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Pattern of Cytoplasmic RNA in Brain and Liver of Immature Rats (39897)

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In the immature rat, protein is synthesized more rapidly than in the adult. Protein synthesis occurs on cytoplasmic ribosomes which contain two RNA components, one associated with a large subunit and the other with a smaller subunit. Ribosomal RNA metabolism undergoes distinctive changes during cellular maturation. Synthesis of rRNA parallels growth rate in both liver (1) and brain (2). This increase in rRNA synthesis is reflected by an enlargement of nucleoli in both liver and brain cells (1). In addition, the electrophoretic profiles of nucleolar and extranucleolar RNA are similar in both tissues (3). Finally, the half-life of rRNA is about 6 days in brain (4) and very close to this in liver (5, 6). In view of the role of ribosomes in protein synthesis and the role of rRNA as an integral component of ribosomes, we have examined the nature and appearance of the major RNA species in rat brain and liver cytoplasm during the actively growing period.

Materials and methods. Liver, cerebrum, and cerebellum were removed from 14- and 20-day-old Sprague-Dawley rats and compared with the same tissues from adult animals (weighing 150 to 200 g). When radioactivity measurements were part of the experimental design, rats were injected with 5 μCi of [^{14}C]orotic acid (49.1 mCi/mmole) per 100 g of body weight via the tail vein. Young rats were decapitated 0.5, 1, 3, and 6 hr after injection, and mature animals were decapitated 25, 45, and 90 min, and 2, 4, and 8 hr after injection. Livers were pooled (four to six), pressed, and homogenized in 3 vol of 0.25 *M* sucrose; TKM buffer (10 m*M* Tris, pH 7.4, 10 m*M* KCl, 2 m*M* $MgCl_2$). Individual cerebra or cerebella were pooled to give at least 5 g of tissue of each and they were treated the same as liver. Homogenate was centrifuged at 12,000*g* for 10 min at 5°. About 80% of the supernatant was aspirated from the middle, 10% sodium deoxycholate was added to the supernatant to a final concentration of 1%, and aliquots were layered over 1.0 *M* sucrose; TKM buffer. Ribosomes were pelleted by centrifugation at 95,000*g* for 2 hr at 5°. RNA was extracted from pellets using a slight modification of the dodecyl sulfate–phenol–chloroform extraction procedure (7, 8). Modifications included shaking the aqueous and organic phases for 15 min at room temperature. Gel electrophoresis was by the method of Loening (9). RNA (20 μg) in phosphate buffer and 10% sucrose was layered on each 2.3% polyacrylamide gel and electrophoresed for 3.5 hr at 4 mA/gel. Gels were removed from their tubes and absorbance at 260 nm was measured with a Gilford model 240 spectrometer with model 2410 linear transport. Gels were then sliced into 2-mm sections and placed in a scintillation minivial (7-ml capacity). Each section was allowed to incubate at 50° overnight in a 0.4 ml of NCS tissue solubilizer (Amersham/Searle). This treatment hydrolyzes the RNA and causes the slices to swell sufficiently to allow nucleotides to diffuse out of the slice. After incubation, 5 ml of Liquifluor–toluene mixture [Liquifluor (New England Nuclear) was diluted in toluene to give 5 g of PPO/liter] was added to each vial for radioactivity determination.

In all enzyme studies, 100 μg of RNA was treated with 20 μg of enzyme. Chymotrypsin (EC 3.4.4.5) was prepared in 0.2 *M* NH_4HCO_3 buffer, pH 8.4. DNase (EC 3.1.4.6) was prepared in 50 m*M* $MgCl_2$ and 50 m*M* $NaC_2H_3O_2$ buffer, pH 4.6. RNase (EC 2.7.7.16) was prepared in 0.1 *M* $NaC_2H_3O_2$ buffer, pH 5.0. KOH was made 0.3 *N*. All incubations were at 37° for 45 min. Enzyme reactions were stopped by placing test tubes in ice water and by adding Na_2EDTA.

Results. Figure 1 shows a typical absorbance and radioactivity scan of liver cytoplas-

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mic RNA in adult and 14-day-old animals. The major cytoplasmic RNA species in adult and growing tissues were 28 and 18S rRNA. When specific activity was calculated from the electropherograms as typified in Fig. 1 and plotted against time after injection of [^{14}C]orotic acid, it was seen (Fig. 2) that the specific activity in both 18 and 28S rRNA was significantly greater in the immature liver.

Two molecules migrating slower than 28S rRNA were consistently noted on the electropherograms of cytoplasm from immature tissues which were not present in the corresponding region of adult tissues (Fig. 1).

To study the temporal occurrence of these RNA species in the cytoplasm of immature animals, liver, cerebral, and cerebellar cytoplasm from 14- and 20-day-old rats were examined. Figure 3 shows that the two peaks migrating slower than 28S were present at both 14 and 20 days in liver, they were present in trace amounts in 20-day-old cerebrum, and they were absent in 20-day-old cerebellum. Since the peaks heavier than 28S were present at 20 days of age, we conducted serial experiments to determine when these peaks disappear in liver. At days 28 and 42 no peaks heavier than 28S were detectable.

To elucidate the chemical nature of these heavier-than-28S peaks, we extracted cytoplasmic RNA from livers of 14-day-old rats and incubated the extract with DNAse, chymotrypsin, KOH, and RNase. Figure 4 demonstrates that DNase and chymotrypsin exhibited no enzymatic activity on the two peaks heavier than 28S. Addition of KOH or RNase to the incubation medium resulted in complete hydrolysis of the two heavier peaks. These data demonstrate that these two heavy peaks are composed of RNA. To eliminate the possibility that the two RNAs heavier than 28S might be nuclear RNAs "leaking" from more fragile immature nu-

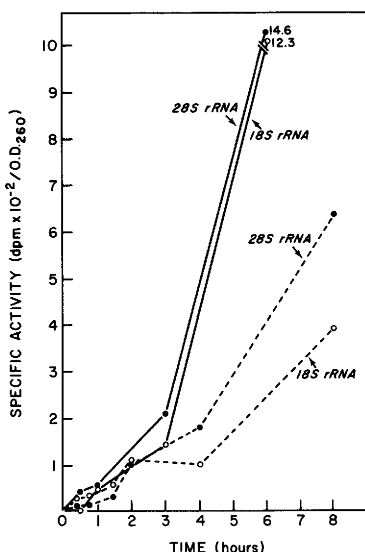

FIG. 2. Specific activities of liver cytoplasmic 18S rRNA (○) and 28S rRNA (●) from adult (- - -) and 14-day-old (——) rats. Experimental conditions are the same as those described in Fig. 1. Specific activities were calculated from the center of the peaks in the absorbance and radioactivity profiles.

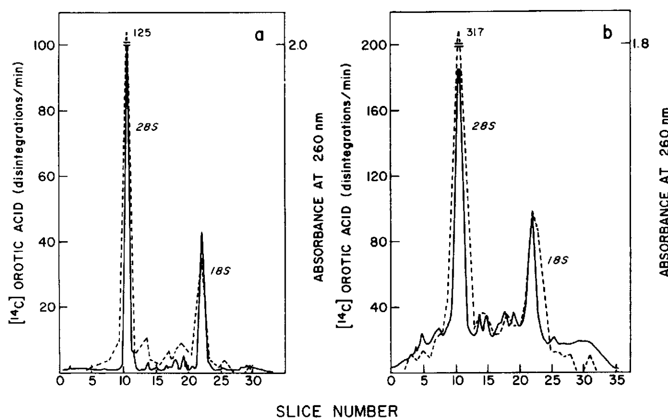

FIG. 1. Gel electrophoresis of liver cytoplasmic RNA from adult (a) and 14 day-old (b) rats. (——) Absorbance at 260 nm; (-----) [^{14}C]orotic acid radioactivity. Rats were injected with 5 μCi of [^{14}C]orotic acid/100g of body weight via the tail vein and were killed 24 hr later. Other conditions are detailed in text.

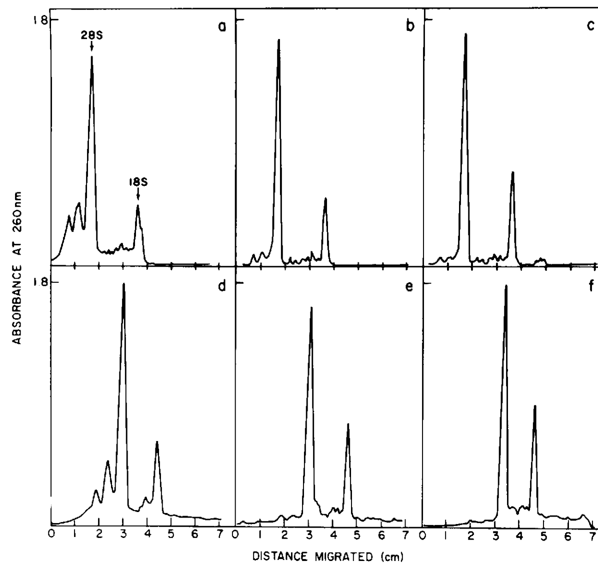

FIG. 3. Gel electrophoresis of cytoplasmic RNA from liver, cerebrum, and cerebellum of 14-day-old rats (a–c) and 20-day-old rats (d–f). Conditions for electrophoresis are described under Materials and Methods.

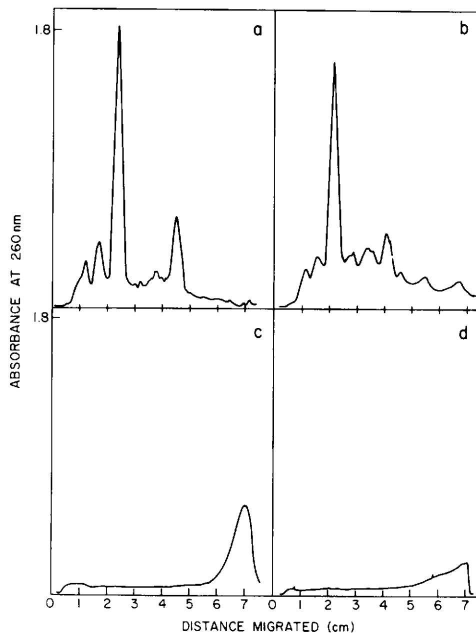

FIG. 4. Incubation of 14-day old-liver cytoplasmic RNA with DNase (a), chymotrypsin (b), KOH (c), and RNase (d). Conditions for electrophoresis are described under Materials and Methods.

clei during the extraction process, [^{14}C]-orotic acid was injected into 20-day-old animals, livers were removed 30 min later, and cytoplasmic RNA was extracted from ribosomes. These data demonstrate no radioactivity in 28 and 18S rRNA and similarly no radioactivity in the two heavier peaks. Since nuclear RNA is markedly labeled by this time, we have concluded that no nuclear leakage is taking place during the extraction period and that these two heavier-than-28S peaks represent cytoplasmic RNA.

Discussion. We have initiated a study of cytoplasmic RNA metabolism by studying the appearance of newly synthesized RNA in cytoplasm of immature liver. Both 28 and 18S rRNA accumulate more rapidly in cytoplasm of liver from immature rats than in that from mature rats. In the mature rat 18S rRNA specific activities of 0.3 and 0.4 were measured at 25 and 45 min after injection of [^{14}C]orotate. The corresponding values for 28S rRNA were 0.1 and 0.2. The time of appearance of label in cytoplasm of the adult rat and the rapid labeling of 18S rRNA agree well with previous reports (10, 11). At 30 and 60 min after labeling, the specific activities of 18 and 28S rRNA were 0.1, 0.5, and 0.4, 0.6, respectively, in the immature rat.

During the course of these experiments two peaks heavier than 28S were consistently noted on the electropherograms of cytoplasm from 14- and 20-day-old liver and cerebrum, and from 14-day-old cerebellum which were not present in the corresponding

adult tissues. Enzyme studies confirmed that the peaks heavier than 28S are indeed RNA molecules. An estimation of S values based upon electrophoretic mobilities places these peaks at approximately 40 and 34S. We have measured nuclear RNA-specific activities of greater than 20 at 25 min after injection of [^{14}C]orotate (unpublished data), and the absence of radioactivity in these peaks 30 min after injection of [^{14}C]orotic acid strongly suggests that these heavy RNAs are cytoplasmic components.

Other workers have reported the existence of cytoplasmic components other than 28 and 18S rRNA in liver (12) and brain (13–18). We extend these observations by reporting the existence in immature liver and brain of cytoplasmic RNAs larger than 28S which could not be found in the same tissues as they mature. Our findings are in agreement with recent studies (13, 19, 20) which suggest that the cytoplasm from brain of immature rats contained RNA heavier than 28S, but are in discrepancy with an electrophoretic study (12) of newborn and fetal liver cytoplasmic RNA which failed to show any RNAs heavier than 28S. In the latter study, the use of concentrated 2.8% polyacrylamide gel and short electrophoresis time may have prevented larger RNAs from migrating into the gels.

Summary. The nature and appearance of RNA species in rat brain and liver cytoplasm have been examined by polyacrylamide gel electrophoresis during the actively growing period. Accumulation of newly synthesized 28 and 18S rRNA is more rapid in immature than in mature liver cytoplasm. Two species of RNA heavier than 28S exist in cytoplasm of immature cerebrum, cerebellum, and liver and disappear from these tissues as they mature. Values of S based upon electrophoretic mobilities are 40 and 34S for these heavy RNAs. Their exact nature and function during development are not known.

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