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Evidence for Post-transcriptional Stabilization of Ribosomal Precursor Ribonucleic Acid by Phenobarbital

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SUMMARY

The administration of phenobarbital (100 mg/kg) for either 16 hr or 4 days failed to increase the specific activity (disintegrations per minute per milligram) of rat hepatic nuclear 45 S RNA after a 20-min pulse of [^{3}H]orotic acid or [^{14}C]adenine. Prior treatment of rats with phenobarbital for 4 days increased the cellular content of nuclear 45 S RNA nearly 2-fold. Because no phenobarbital-induced elevations occurred in the labeling of nuclear 45 S RNA, an incubation system was devised to test for increased stability of 45 S RNA in phenobarbital-treated rats. Liver nuclei isolated from rats receiving 0.9% NaCl or phenobarbital for either 16 hr or 4 days were suspended in 0.25 M sucrose containing 5 mM $MgCl_2$ and incubated at 37° for 2 min. Planimetric analysis of sucrose gradient patterns of RNA extracted from these nuclei showed a 25% decrease in the optical density of the 45 S RNA from phenobarbital-treated rats, whereas the 45 S RNA from control rats was degraded by more than twice that amount. Increased metabolic stability of ribosomal precursor RNA, rather than enhanced synthesis, is associated with phenobarbital administration.

Labeling of cytoplasmic microsomal 18 S and 28 S RNA increased approximately 50% within 16 hr after a single dose of phenobarbital but was not significantly altered by 4 days of treatment. These results suggest that different mechanisms may exist for the effects of single and multiple doses of phenobarbital on ribosomal RNA metabolism.

INTRODUCTION

The stimulatory effects of a single dose or multiple doses of phenobarbital on the activity of drug-metabolizing enzymes in hepatic smooth endoplasmic reticulum may arise from reduced degradation of these enzymes, their enhanced synthesis, or both (1–5). Increased synthesis or decreased destruction of the membranes of the smooth endoplasmic reticulum (3, 6, 7) and ribosomes (8, 9) has been reported. Most of these phenobarbital-induced alterations have been measured after 3 or 4 days of drug treatment. The observation that such inhibitors of protein or RNA synthesis as ethionine, puromycin, and actinomycin D prevent induction of the drug-metabolizing enzymes suggests their enhanced synthesis *de novo* (6, 10–13). Controversy exists whether phenobarbital increases transcription of messenger RNA (14–18), ribosomal RNA (16, 19–21), or both.

Within 12 hr after a single dose of phenobarbital, Cohen and Ruddon (19) observed no increase in labeling of liver nuclear 45 S RNA (the ribosomal RNA precursor) that would account for the enhanced labeling of cytoplasmic ribosomal RNA (19, 20). On the other hand, after treatment of rats with phenobarbital for 4 days, Wold and Steele

(16) reported significant increases in the labeling and amount of liver nuclear and nucleolar 45 S RNA. To clarify these conflicting reports (16, 19, 21), this study was designed (a) to determine whether the increased amounts of nuclear 45 S RNA measured after 4 days of phenobarbital treatment result from increased synthesis, decreased breakdown, or both, and (b) to examine the dynamics of ribosomal RNA metabolism during the first 4 days of treatment with phenobarbital. Our findings suggest that phenobarbital does not stimulate the synthesis of ribosomal RNA, but enhances the stability of ribosomal precursor RNA and may subsequently increase the transport of ribosomal RNA from the nucleus to the cytoplasm.

MATERIALS AND METHODS

Drug treatment and labeling. Male Sprague-Dawley rats from Charles River Laboratories, weighing 100 or 200–250 g, received daily intraperitoneal injections of phenobarbital (75 mg/kg and 100 mg/kg, respectively) in 0.9% NaCl, or of NaCl alone, for 3 or 4 days. Under these conditions phenobarbital treatment caused liver weight to increase by 20–30%. All animals were fasted for 16 hr preceding death. All isotope and drug injections were given intraperitoneally. At various times before decapitation, for nuclear RNA studies, each rat that was treated as above received an injection of 20 μCi of [5-^{3}H]orotic acid (19.3 Ci/mmole, Schwarz BioResearch), 20 μCi of [8-^{14}C] adenine (50 mCi/mmole, New England Nuclear), or 40 μCi of [8-^{14}C]guanine (52 mCi/mmole, New England Nuclear). In short-term experiments rats were treated with a single injection of phenobarbital (100 mg/kg) and killed 16 hr later; for nuclear RNA studies, each rat received 20 μCi of [^{3}H]orotic acid 15 min before death or 20 μCi of [8-^{14}C]adenine 20 min before death. For microsomal RNA studies, each rat received 100 μCi of [5-^{3}H]orotic acid or 50 μCi of [8-^{14}C]adenine 4 hr before death. Excised livers were placed in ice-cold 0.25 M sucrose. All further steps were carried out at 4°.

Isolation of nuclei. For each time point or experimental group the livers from three or four rats were pooled and homogenized in 2.4 M sucrose containing 3.3 mM $CaCl_2$ (1:10, w/v) with four up-and-down strokes in a Teflon-glass homogenizer (0.015–0.020-inch pestle clearance). After filtration through four layers of cheesecloth, the homogenate was centrifuged at 40,000 × *g* for 75 min to sediment the nuclei (22–24). Nuclear preparations examined by phase contrast microscopy were found to be highly purified without cytoplasmic contamination or whole cells. No difference was observed between the purity of the isolated nuclei of phenobarbital-treated and NaCl-treated rats.

Chemical determination of RNA and DNA of nuclei. The extractions were performed on isolated nuclei in accordance with the technique of Fleck and Munro (25). The amounts of RNA and DNA were determined by the orcinol (26) and indole (27) reactions, respectively. From these values the RNA: DNA ratios were calculated.

Acid-soluble pool. Before centrifugation at 40,000 × *g*, 1 ml of the 2.4 M sucrose homogenate was homogenized in 4.5 ml of water and 2.8 ml of 0.6 N perchloric acid and then centrifuged at 2000 × *g* for 15 min. The precipitate was washed once with 0.2 N perchloric acid and centrifuged again. The supernatant solutions were pooled and assayed for absorbance at 260 nm and count rate (28); the specific activity of the acid-soluble pool was computed as disintegrations per minute/A_{260}.

In some experiments with labeled orotic acid, UMP was separated from the acid-soluble nucleotides by gradient elution chromatography on Dowex 1-formate columns, using 4 N formic acid and 4 N ammonium formate (29). The specific activity of this material was also computed (disintegrations per minute/A_{260}).

Isolation of nucleoli. The sedimented nuclei were suspended in 0.25 M sucrose (1:1, w/v) and sonicated for 30–40 sec in a Branson Sonifier (9–10 amp) (30). Then 40 ml of this suspension were layered over 40 ml of 0.88 M sucrose and centrifuged at 2000 × *g* for 20 min to sediment the nucleoli.

Isolation of microsomes. To prepare the cytoplasmic microsome fraction, minced livers from three or four rats were homogenized using a loosely fitting Teflon pestle

(0.030–0.040-inch pestle clearance) with three strokes in an ice-cold solution of 0.05 M Tris-HCl (pH 7.6) containing 0.005 M magnesium acetate, 0.025 M potassium chloride, and 0.25 M sucrose (1:10, w/v). After filtration through four layers of cheesecloth, an aliquot (10%) of the suspension was centrifuged twice at 12,000 × *g* for 20 min to remove nuclei, whole cells, and mitochondria. The remaining supernatant fluid was centrifuged at 100,000 × *g* for 2 hr to sediment the cytoplasmic microsomes.

RNA extraction. Nuclei, nucleoli, or microsomes were homogenized in 0.3% sodium dodecyl sulfate, 0.14 M NaCl, and 0.05 M sodium acetate, pH 5.1 (31, 32), for 1 min (15 strokes) with a loosely fitting Teflon pestle. After addition of 0.05 M sodium acetate-saturated phenol containing 0.1% 8-hydroxyquinoline (32), the sample was homogenized again for 1 min. The suspension was shaken successively at 65° for 10 min, and then for 20 min at room temperature (31, 33). For extraction of cytoplasmic microsomal RNA, heating at 65° was omitted.

The mixture was centrifuged at 17,000 × *g* for 10 min, and the aqueous phase was removed; this was followed by re-extraction of the aqueous phase with phenol at room temperature. The RNA was precipitated overnight at −20° with 2.5 volumes of ethanol containing 2% potassium acetate (34). The precipitated RNA was then dissolved in 0.01 M sodium acetate, pH 5.1, or H_2O, and analyzed spectrophotometrically for absorbance at 260 nm.

Sucrose gradients. Between 1 and 2 mg of RNA were layered over 10–40% sucrose gradients (38 ml) made up in 0.1 M NaCl, 1.0 mM EDTA, and 0.01 M sodium acetate, pH 5.1 (35). The gradients were centrifuged in a Spinco SW 27 rotor at 26,000 rpm for 15 hr at 5°. Analysis of gradients was carried out with the aid of an ISCO automatic fractionator system. Absorbance at 254 nm was transcribed with a Honeywell recorder in order to obtain enlarged, sharp peaks for planimetric analysis. Increases in the optical density baseline occurred as a function of increasing sucrose density. This was corrected for by subtraction of blank gradients. The slight upward drift of the baseline that occurs in sucrose density gradients was the same for RNA obtained from NaCl-treated and phenobarbital-treated rats.

Several parameters were measured. (a) Radioactivity patterns for the gradients were determined by hydrolyzing the individual 1-ml fractions in 0.25 M perchloric acid for 20 min at 70°. The samples were then counted in a liquid scintillation counter. (b) The specific activity of the 45 S RNA peak was determined by pooling the three gradient fractions which constituted the peak and by precipitating them in ethanol overnight as above. The precipitate was dissolved in water and its absorbance at 260 nm was measured; the radioactivity of the solution was then measured in fluor as above (28). (c) The proportion of 45 S RNA in each of the gradient profiles was calculated by determining the following: the total area included under the profile was either measured planimetrically (36, 37) or cut out and weighed on an analytical balance; the area under the 45 S RNA peak was determined similarly, and the ratio of the 45 S RNA to total RNA was then computed.

Incubation mixtures. Isolated nuclei or nucleoli from control and drug-treated animals were resuspended in 0.25 M sucrose containing 5mM $MgCl_2$ (0.4 ml/g of original wet weight of liver for nuclei and 0.08 ml/g for nucleoli) and divided into two fractions. One fraction remained in an ice bath, and the other was incubated at 37° for 2 min. The nuclei or nucleoli were pelleted by centrifugation; RNA was then extracted and analyzed on sucrose density gradients as described above.

RESULTS

Sucrose gradient profiles of nuclear RNA from phenobarbital-treated (250-g rats, 100 mg/kg, 4 days) and control animals are presented in Fig. 1. The mean ratios of the area of 45 S RNA to total optical density were 0.096 and 0.067 for drug-treated and control samples, respectively. The difference between these means is significant at the $p < 0.05$ level (Table 1) at day 4 of treatment, but not at earlier times.

To relate this increase in the proportion of 45 S RNA to amounts of ribosomal precursor

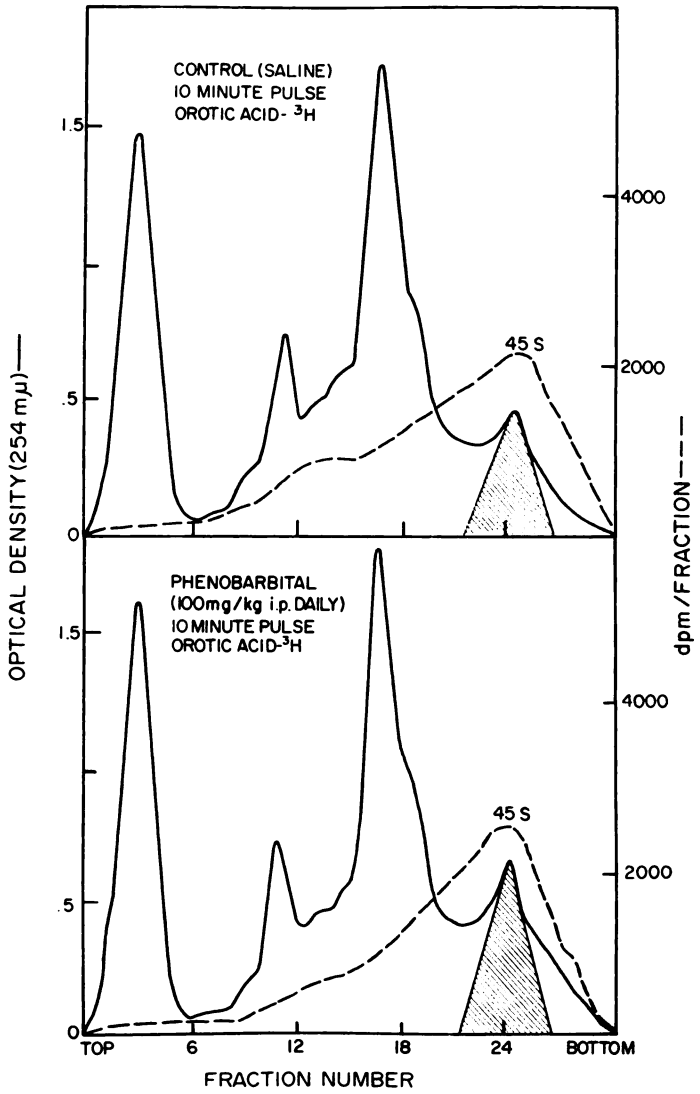

FIG. 1. *Sucrose gradient sedimentation patterns of liver nuclear RNA of phenobarbital- and NaCl-treated rats after labeling with [5-^{3}H]orotic acid for 10 min*

Rats weighing 250 g received phenobarbital (100 mg/kg) or 0.9% NaCl for 4 days. RNA extracted from the nuclei of control and drug-treated rats was layered on 10–40% linear sucrose density gradients as described in MATERIALS AND METHODS. Gradients were centrifuged at 26,000 rpm for 15 hr, analyzed for absorbance at 254 nm with an ISCO density gradient fractionator, and transcribed with a Honeywell recorder. Aliquots were taken for determination of radioactivity.

per cell, the RNA:DNA ratios were determined colorimetrically for experimental and control nuclei; these values were 0.094 and 0.069, respectively (Table 1). Multiplication of the 45 S RNA to total RNA and the RNA:DNA ratios yields the 45 S RNA:DNA ratio (Table 1, last column); the ratio of phenobarbital to control is 1.95. Therefore, in phenobarbital-treated nuclei, a 2-fold increase occurs in the amount of 45 S RNA per cell. Similar changes were observed with young (100-g) rats treated for 4 days (75 mg/kg).

Support for the view that the increase in nuclear 45 S RNA represents primarily ribosomal RNA precursor rather than

messenger RNA precursor is derived from analyses of nucleolar RNA gradient profiles. Increases of a similar magnitude were found for nucleolar 45 S RNA of 250-g rats treated with phenobarbital for 4 days.

Experiments were undertaken to determine the source of the increased amounts of nuclear 45 S RNA in phenobarbital-treated animals. Either increased synthesis or decreased destruction of 45 S RNA could account for the observed effect; synthesis was examined first. The following time periods of phenobarbital administration were analyzed: 16 hr after a single injection and 3 and 4 days after single daily doses. The specific activity of 45 S RNA isolated from sucrose gradients was 67% of the control value in 250-g rats (10-min or 60-min pulse of [^{3}H]orotic acid) and 100-g rats (10-min pulse of [^{3}H]orotic acid) treated for 4 days (Table 2, experiment A). This decrease in labeling was also found in nucleolar 45 S RNA from 250-g rats treated for 4 days (10-min pulse of [^{3}H]orotic acid). No such decreases in labeling of 45 S RNA were found in any of the other experiments.

It was possible that apparent decreases in labeling of 45 S RNA in phenobarbital-treated rats might be due to a drug-induced depression of the incorporation of [5-^{3}H] orotic acid into the acid-soluble nucleotides (38). However, phenobarbital had no effect on the incorporation of [5-^{3}H]orotic acid into the acid-soluble nucleotides (Table 2, experiment A) or into isolated UMP. After a 10-min labeling of 4-day-treated animals, the mean specific activities of UMP (disintegrations per minute/$A_{260} \times 10^{-3}$) were 20.0 ± 2.9 (SE) for experimental and 19.4 ± 2.3 for control samples, computed from five determinations of each. Therefore 4-day treatment with phenobarbital did not result in increased synthesis of 45 S RNA.

To investigate the effect of phenobarbital administration on the incorporation of isotopically labeled purines into 45 S RNA, [8-^{14}C]adenine or [8-^{14}C]guanine was injected into 200-g rats treated for 4 days with phenobarbital (Table 2, experiments B and C). After 20 min of labeling with these isotopes in phenobarbital-treated rats, no significant increases in the labeling of nuclear 45 S RNA or acid-soluble nucleotides over controls were observed.

Although 4-day treatment with phenobarbital did not stimulate the labeling of 45 S RNA, such an effect may occur but only after shorter exposures to phenobarbital. Treatment for 3 days with the drug had no effect on the incorporation of [^{3}H]orotic acid into nuclear 45 S RNA (Table 2, experiment A). Moreover, no increase in the labeling of 45 S RNA with [^{14}C]adenine or [^{3}H]orotic acid was found 16 hr after a single injection of phenobarbital (Table 2, experiments A and B).

The absence of enhanced labeling of nuclear 45 S RNA at 4 days, when the amount of nuclear 45 S RNA has increased, suggests that phenobarbital administration may inhibit the degradation of 45 S RNA. To investigate this possibility of enhanced metabolic stability of 45 S RNA in rats treated with phenobarbital, experiments

TABLE 1

Concentration of nuclear 45 S RNA in hepatic cells of rats treated with phenobarbital or NaCl for 4 days

RNA was extracted from liver nuclei of NaCl- and phenobarbital-treated rats and analyzed on sucrose density gradients as described in Fig. 1. The mean ratio of the area of 45 S RNA to total nuclear RNA (determined from Fig. 1 as described in the text) was increased by 43 ±2.6% (SE) in phenobarbital-treated rats. The RNA:DNA ratio was obtained by chemical determination of RNA and DNA as described in MATERIALS AND METHODS. The number of experiments is shown in parentheses.

Treatment	45 S RNA:total RNA (A)[a]	RNA:DNA (B)	45 S RNA:DNA (A × B)	Ratio of phenobarbital to control
Phenobarbital	0.096 ± 0.007 (7)	0.094 ± 0.010 (4)	9.02×10^{-3}	1.95
Control (NaCl)	0.067 ± 0.009 (4)	0.069 ± 0.005 (4)	4.62×10^{-3}	

[a] Difference between experimental and control means, $p < 0.05$.

TABLE 2

Effects of single and multiple doses of phenobarbital on labeling of rat liver nuclear 45 S RNA with various purine and pyrimidine radioisotopes

Rats were given a single dose or multiple doses of phenobarbital. [5-^{3}H]Orotic acid, [8-^{14}C]adenine, or [8-^{14}C]guanine was injected at various times before death. The 45 S RNA was precipitated from the sucrose gradients and dissolved in 1 ml of water; absorbance was determined at 260 nm, and radioactivity was measured by liquid scintillation counting as described in MATERIALS AND METHODS. Each value is the mean ± standard error of three to five experiments, and three or four rats were used for each experiment. The specific activity of the acid-soluble nucleotides was determined as described in MATERIALS AND METHODS.

Isotope; phenobarbital treatment	Rat weight	Pulse	Specific activity of 45 S RNA			Specific activity of acid-soluble nucleotides: ratio of phenobarbital to control
			Phenobarbital	Control	Ratio	
	g	*min*	*dpm/mg RNA* × 10^{-3}			
A. [^{3}H]Orotic acid						
100 mg/kg, 4 days	250	10	732 ± 51	1087 ± 75	0.67	1.00
100 mg/kg, 4 days	250	60	1398 ± 173	2113 ± 78	0.66	1.02
75 mg/kg, 4 days	100	10	368 ± 23	518 ± 5	0.71	1.05
100 mg/kg, 3 days	250	10	902 ± 80	955 ± 120	0.94	
100 mg/kg, 16 hr	250	15	1608 ± 294	1643 ± 282	0.98	1.02
B. [^{14}C]Adenine						
100 mg/kg, 16 hr	200	20	898 ± 75	900 ± 119	1.00	1.08
100 mg/kg, 4 days	200	20	847 ± 83	817 ± 77	1.04	1.12
C. [^{14}C]Guanine						
100 mg/kg, 4 days	200	20	642 ± 54	556 ± 60	1.15	1.15

were performed in which liver nuclei from phenobarbital-treated and control animals were incubated in 0.25 M sucrose containing 5 mM $MgCl_2$ at 37° for 2 min (Fig. 2). In NaCl-treated controls, planimetric analysis of RNA from sucrose gradients [(45 S RNA:total RNA at 2 min)/(45 S RNA: total RNA at 0 min)] revealed that after 2 min of heating at 37° only 41% of the optical density in the 45 S RNA remained. This decrease in optical density was paralleled by a loss of label in the 45 S RNA region, and there was a corresponding increase in optical density and labeling in the 18–28 S regions of the gradients. In contrast to these results in the controls, 75% of the 45 S RNA remained in phenobarbital-treated samples after the 2-min incubation. Similar results occurred in incubation studies with isolated nucleoli. Moreover, these same results were found 16 hr after a single injection of the drug, when no increased accumulation of 45 S RNA had occurred as determined by planimetric analysis. Therefore increased metabolic stability of nuclear 45 S RNA may be an early as well as a late effect of phenobarbital. In this incubation system the addition of phenobarbital (0.075 or 0.75 mg/ml) to isolated liver nuclei of NaCl-treated rats exerts no protective effect.

After 2 min of incubation in our system, nonspecific breakdown of nuclear 45 S RNA of control rats was observed, as indicated by the broad distribution of label and optical density throughout the gradient (Fig. 2). The nonspecific nature of breakdown of 45 S RNA was substantiated after incubation times of 4 min and longer, when the bulk of labeled RNA had been degraded to low molecular weight material sedimenting at the top of the gradient.

The RNA patterns from nuclei kept in an ice bath for 2 min were not affected by the addition of Mg^{++} to the 0.25 M sucrose. Without Mg^{++} in the incubation system, no consistent difference occurred between experimental and control 45 S RNA. Nuclear exonucleases believed to be involved in physiological cleavage of newly formed ribosomal RNA (39) and messenger RNA (40) require Mg^{++}. However, nonspecific degradation of newly formed nuclear RNA

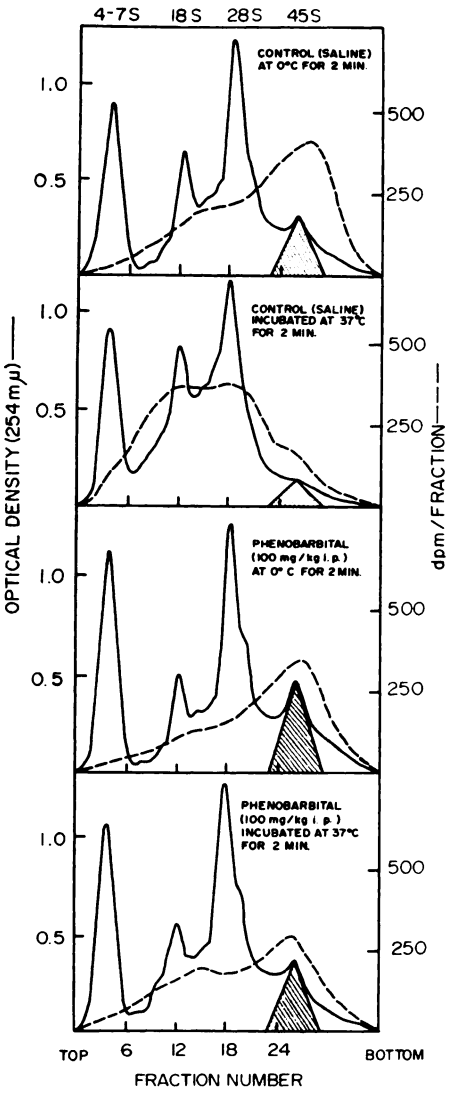

FIG. 2. *Effect of treatment with phenobarbital for 4 days on stability of liver nuclear 45 S RNA*

Rats weighing 250 g received phenobarbital (100 mg/kg daily) or 0.9% NaCl for 4 days. Each rat received an intraperitoneal injection of 20 μCi of [5-^{3}H]orotic acid 10 min before death. Liver nuclei of NaCl- and drug-treated animals were suspended in 0.25 M sucrose containing 5 mM $MgCl_2$ and left in an ice bath or incubated at 37° for 2 min as described in MATERIALS AND METHODS. RNA was extracted from the nuclei with hot sodium dodecyl sulfate–phenol and analyzed on 10–40% sucrose gradients as described in Fig. 1. Planimetric analysis of five experiments showed a mean decrease of 45 S RNA optical density of 58.9 ± 4.6% in control incubations. Incubation of phenobarbital-treated nuclei showed a mean decrease of 25.3 ± 2.4%. These analyses were carried out as described in the text.

also requires Mg^{++}; this cation stimulates the breakdown of labeled RNA to acid-soluble products in incubated HeLa cell nuclei (41).

Hypertonic sucrose apparently inhibits ribonuclease activity. Liver nuclei isolated in 2.4 M sucrose may be kept in ice for at least 1 hr, and the 2.4 M sucrose liver homogenate may be kept in ice for up to 3 hr, without any subsequent effect on the sedimentation properties of 45 S RNA.[1]

Even though no evidence exists for increased labeling of the ribosomal precursor RNA, Cohen and Ruddon (19) reported a 20–30% increase in the specific activity of microsomal RNA after single injections of phenobarbital and labeling times of 2–12 hr with orotic acid. In agreement with these and other observations (20), we found that 16 hr of phenobarbital treatment, including administration of [^{3}H]orotic acid or [^{14}C]-adenine for the last 4 hr, results in an increase of approximately 50% in the specific activity of microsomal RNA (Table 3). Such an increase was not observed in rats treated with four daily doses of phenobarbital (Table 3). Initially, then, phenobarbital may affect the transport of ribosomal RNA from the nucleus to the cytoplasm, but later this effect disappears.

DISCUSSION

The present study reveals that phenobarbital administration to rats for 4 days quantitatively increases nuclear 45 S RNA, confirming observations by Wold and Steele (16). However, contrary to that report (16), the increase in the concentration of nuclear 45 S RNA appears to arise from enhanced stabilization of the ribosomal precursor molecules rather than from their increased synthesis. Two observations support this conclusion: (a) no increase in the labeling of 45 S RNA was detected with radioactive purines and pyrimidines, and (b) the 45 S RNA in nuclei of phenobarbital-treated rats was protected from degradation in an incubation system *in vitro*.

[1] Unpublished observations.

TABLE 3

Effect of phenobarbital treatment on labeling of microsomal RNA with [5-³H]orotic acid and [8-¹⁴C]adenine

Rats weighing 200 g were given a single injection or daily injections of phenobarbital (100 mg/kg) for 16 hr and 4 days, respectively. Each rat received 100 μCi of [5-³H]orotic acid or 50 μCi of [8-¹⁴C]-adenine intraperitoneally 4 hr before death, and specific activity was determined for the 18 S and 28 S RNA as described in MATERIALS AND METHODS. Each value is the mean ± standard error of four to eight experiments, and three or four rats were used for each experiment.

Isotope and treatment	28 S RNA		18 S RNA	
	Specific activity	Ratio of phenobarbital to control	Specific activity	Ratio of phenobarbital to control
	dpm/mg RNA		*dpm/mg RNA*	
[5-³H]Orotic acid				
Phenobarbital, 16 hr	8,272 ± 350	1.59	6,394 ± 330	1.46
Control	5,202 ± 360		4,378 ± 410	
[8-¹⁴C]Adenine				
Phenobarbital, 16 hr	19,660 ± 1,500	1.42	17,740 ± 2,240	1.45
Control	13,860 ± 1,280		12,200 ± 1,040	
[5-³H]Orotic acid				
Phenobarbital, 4 days	2,006 ± 266	0.82	1,738 ± 202	0.81
Control	2,452 ± 130		2,154 ± 124	

Concerning the first observation, phenobarbital at no time significantly increased the labeling of nuclear 45 S RNA after short pulses of labeled orotic acid, adenine, or guanine. These results with orotic acid confirm two other studies using this labeled precursor (19, 21). Moreover, Gielen and Nebert (42) found no significant increases in the incorporation *in vitro* of [³H]uridine into the total RNA of fetal rat liver cells that had been exposed to inductive levels of phenobarbital for 3 days. Therefore our data do not support the contention that phenobarbital produces changes in synthesis of this RNA that may be related to the inductive effects of the drug (42, 43).

After 4 days of phenobarbital treatment, decreased incorporation of [³H]orotic acid occurred in both nuclear (Table 2) and microsomal RNA (Table 3). Decreases in labeling of RNA were not observed at earlier times or with the labeled purines. The barbital structure of phenobarbital is quite similar to that of orotic acid and pyrimidines in general. Some metabolite of phenobarbital might accumulate after 4 days of treatment and interfere with orotate disposition, leading to its incorporation into RNA. Since the specific activity of the acid-soluble pool and UMP remains at control values after 4 days of phenobarbital, a step beyond the formation of UMP could be involved. In bacteria, Mandel and Riis (44) have demonstrated that phenobarbital and other barbiturates decrease the uptake of labeled orotate. Drug-induced alterations in the incorporation of different labeled RNA precursors by eukaryotic cells is not uncommon (38, 41, 45).

Protection *in vitro* of 45 S RNA in nuclei of phenobarbital-treated rats indicates a stabilizing effect of the drug on newly synthesized RNA molecules. Our experiments suggest that different mechanisms may exist for the effects produced by single as contrasted to multiple doses of phenobarbital. No increase in concentration of nuclear 45 S RNA occurs after a single dose of the drug, but does occur after four daily doses. It has been estimated that approximately 80% more 45 S RNA is synthesized in normal rat liver than is needed to replace ribosomes lost by turnover (46). A single dose of phenobarbital may enhance the metabolic stability of newly formed 45 S RNA, thereby allowing a greater number of molecules of ribosomal RNA to reach the cytoplasm. This situation would be analogous to the enhanced stability of ribosomal RNA precursors in resting lymphocytes exposed to phytohemagglutinin (47, 48). Possibly independent of changes in rates of processing

of the ribosomal precursor and/or transport of ribosomal RNA, this effect would account for increased labeling of cytoplasmic RNA within 16 hr after a single dose of phenobarbital (Table 3) (19, 20). The mechanisms responsible for phenobarbital-enhanced stability of newly formed 45 S RNA are still operative after 4 days of drug treatment. Therefore accumulation of 45 S RNA at this time may indicate that enhanced production of new ribosomes no longer occurs. In support of this idea, no increase in the labeling of cytoplasmic ribosomal RNA was observed after 4 days of drug treatment (Table 3). Also, Stäubli *et al.* (7) reported that phenobarbital increased the number of membrane-bound ribosomes to maximal levels 16 hr after a single dose of the drug; these values approached normal by 4 days of treatment.

The mechanism of enhanced metabolic stability of 45 S RNA needs further investigation, but may involve alterations of the structure or conformation of newly formed nuclear 45 S RNA or alterations in the activity of specific cellular nucleases or other enzymes. For example, if methylation of newly formed ribosomal precursor RNA plays a role in the prevention of nonspecific degradation, then a possible mechanism for phenobarbital-induced stability may involve an effect on the methylating process (49–52). Alternatively, changes in the activity of certain nuclear nucleases may play a role. Phenobarbital administration to rats for 4 or 5 days decreases nuclear (9) and cytoplasmic (53, 54) RNase activity. Whether or not increased activity of cytoplasmic (55) or nuclear (56) RNase inhibitors occurs after phenobarbital treatment is not known. Our data suggest that nuclear rather than cytoplasmic factors play the more important role (Fig. 2).

The present findings do not rule out the possibility that phenobarbital increases transcription (17, 18) or transport (57–62) of messenger RNA. Since messenger RNA synthesis accounts for only a small percentage of labeled nuclear RNA after 20 min of isotope incorporation, no increases in labeling of nuclear RNA would be observed even if phenobarbital markedly stimulated the synthesis of specific classes of messenger RNA.

These data, as well as observations by others (9, 19, 20, 53, 54, 63), suggest that phenobarbital produces by various mechanisms post-transcriptional stabilization of nuclear ribosomal RNA precursor or cytoplasmic ribosomal RNA. Enhanced metabolic stability of ribosomal RNA may be more important than enhanced transcription in relating ribosomal RNA metabolism to the enzyme-inductive effects of phenobarbital. The stimulatory effect of various hormones and other agents also may be mediated by alterations in the stability of newly formed ribosomal (47, 48, 64, 65) as well as messenger RNA (57–62). In particular, changes in nuclear RNA metabolism related to carcinogenesis may be due to selective effects on RNA stabilization and subsequent transport rather than gene derepression (58–60).

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