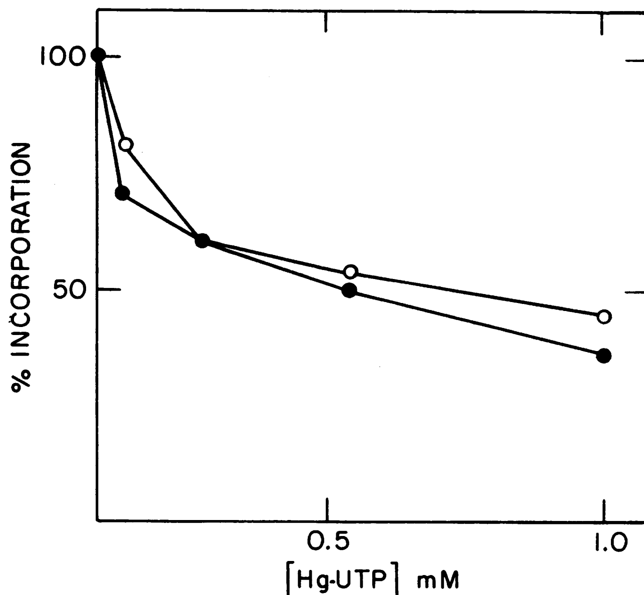

Figure 2. Inhibition of RNA synthesis by Hg-UTP. Isolated nuclei were incubated for 60 minutes under conditions for RNA synthesis in a 0.1 ml standard reaction mixture that contained [^{3}H]GTP and the indicated concentration of Hg-UTP. After incubation (30 minutes) the nuclei were pelleted and the supernatant was withdrawn. The nuclei were resuspended into 0.1 ml of 25% glycerol containing 5 mM Mg acetate, 0.1 mM EDTA, 5 mM 2-mercaptoethanol and 50 mM Hepes NaOH pH 7.5. The samples were made up to 1% SDS, 50 mM Tris pH 7.5, 200 mM NaCl and 50 mM EDTA, and diluted 10-fold with the same buffer. Aliquots from each sample were precipitated with TCA and radioactivity was measured. One hundred percent incorporation is the incorporation achieved with 250 μM UTP as a substrate when no Hg-UTP is added. •——• Percent incorporation into nuclei; o——o percent incorporation into supernatants.

D) and assuming that UTP and Hg-UTP are incorporated at the same rate, we can calculate the effective UTP pool size. This analysis suggests a pool size of 0.4 mM UTP.

We have measured the pool size of UTP by an independent method using isotope dilution. This was done by monitoring GTP incorporation relative to UTP incorporation as a function of radioactive UTP concentration. This analysis suggests an effective pool size of 0.05 mM. The discrepancy between these two results suggests that there is a preferential utilization of UTP over Hg-UTP by a factor of 8. However, we demonstrate below that the preferential utilization of UTP is constant during the entire incubation period and that UTP is not depleted prior to Hg-UTP incorporation. This can be seen by

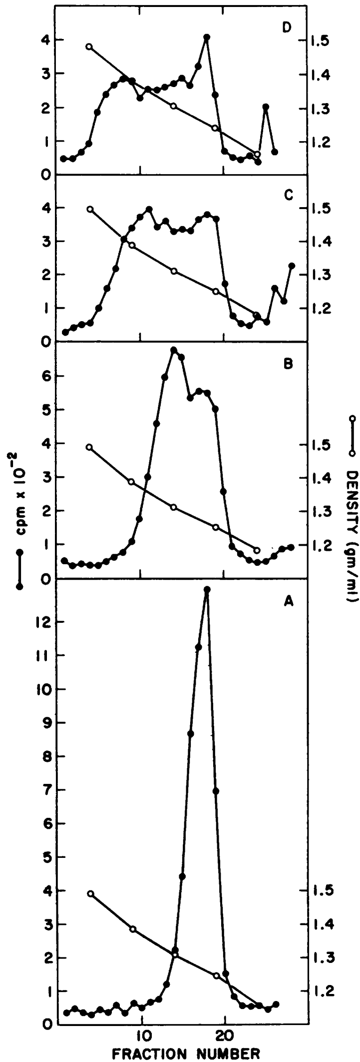

Figure 3. Buoyant density analysis of RNA synthesized in the presence of increasing concentrations of Hg-UTP. RNA was extracted with phenol/chloroform from each of the samples prepared for experiment of Fig. 1. Aliquots containing the same amount of radioactivity were analyzed by equilibrium sedimentation in sodium iothalamate gradients as described under Materials and Methods. A) RNA synthesized in the presence of 50 μM Hg-UTP; B) 220 μM Hg-UTP; C) 550 μM Hg-UTP; D) 1 mM Hg-UTP.

examining the results in Figure 4. The density value of the "heaviest" RNA appearing early during incubation (presumably synthesized almost entirely *de novo*) is the density at which all subsequent RNA appears during further incubation (compare panel B with C and D). Since the rate of RNA synthesis as measured by GTP incorporation is linear for 120 min and "heavy" RNA appears at 30 min of incubation, we would expect RNA of a heavier density than that seen at 30 min to appear at 120 min; this is not the case. Thus we can assume that employing any particular Hg-UTP concentration, the "heavy" density material (90 min of incubation) arising by the appropriate mixing of endo-

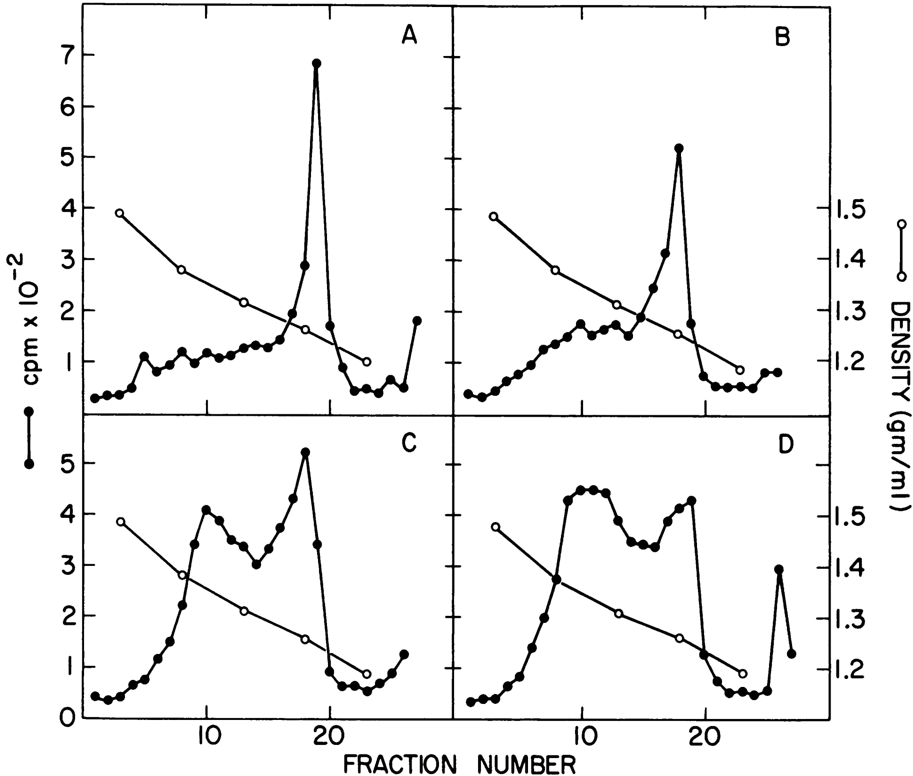

Figure 4. Kinetics of Hg-UTP incorporation. Nuclei were incubated in reaction mixtures that contained 500 µM Hg-UTP. At the indicated times the reactions were stopped, the nuclei were pelleted and RNA prepared. Aliquots were mixed with the iothalamate solution as indicated under Materials and Methods and centrifuged to equilibrium. Panels A, B, C and D show the density distribution of RNA synthesized at 15, 30, 60 and 120 minutes respectively.

genous UTP and Hg-UTP is a measure of de novo synthesized RNA. Typically, the Hg-UTP concentration used was 0.5 mM which usually gave a "heavy" density of 1.35 g/cc. This density difference is sufficient for analytical purposes (see below).

Kinetic analysis of in vitro synthesized RNA -- The accumulation of heavy RNA synthesized in vitro in the presence of Hg-UTP requires prolonged synthesis. Nuclei were incubated in standard reaction mixtures that contained 500 µM Hg-UTP; RNA accumulated linearly til 120 min. At different times of incubation, RNA was prepared from the nuclei and analyzed by equilibrium density centrifugation in iothalamate gradients (Fig. 4). At fifteen minutes of incubation most of the radioactivity bands with the

density expected for unmercurated RNA. With increasing time of incubation, the density of a portion of the *in vitro* synthesized RNA increases. When the heaviest density achievable under the experimental conditions is reached (1.35 g/cc), RNA accumulates continually at this density. This result is what is expected if *in vitro* synthesis consists of completion of pre-existing molecules (banding light), considerable extension of shorter molecules (banding at intermediate density), and elongation of molecules just initiated or ones initiated *de novo* (banding heavy). Further support for the above conclusion was obtained by examining the density of various size classes of RNA synthesized for a fixed period of time.

If all size classes of RNA molecules are synthesized and elongated at the same rate, we should expect short molecules synthesized in the presence of Hg-UTP to be denser than larger molecules synthesized under the same conditions for the same period of time. The larger molecules will only have a "tail" of "heavy" label, shorter molecules will have a higher proportion of heavy nucleotide and molecules just begun will be completely "heavy". In order to verify this hypothesis, nuclei were incubated for 60 minutes in the presence of Hg-UTP, RNA was extracted from the nuclei, sedimented through 5 to 20% sucrose gradients and different size classes of RNA were isolated as indicated in the insert of Figure 5. Each RNA fraction was then analyzed by equilibrium sedimentation in sodium iothalamate gradients. As can be seen from Figure 5, shorter RNA molecules do indeed have an average density greater than longer molecules. Molecules of 40S in size are essentially light (panel A), and molecules of 18S in size have approximately 50% fully heavy RNA. Molecules in panel F show an aberrant density distribution because they are too low in molecular weight to form condensed bands.

To verify that Hg-UTP is incorporated into RNA molecules as heavy "tails" on light molecules, the following experiment was performed. Nuclei were incubated for 15 minutes in the presence of Hg-UTP, RNA was extracted and sedimented through a sucrose gradient. All the RNA sedimenting faster than 30S was pooled. This RNA,which has a density of 1.25 g/ml (identical to the density of unmercurated RNA, Figure 6A) was partially digested with alkali and analyzed by equilibrium density gradient. Mild alkali treatment of large RNA molecules elongated in the presence of Hg-UTP but still having a density of, or close to that of unmercurated RNA, release molecules with densities up to 1.35 g/ml. This is the density of fully mercurated RNA synthesized under the conditions described in the legend to Figure 6. In fact, after the appropriate digestion time (6C), none of the radioactivity bands at a light posi-

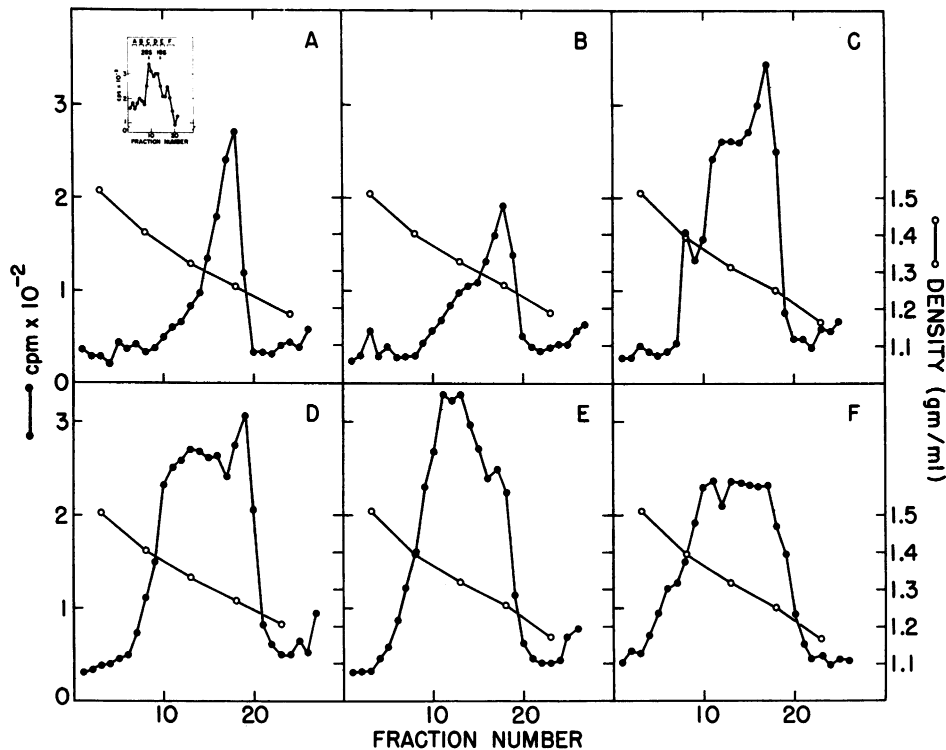

Figure 5. Density of mercurated RNA as a function of size. Nuclei were incubated for 60 min in a reaction mixture that contained Hg-UTP in a final concentration of 500 μM. After incubation, RNA was extracted from the nuclei and sedimented through a 5 to 20% sucrose gradient. Fractions were pooled as indicated in the insert,ethanol precipitated and sedimented to equilibrium in iothalamate gradients. Each panel shows the density distribution of the RNA from the corresponding fraction of the sucrose gradient; panel A greater than 40S to panel F less than 10S.

tion. This result further supports the assumption that Hg-UTP and endogenous UTP are incorporated at constant rates. If this were not the case, the radioactivity in this experiment (GTP),which is indifferent to the selection of Hg-UTP or UTP,would show a "light" component. Thus it appears that the density of an RNA molecule may be used to measure the fractional length synthesized *in vitro* using Hg-UTP.

It follows from the above result, that the rate of chain elongation in this system may be measured by analyzing the size and density of *in vitro* synthesized molecules.

Rate of chain elongation -- The shortest time in which an RNA molecule

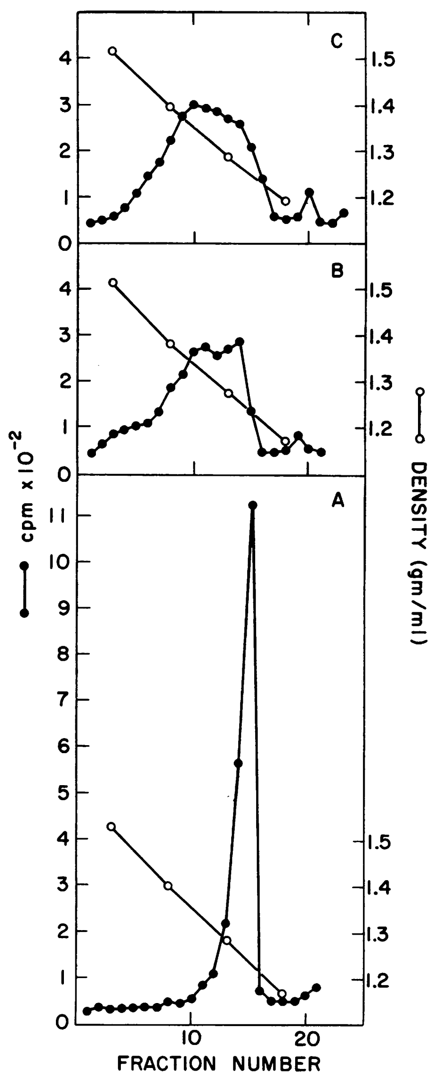

Figure 6. Alkali digestion of mercurated RNA. Nuclei were incubated for 15 min in a 0.2 ml reaction mixture containing 350 µM Hg-UTP. RNA was extracted from the nuclei and sedimented through a 5 to 20% sucrose gradient. All the RNA sedimenting faster than 30S was pooled, ethanol precipitated, and resuspended into 1.2 ml of 10 mM Tris HCl pH 7.5, 10 mM EDTA, 10 mM NaCl, 0.1% SDS. The RNA was divided in three equal aliquots which were chilled on ice for 10 min. One aliquot was mixed with 0.2 ml H_2O and 0.15 ml of Na acetate pH 5.1 and kept on ice (0 time). The second and third aliquots were mixed with 0.1 ml of 1 N NaOH and incubated on ice for 10 and 20 min respectively. Digestion was stopped by adding 0.1 ml of 1 N HCl and 0.15 ml of Na acetate pH 5.1. The RNAs from the three samples were precipitated with ethanol, dried and resuspended into 0.1 ml of 10 mM Tris HCl pH 7.5, 10 mM NaCl, 10 mM EDTA and 0.5% SDS and sedimented to equilibrium in sodium iothalamate gradients. A) 0 time; B) 10 minutes digestion; C) 20 minutes digestion.

of given length reaches the density of fully mercurated RNA, when the synthesis is carried out in the presence of Hg-UTP, is a measure of the rate of chain elongation. We performed such an analysis for 18S and 28S RNA molecules. Nuclei were incubated in the presence of Hg-UTP and at the indicated times RNA was extracted and sedimented through sucrose gradients. RNAs cosedimenting with 18S and 28S markers were isolated and analyzed by equilibrium density centrifugation. As is evident from Figure 7, already at 15 minutes of incubation, 28S RNA molecules begin to accumulate at a density of 1.36 g/ml

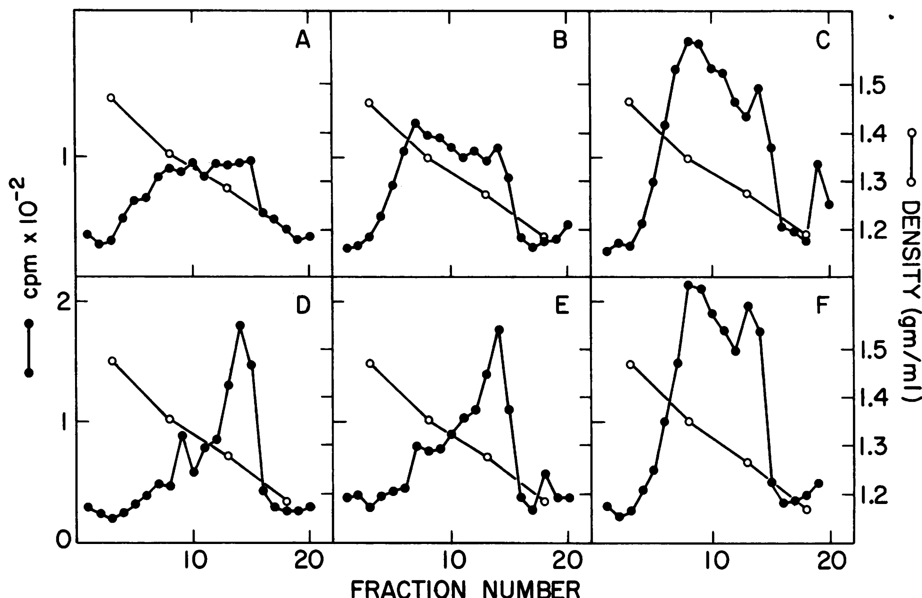

Figure 7. Rate of chain elongation. Nuclei were incubated for 15, 30 and 60 minutes in 0.1 ml reaction mixtures containing 500 μM Hg-UTP. RNA was extracted from the nuclei and sedimented through a 5 to 20% sucrose gradient. Two fractions from each gradient corresponding to 18S and 28S respectively were collected and resuspended in 0.1 ml of 10 mM Tris HCl pH 7.5, 10 mM EDTA, 10 mM NaCl, 0.1% SDS. The RNAs were sedimented to equilibrium in iothalamate gradients. Panels A, B, C show the density profile of 18S at 15, 30 and 60 minutes of incubation respectively; panels D, E, F show the density profile of 28S RNA at 15, 30 and 60 minutes of incubation respectively.

(fully mercurated RNA). As expected, it takes twice as long for 28S molecules that are heavy to accumulate. Making the necessary corrections due to the different sedimentation properties of mercurated and unmercurated RNA, we calculate that the rate of chain elongation is 240 nucleotide per minute when synthesis is carried out in the presence of 500 μM Hg-UTP. Under these conditions the rate of RNA synthesis is 50% inhibited (Figure 2). We therefore conclude that RNA synthesis in the isolated nuclei system proceeds at a rate of 480 nucleotide per minute. Synthesis is continuous for some molecules at the high chain elongation rate in that "fully dense" molecules of 10-20,000 nucleotides in length are observed in this system.

DISCUSSION

We have described a method whereby RNA synthesized *in vitro* may be analyzed in a new way. The method allows for quantitative separation, based on bouyant density, of RNA synthesized in whole, or in large part, *in vitro*.

With greater care, molecules may be separated according to their fractional content of nucleotide added *in vitro*. New RNA is synthesized substituting Hg-UTP for UTP and is therefore more dense than pre-existing RNA molecules. RNA fractionally synthesized *in vitro*, *i.e*. end addition to pre-existing molecules, has a density intermediate between "heavy" and "light". The former class is readily separated from other species. Molecules of intermediate density may result from end addition or from aggregation of new (heavy) RNA to pre-existing (light) RNA. Under the experimental conditions outlined however, we have not seen evidence of aggregation. Molecules obtained from a given portion of an iothalamate gradient can be re-banded and show no shift from their original density even after mild denaturation conditions. RNA obtained containing any Hg-UTP can be demercurated without degradation and used for further analysis. Thus the use of Hg-UTP has advantages over other "heavy" nucleotide analogues.

Problems that have been encountered with the system are related to the reactivity of Hg-containing RNA. Irreversible aggregation with protein and other RNA has been seen. This can be avoided, however, by working in the presence of low concentrations of monofunctional thiol reagents. High concentrations of thiols and elevated temperatures prevent aggregation but lead to demercuration. A detailed examination of this problem has been reported (14). We have found that following determination of "heavy" density for a particular preparation of nuclei, that density is invariant under the experimental conditions reported. We do not specify the mole percent substitution of Hg-UTP for UTP for a given density but operationally define "fully heavy".

An obvious problem is the use of Hg-UTP in place of UTP. It has been reported (10) that the use of Hg-UTP impairs elongation as well as post transcriptional modifications of RNA. In the system described here, molecules of greater than 10,000 nucleotides in length have been recovered from sucrose gradients and shown to be fully heavy. We have not yet examined the maturation of RNA into which Hg-UTP had been incorporated. We have previously shown (1) that this system does give rise to polyadenylated and "capped" RNA as well as polysomal mRNA-like molecules. Recent studies (in preparation) indicate that indeed *de novo* initiation is occuring in this system. We are presently studying the effects of Hg-UMP containing RNA on all of these processes.

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REFERENCES

1 Mory, Y.Y. and Gefter, M.L. (1977) Nucleic Acids Res. 4, 1739-1757.
2 Dale, R.M.K., Livingston, D.C. and Ward, D.C. (1973) Proc. Nat. Acad. Sci. USA 70, 2238-2242.
3 Dale, R.M.K., Martin, E., Livingston, D.C. and Ward, D.C. (1975) Biochemistry 14, 2447-2457.
4 Dale, R.M.K. and Ward, D.C. (1975) Biochemistry 14, 2458-2469.
5 Smith, M.M. and Huang, R.C.C. (1976) Proc. Nat. Acad. Sci. USA 73, 775-779.
6 Crouse, G.F., Fodor, E.J.B. and Doty, P. (1976) Proc. Nat. Acad. Sci. USA 73, 1564-1567.
7 Beissmann, H., Gjerset, R.A., Levy, W.B. and McCarthy, B.J. (1976) Biochemistry 15, 4356-4362.
8 Beebe, T.J.C. and Butterworth, P.H.W. (1976) Eur. J. Biochem. 66, 543-548.
9 Zasloff, M. and Felsenfeld, G. (1977) Biochemistry 16, 5135-5145.
10 Schafer, K.P. (1977) Nucleic Acids Res. 4, 4465-4479.
11 Penman, S. (1966) J. Mol. Biol. 17, 117-130.
12 Serwer, P. (1975) J. Mo.. Biol. 92, 433-448.
13 Berns, K.I. and Thomas, C.A. (1965) J. Mol. Biol. 11, 476-490.
14 Van Broeckhoven, C. and De Wachter, R. (1978) Nucleic Acids Res. 5, 2133-2152.