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DIFFERENTIAL EFFECTS OF PHENOBARBITAL AND 3-METHYLCHOLANTHRENE ON RAT HEPATIC RIBOSOMAL PRECURSOR RNA

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Abstract—Synthesis of hepatic ribosomal precursor RNA (45S rRNA) was investigated in rats treated with single intraperitoneal injections of methylcholanthrene (MC) (30 mg/kg) or phenobarbital (PB) (100 mg/kg). Labeling *in vivo* of total nuclear RNA and of nuclear 45S rRNA with [^{3}H]orotic acid was unchanged in rats sacrificed 3 or 20 hr after reveiving a single dose of MC. These results agreed with measurements *in vitro* of nucleolar RNA polymerase activity, which was unaffected by either MC or PB pretreatment. In rats sacrificed 6 hr after receiving an injection of PB or 3 or 20 hr after receiving an injection of MC, incorporation of [^{3}H]UTP into isolated nucleoli was not enhanced. Labeling *in vivo* of microsomal 28S and 18S rRNA with [^{3}H]orotic acid increased 50 per cent 6 hr after a single dose of PB but was unchanged 3 or 20 hr after a single dose of MC. The stability of 45S rRNA in rats treated with PB or MC was tested in a nuclear incubation system. After incubation of nuclei at 37° for 2 min in 0.25 M sucrose containing 5 mM $MgCl_2$, RNA was extracted and layered on 10–40% sucrose gradients. Planimetric analysis of these sucrose gradients demonstrated that, 16 hr after PB treatment, the stability of 45S rRNA was increased over that of controls, whereas 20 hr after MC, 45S rRNA stability was unchanged. These results suggest that PB, but not MC, enhances post-transcriptional stability of 45S rRNA and labeling *in vivo* of microsomal RNA. Neither MC nor PB increases synthesis of ribosomal precursor RNA.

Phenobarbital (PB) and methylcholanthrene (MC), potent inducers of hepatic microsomal mixed-function oxidases, have served as prototypes of two distinct classes of compounds that stimulate the enzyme system in different ways. For example, PB enhances the metabolism of a wide variety of xenobiotics and endogenous compounds, whereas polycyclic hydrocarbons, such as MC, increase the metabolism of relatively few chemicals. Each inducer stimulates the formation of spectrally distinct carbon monoxide-binding hemoproteins in liver microsomes [1–5]. MC stimulates primarily the metabolism of polycyclic aromatic hydrocarbons [6]. Unlike PB, MC is a carcinogen in a variety of animals [6, 7]. Furthermore, the RNA synthesized in the presence of chromatin from MC-treated animals differs in base composition from that produced by chromatin of PB-treated animals [8].

Although effects of PB and MC on RNA metabolism have been extensively studied, the precise relationship between the inductive effects of these compounds and RNA metabolism remains controversial. It has been postulated that PB or MC stimulates transcription [9–14] of messenger as well as ribosomal RNA [9, 11–13, 15]. However, some investigators failed to observe significant alterations in transcription after administration of either PB or MC [16, 17–19]. Conflicting reports exist concerning the particular species of cellular RNA primarily affected by PB or MC treatment [12–20].

Thus, the mechanisms by which PB and MC exert their inductive effects at the molecular level remain to be defined. Among the possible sites for their action are transcription, translation and direct effects on the microsomal drug-metabolizing system. Our previous data [17] and that of Cohen and Ruddon [19] suggested that PB does not affect the rate of transcription of ribosomal precursor RNA but produces post-transcriptional stabilization of this RNA. In the present study, we compare PB and MC with respect to transcription and post-transcriptional stabilization. Under the conditions of our study, neither MC nor PB affected nucleolar RNA polymerase activity. PB, but not MC, enhanced 45S rRNA stability and cytoplasmic RNA labeling.

MATERIALS AND METHODS

Drugs and isotopes

PB (Merck) or MC (Sigma) was injected intraperitoneally (i.p.) in male Sprague–Dawley rats (Charles River) as follows: a single dose of PB (100 mg/kg) in 0.9% NaCl was injected in rats weighing 170–200 g. Animals were sacrificed 6 or 16 hr later. MC (30 mg/kg) was injected in 0.3 to 1 ml corn oil in rats weighing 40–60 or 170–200 g; rats were sacrificed 3, 6, 12 or 20 hr after MC injection. In some experiments, rats were fasted for 20 hr before decapitation at 9:00 a.m. Overnight fasting did not affect the results. Control rats were injected with 0.9% saline or corn oil alone. All rats were sacrificed at 9:00 a.m. The different treatment schedules for rats injected with PB or MC were based on previous studies, suggesting that a single dose of MC [10–14, 21] or PB [11–13, 15] increased synthesis of 45S rRNA and/or ribosomal RNA at these times.

[5-^{3}H]orotic acid (12.2 Ci/m-mole) and [^{3}H]UTP (36 Ci/m-mole) were obtained from New England Nuclear Corp. For labeling *in vivo* of nuclear RNA or microsomal RNA, each rat received 20–30 or 100 μCi of [^{3}H]orotic acid, respectively, at various times before sacrifice. Excised livers were placed in ice-cold 0.25 M sucrose. All further steps were performed at 4°.

Isolation of nuclei

Isolation of nuclei for labeling and stability studies. For each time point or experimental group, livers from three to six rats were pooled. Minced liver tissue was homogenized in 2.4 M sucrose (1:11, w/v) containing 3.3 mM $CaCl_2$ with four up-and-down strokes in a Teflon–glass homogenizer (0.015 to 0.020 in. pestle clearance). After successive filtrations through two and four layers of cheesecloth, the homogenate was centrifuged at 40,000 *g* for 75 min to sediment the nuclei [22, 23].

Isolation of nucleoli for RNA polymerase assay. Minced liver tissue from six to eight rats placed in a freshly prepared solution of 2.3 M sucrose containing 15 mM $MgCl_2$ and 0.25 mM spermine was homogenized as above [23, 24]. The homogenate was centrifuged at 40,000 *g* for 65 min to sediment the nuclei. Nucleoli were prepared using the sonication technique [25].

Phase contrast microscopy revealed that the nuclear and nucleolar preparations obtained by either the sucrose–calcium or sucrose–magnesium procedure were highly purified. No differences were observed between drug-treated and control rats in the purity or gross morphology of isolated nuclei or nucleoli.

Isolation of hepatic cytoplasmic microsomes. Minced livers from three rats were homogenized using a loosely fitting Teflon pestle, and microsomes were prepared as described previously [17].

Determination of nucleolar RNA polymerase activity

RNA polymerase was assayed in low ionic media containing Mg^{2+} [26]. The assay carried out for 30 min at 37° contained the following in a final volume of 0.5 ml: Tris–HCl buffer, pH 8.5, 50 μmoles; $MgCl_2$, 2.5 μmoles; NaF, 3 μmoles; dithiothreitol, 5 μmoles; ATP, GTP and CTP, 0.3 μmole each; UTP, 0.05 μmole; [^{3}H]UTP (36 Ci/m-mole), 33.6 pmoles; and nucleoli (0.1 to 0.15 mg DNA).

The reaction was terminated by adding 400 μg UTP and 1.5 ml of ice-cold 10% trichloroacetic acid containing 0.04 M $Na_4P_2O_7$. Each enzyme assay was performed in quadruplicate. The reaction mixtures were filtered under light vacuum through Whatman GF/C filters and washed four times with 2 ml of ice-cold 5% trichloroacetic acid containing 0.02 M $Na_4P_2O_7$ followed by four washes with 2 ml of 95% ethanol containing 2% potassium acetate. Dried filter discs were placed in glass vials, and 8 ml of Omnifluor–toluene scintillation fluid was added. Samples were counted in a Beckman LS-100 scintillation counter. Data were expressed as pmoles UMP incorporated/mg DNA.

Chemical determination of DNA

DNA was extracted from aliquots of nucleoli by acid hydrolysis; DNA content was determined by Burton's modification of the diphenylamine method [27].

RNA extraction

RNA was extracted from nuclei or microsomes using the sodium dodecyl sulfate-phenol (SDS-phenol) procedure [28–30]. For extraction of "cold" nuclear RNA or cytoplasmic microsomal RNA, heating at 65° was omitted.

Sucrose gradients

Between 1 and 2 mg RNA was layered over 10–40% sucrose gradients (38 ml) containing 0.1 M NaCl, 1.0 mm EDTA and 0.1 M sodium acetate, pH 5.1 [31]. The gradients were centrifuged in a Spinco SW 27 rotor at 122,000 *g* for 16 hr at 5°. Analysis of gradients was performed with the aid of an ISCO automatic fractionator system. Absorbance at 254 nm was transcribed with a Honeywell recorder to obtain enlarged, sharp peaks for planimetric analysis [32, 33]. Elevations of the baseline occurred as a function of increasing sucrose density, a phenomenon corrected by subtraction of blank gradients.

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 by liquid scintillation [34].

Table 1. Effect of treatment with MC on labeling of rat hepatic nuclear 45S rRNA with [^{3}H]orotic acid*

Treatment	Time (hr)	Sp. act. of 45S rRNA (dis./min/mg, RNA × 10^{-2})	Ratio of MC to control	Sp. act. of acid-soluble nucleotides: ratio of MC to control (dis./min/O.D.$_{260}$)
MC	3	1700 ± 666†	0.98	1.00
Control		1740 ± 260		
MC	20	1360 ± 208†	0.78	0.86
Control		1742 ± 307		

* Rats weighing 170–200 g were treated, i.p. with MC (30 mg/kg) in corn oil at 3 hr or 20 hr before sacrifice. Controls received corn oil alone at these same times. Animals were sacrificed 20 min after receiving, i.p., 20 μCi [^{3}H]orotic acid. Nuclear 45S rRNA was extracted and specific activity of 45S rRNA was determined as described in Materials and Methods. Each value is the mean ± S.E.M. of four to six experiments, and three to four rats were used for each experiment.

† No significant difference between experimental and control means.

Determination of specific activity

The specific activities of the nuclear 45S rRNA and of the microsomal 18S and 28S rRNA were determined by pooling the gradient fractions that constituted each peak and precipitating them in ethanol overnight. The specific activity of total nuclear RNA was determined prior to layering the samples on sucrose gradients. Each precipitate was dissolved in water and its absorbance measured at 260 nm; the radioactivity of the solution was then measured as described above.

Acid-soluble pool

From aliquots of the sucrose homogenates, the total acid-soluble nucleotides were extracted with perchloric acid [35].

Determination of the stability of 45S rRNA in a nuclear incubation system in vitro

Isolated nuclei from control or drug-treated animals were resuspended in 0.25 M sucrose containing 5 mM $MgCl_2$ (0.4 ml/g original wet weight of liver) and divided into two fractions. One fraction remained in an ice bath, and the other was incubated at 37° for 2 min [17]. From nuclei pelleted by centrifugation, RNA was extracted and analyzed on sucrose density gradients as described above. In some experiments, hepatic nucleoli were isolated and incubated as described above in 0.25 M sucrose containing 5 mM $MgCl_2$ (0.08 ml/g original wet weight of liver).

The proportion of 45S rRNA in each of the gradient profiles was estimated as follows. The total area included under the profile was either measured planimetrically [17, 32, 33] or cut out and weighed on an analytical balance; the area under the 45S rRNA peak was determined similarly, and the ratio of the 45S rRNA to total RNA was then computed. The percentage of 45S rRNA (45S rRNA/total nuclear RNA) in the incubated samples was divided by the percentage of 45S rRNA in the unincubated samples. This value represented the percentage of 45S rRNA remaining after incubation of nuclei.

RESULTS

Effect of MC treatment on labeling of rat hepatic nuclear 45S rRNA with [^3H]orotic acid

Labeling of nuclear 45S rRNA *in vivo* was not significantly changed in rats sacrificed either 3 or 20 hr after receiving a single dose of MC (Table 1). Moreover, treatment of rats with MC for either 3 or 20 hr had no effect on labeling of total hepatic nuclear RNA. No alterations had previously been observed in nuclear 45S rRNA labeled *in vivo* 16 hr after a single dose of PB [17]. In the present experiments, no significant alterations were obtained in hepatic 45S rRNA labeling 5 or 10 hr after a single dose of MC or after three daily injections of MC. Previously, four daily doses of PB were reported not to affect labeling of 45S rRNA *in vivo* [17]. Labeling of the total acid-soluble nucleotides (see Tables 1 and 3) or of acid-soluble UMP is unaffected under these conditions in rats treated with PB [17] or MC [16].

Figure 1 demonstrates, for cold phenol-extracted RNA, similarity between MC-treated and control rats with respect to sucrose gradient sedimentation profiles, labeling patterns, and specific activity of 45S rRNA (dis./min/O.D.$_{260}$). Only hot phenol extraction has been considered to be capable of releasing interphase-bound 45S rRNA into the aqueous phase [29, 30]. However, Fig. 1 shows that some 45S rRNA is extracted from isolated nuclei even in the absence of heating at 65°.

Effects of PB or MC on nucleolar RNA polymerase activity

Fluctuations in precursor pool size [17, 18] could produce errors in labeling *in vivo* of 45S rRNA in rats treated with PB or MC. This possibility was investigated by determining the effect of PB or MC on nucleolar RNA polymerase activity under conditions where precursors are not rate limiting, but present in excess concentrations. Table 2 shows that 6 hr after a single dose of PB or 3 hr or 20 hr after a single dose of MC in young adult rats or 6 hr after MC treatment of immature rats, no alterations occurred in nucleolar RNA polymerase activity assayed in low ionic media containing Mg^{2+}. Previously, similar results were obtained for rats 16 hr after PB [18]. These results agree with those of labeling studies *in vivo* in MC-treated rats (Table 1) and PB-treated rats [17].

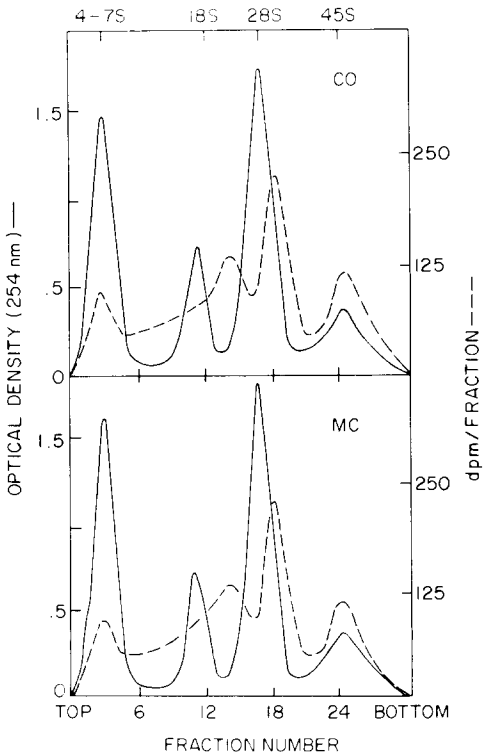

Fig. 1. Sucrose gradient analysis of nuclear RNA extracted by cold SDS-phenol from rats treated with MC. Rats weighing 170–200 g were sacrificed 3 hr after a single i.p. injection of MC (30 mg/kg). Each rat received, i.p., 30 μCi [^{3}H]orotic acid 20 min before death. Nuclei were isolated, cold nuclear RNA was extracted and sucrose gradient analysis was carried out, as described in Materials and Methods. The sucrose gradients in this figure and in Figs. 2 and 3 give mean values of four to seven experiments; three to five rats were used for each control and each experimental group.

Table 2. Effects of single doses of PB or MC on hepatic nucleolar RNA polymerase activity*

Treatment	Rat weight (g)	Time (hr)	UMP incorporated in the presence of Mg^{2+} and low ionic media (pmoles/mg DNA)
PB	170–200	6	2835 ± 387†
Control			2676 ± 492
MC	170–200	3	5324 ± 340†
Control			6283 ± 850
MC	170–200	20	6726 ± 962†
Control			6880 ± 576
MC	40–60	6	6020 ± 840†
Control			6320 ± 630

* Rats were treated, i.p., with a single dose of PB (100 mg/kg) or 0.9% NaCl 6 hr before death. Treatment of rats with MC was carried out as described in Table 1. Nucleoli from five to eight rat livers were isolated and the assays were performed as described in Materials and Methods. Enzyme activity was linear with increasing concentrations of nucleoli under the conditions of our assay. The results are means ± S.E.M. of four to six separate experiments.

† No significant difference between experimental and control means.

Effect of PB or MC on labeling of microsomal RNA with [^3H]orotic acid

Table 3 shows that in young adult rats treated with MC for 3 or 20 hr, or in immature rats treated with MC for 12 hr, no increase occurred in labeling *in vivo* of microsomal 28S or 18S rRNA. In contrast, 6 hr (Table 3) or 16 hr [17] after a single dose of PB, labeling of microsomal rRNA was enhanced approximately 50 per cent. The specific activity of the total acid-soluble nucleotides was not significantly affected in rats treated with MC or PB under these conditions (Table 3). After a single dose of PB, enhanced labeling of cytoplasmic microsomal RNA appears not to result from transcriptional effects [17] (Table 2), but from decreased degradation of 45S rRNA [17, 18]. To investigate the possibility that 45S rRNA from PB-treated rats is more stable than 45S rRNA from MC-treated rats or from saline- or corn oil-treated rats, a nuclear incubation system *in vitro* was devised [17].

Effects of PB or MC on stability of nuclear 45S rRNA

Liver nuclei from rats treated with PB or MC or from saline-treated rats were suspended in 0.25 M sucrose containing 5 mM $MgCl_2$. Nuclear RNA extracted from incubated and non-incubated nuclei of saline-treated rats or drug-treated rats was analyzed on sucrose gradients. Figure 2 shows that, 16 hr after PB treatment, enhanced metabolic stability of 45S rRNA occurred compared to its stability in nuclei of control rats. Similar results occurred in rats sacrificed 6 hr after PB. Figure 3 shows that MC failed to alter 45S rRNA stability. Nucleoli isolated from PB- or MC-treated rats were incubated and similar results were obtained as shown in Figs. 2 and 3. Unaltered 45S rRNA stability in hepatic nuclei from MC-

Table 3. Effects of PB or MC on labeling of microsomal RNA with [^{3}H]orotic acid*

Treatment	Sp. act. of acid-soluble nucleotides: ratio of experimental to control (dis./min/O.D.$_{260}$)	28S RNA		18S RNA	
		Sp. act. (dis./min/mg RNA)	Ratio of experimental	Sp. act. (dis./min/mg RNA	Ratio of experimental to control
PB, 6 hr	1.01	7960 ± 760†	1.53	7140 ± 460†	1.55
Control		5200 ± 400		4600 ± 500	
MC, 3 hr	1.02	2220 ± 40‡	1.04	2080 ± 230‡	1.06
Control		2140 ± 150		1920 ± 514	
MC, 20 hr	0.97	4640 ± 137‡	1.06	3940 ± 370‡	1.08
Control		4360 ± 374		3640 ± 359	
MC, 12 hr	0.98	38600 ± 5200‡	1.08	31260 ± 3840‡	0.98
Control		35600 ± 840		31760 ± 5620	

* Rats weighing 40 or 170–200 g were treated with MC as described in Tables 1 and 2 and in Materials and Methods. Immature rats were treated with MC 12 hr before sacrifice and received, i.p., 100 μCi [^{3}H]orotic acid 4 hr before death. Rats treated with MC 3 hr before sacrifice received 100 μCi [^{3}H]orotic acid at 90 min before death. Rats weighing 170–200 g received PB 6 hr before sacrifice as described in Table 2 and were injected, i.p., with 100 μCi [^{3}H]orotic acid 3 hr before sacrifice. Microsomes were isolated and specific activity of microsomal 28S and 18S rRNA was determined as described in Materials and Methods. Each value is the mean ± S.E.M. of five to six experiments, and three to five rat livers were used for each experiment.

† Difference between experimental and control means, $P < 0.05$.

‡ No significant difference between experimental and control means.

treated animals (Fig. 3) correlates with the observation that MC produced no increase in labeling of microsomal RNA *in vivo* (Table 3), whereas PB enhanced metabolic stability of 45S rRNA (Fig. 2) and increased labeling of microsomal RNA *in vivo* (Table 3). Planimetric analysis of the sucrose gradients presented in Figs. 2 and 3 showed that the ratio of 45S rRNA to total nuclear RNA increased 50 per cent in PB-treated rats compared to saline-treated controls; this ratio was unaltered by MC treatment.

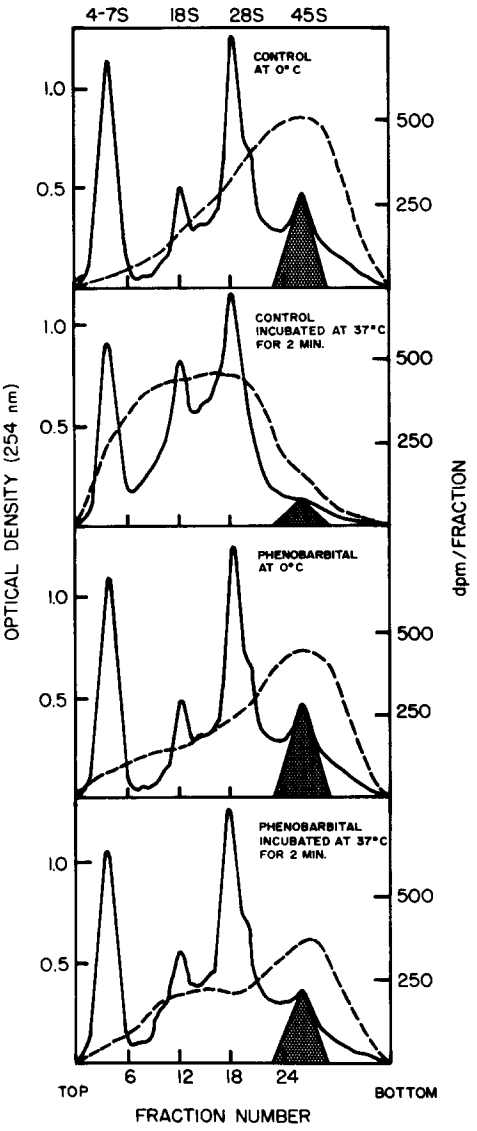

Fig. 2. Effects of a single dose of phenobarbital on stability of hepatic nuclear 45S rRNA. Rats weighing 170–200 g were treated with PB for 16 hr as described in Materials and Methods. Each rat received, i.p. 20 μCi [^{3}H]orotic acid 10 min before death. Liver nuclei of control and drug-treated rats 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. RNA was extracted from the nuclei with hot sodium dodecyl sulfate-phenol and analyzed on 10–40% sucrose gradients as described in Materials and Methods. The area of the gradient represented by 45S rRNA and total RNA was estimated by planimetric analysis as described in Materials and Methods. The solid line represents optical density; the dashed line represents dis./min; the shaded area represents the 45S RNA as estimated by planimetric analysis.

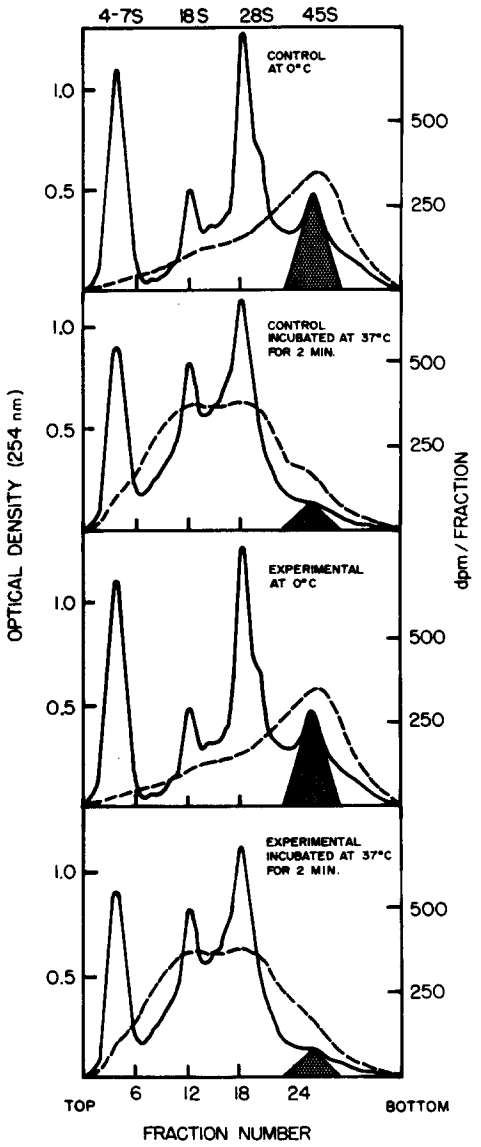

Fig. 3. Effects of a single dose of MC on hepatic nuclear 45S rRNA stability. Rats weighing 170–200 g were treated with MC for 20 hr as described in Table 1. Isotope injection, incubation of nuclei, RNA extraction and planimetric analysis of RNA on sucrose gradients were performed as described in the legend of Fig. 2. The solid line represents optical density; the dashed line represents dis./min; the shaded area represents the 45S rRNA as estimated by planimetric analysis.

DISCUSSION

Neither PB nor MC appears to increase synthesis of 45S rRNA (see Tables 1 and 2). Our data suggest that PB, but not MC, increases post-transcriptional stability and enables transport of some 45S rRNA molecules that, in the absence of PB, would have been degraded in the nucleus [17, 18]. The mechanisms responsible for PB-induced increases in stability of 45S rRNA are unclear, but could be related to enhanced

methylation or decreased nuclear RNase activity [18]. PB treatment of rats exerted no effect on total nuclear alkaline RNase activity [18], but increased incorporation *in vivo* of labeled methionine into total nuclear RNA [18]. Recent studies carried out in our laboratory show that, in rats sacrificed 6 or 16 hr after receiving a single dose of PB, the incorporation of [^{3}H]*S*-adenosylmethionine into isolated nucleoli was significantly enhanced over that of controls [36].

Investigations of the effects of various drugs on RNA labeling *in vivo* present methodological difficulties, particularly with respect to possible drug-induced changes in precursor pool size [17, 18, 37–41]. Although absence of changes in the labeling of total acid-soluble nucleotides by either PB or MC is inconclusive by itself, evidence from many sources supports our labeling data *in vivo* (see Tables 1 and 3):

(1) Results of studies on nuclear RNA labeled *in vivo* agree with those of RNA polymerase data *in vitro* [17, 18] (see Tables 1 and 2). These investigations reveal that neither MC nor PB increases labeling of 45S rRNA *in vivo* or *in vitro*.

(2) In rats sacrificed within 24 hr after receiving a single dose of PB, labeling *in vivo* of membrane-bound ribosomes was significantly greater than that of free ribosomes [42]. PB increased the number of membrane-bound ribosomes to maximal levels 16 hr after a single dose of the drug [43], but four daily doses of PB decreased the number of bound ribosomes toward normal [43] and failed to change significantly incorporation of [^{3}H]orotic acid into microsomal RNA [17].

(3) In rats sacrificed 3, 12 or 24 hr after a single dose of MC, no change occurred in the incorporation of [^{3}H]orotic acid into microsomal RNA or acid-soluble UMP [16]. Moreover, in rats sacrificed within 24 hr after a single dose of PB, labeling of microsomal rRNA *in vivo* with [^{3}H]orotic acid increased, but labeling of total acid-soluble nucleotides was unaffected [17, 19].

(4) A single dose of MC does not alter the stability of 45S rRNA extracted from incubated nuclei compared to controls, whereas PB enhances the stability of 45S rRNA under identical conditions of incubation of nuclei (see Figs. 2 and 3).

These data are all compatible with the hypothesis that MC exerts no effect on the synthesis or stability of ribosomal precursor RNA. In most earlier studies [9, 10, 13, 16, 44], MC treatment enhanced nuclear RNA labeling *in vivo*; however, in these earlier studies hot phenol extraction was not used and, furthermore, in contrast to the results shown in Fig. 1, sucrose gradient analysis showed very low amounts of 45S rRNA suggestive of appreciable degradation [9, 10, 13, 44]. Using hot SDS and phenol, Bresnick *et al.* [45] reported that MC failed to alter nuclear RNA labeling *in vivo*, an observation confirmed by our results.

After MC treatment, no increase was observed in labeling with [^{3}H]orotic acid of hepatic microsomal RNA from immature rats (see Table 3) or young adult rats [16] (see Table 3), whereas other investigators reported increased labeling of microsomal RNA under these same conditions [21].

The apparent lack of any significant effect of MC or PB on the activity of nucleolar RNA polymerase I (see Table 2) also contrasts with certain published studies [9, 11–14]. The reasons for some of the discrepancies enumerated above are unclear, but may be related to differences in dosage, animal age, techniques of isolation of nuclei and/or extraction of RNA, use of nuclei or nucleoli as sources of enzyme, and method of assay of RNA polymerase activity.

MC and PB have been reported to increase incorporation of labeled amino acid precursors into protein both *in vivo* and *in vitro* [46–53]. In addition, recent studies indicate that MC [51, 52] and PB [53] enhance initiation of protein synthesis. Treatment with PB, MC or both may produce cytoplasmic changes in the efficiency of translation or directly affect drug-metabolizing enzymes in the endoplasmic reticulum. The present data and those of others [9, 51, 52] suggest that MC-induced alterations in rRNA metabolism may not be required for induction of microsomal drug-metabolizing enzymes.

Reports that MC-treated [8, 54, 55] or PB-treated rats exhibit increased template efficiency are incompatible with our observation that nucleolar RNA polymerase activity remains unaltered after MC or PB. However, these prior studies [8, 54, 55] on template efficiency did not distinguish between segments of the genome responsible for ribosomal and messenger RNA synthesis. Although neither PB nor MC appears to increase synthesis of 45S rRNA, these studies do not deal with the possibility that either or both of these agents increase synthesis of messenger RNA. In this regard, Lanclos and Bresnick [56] observed that labeling *in vivo* of hepatic cytoplasmic poly(A) containing RNA was increased in MC-treated rats.

REFERENCES

1. N. E. Sladek and G. J. Mannering, *Molec. Pharmac.* **5**, 186 (1969).
2. W. Levin and R. Kuntzman, *Life Sci.* **8**, 305 (1969).
3. A. Alvares, C. J. Parli and G. J. Mannering, *Biochem. Pharmac.* **22**, 1037 (1973).
4. A. Alvares, G. Schilling and R. Kuntzman, *Life Sci.* **10**, 129 (1971).
5. J. R. Gillette, D. C. Davis and H. A. Sasame, *A. Rev. Pharmac.* **12**, 57 (1972).
6. A. H. Conney, *Pharmac. Rev.* **19**, 317 (1967).
7. A. H. Conney, E. C. Miller and J. A. Miller, *Cancer Res.* **16**, 450 (1956).
8. E. Bresnick and H. Mosse, *Molec. Pharmac.* **5**, 219 (1969).
9. L. R. Younger, R. Salomon, R. W. Wilson, A. C. Peacock and H. V. Gelboin, *Molec. Pharmac.* **8**, 452 (1972).
10. L. A. Loeb and H. V. Gelboin, *Biochemistry* **52**, 1219 (1964).
11. H. V. Gelboin, J. S. Wortham and R. G. Wilson, *Nature Lond.* **214**, 281 (1967).
12. R. G. Wilson, J. S. Wortham and H. V. Gelboin, *Adv. Enzyme Regulat.* **5**, 385 (1967).
13. H. V. Gelboin, in *Fundamentals of Drug Metabolism and Drug Disposition* (Eds. B. N. LaDue, H. G. Mandel and E. L. Way), p. 279. Williams & Wilkins, Baltimore, Md. (1971).
14. E. Bresnick, *Molec. Pharmac.* **2**, 406 (1966).
15. J. S. Wold and W. J. Steele, *Molec. Pharmac.* **8**, 681 (1972).
16. J. Hopkinson and T. S. Argyris, *Lab. Invest.* **29**, 342 (1973).
17. S. J. Smith, R. N. Hill, R. A. Gleeson and E. S. Vesell, *Molec. Pharmac.* **8**, 691 (1972).

18. S. J. Smith, S. T. Jacob, D. K. Liu, B. Duceman and E. S. Vesell, *Molec. Pharmac.* **10,** 248 (1974).
19. A. M. Cohen and R. W. Ruddon, *Molec. Pharmac.* **6,** 540 (1970).
20. N. E. Sladek and G. J. Mannering, *Molec. Pharmac.* **5,** 174 (1969).
21. E. Bresnick, M. E. Synerholm and G. T. Tizard, *Molec. Pharmac.* **4,** 218 (1968).
22. S. J. Smith, H. R. Adams, K. Smetana and H. Busch, *Expl. Cell Res.* **55,** 185 (1969).
23. H. Busch, *Meth. Enzym.* **12,** 421 (1967).
24. H. Busch, K. S. Narayan and J. Hamilton, *Expl. Cell Res.* **47,** 329 (1967).
25. H. Busch, *Meth. Enzym.* **12,** 448 (1967).
26. S. T. Jacob, *Prog. nucleic Acid Res. Mol. Biol.* **13,** 93 (1973).
27. K. Burton, *Biochem. J.* **62,** 315 (1956).
28. W. J. Steele, N. Okamura and H. Busch, *J. biol. Chem.* **240,** 1742 (1965).
29. W. B. Dingman and M. B. Sporn, *Biochim. biophys. Acta* **61,** 164 (1962).
30. W. J. Steele and H. Busch, *Meth. Cancer Res.* **3,** 61 (1967).
31. K. Scherrer and J. E. Darnell, *Biochem. biophys. Res. Commun.* **7,** 486 (1962).
32. T. Svedberg and K. O. Pedersen, in *The Ultracentrifuge* (Eds. R. H. Fowler and P. Kapitza), p. 296. Clarendon Press, Oxford, England (1940).
33. S. T. Jacob, W. J. Steele and H. Busch, *Cancer Res.* **27,** 52 (1967).
34. G. A. Bruno and J. E. Christian, *Analyt. Chem.* **33,** 1216 (1961).
35. A. Fleck and H. N. Munro, *Biochim. biophys. Acta* **55,** 571 (1962).
36. S. J. Smith, D. K. Liu, T. Leonard, B. Duceman and E. S. Vesell, *Molec. Pharmac.* **12,** 820 (1976).
37. M. Feigelson and P. Feigelson, *J. biol. Chem.* **241,** 5819 (1966).
38. J. W. Watts, *Biochem. J.* **112,** 71 (1969).
39. T. W. Munns and P. A. Katzman, *Biochemistry* **10,** 4941 (1971).
40. M. Muramatsu and H. Busch, *J. biol. Chem.* **240,** 3960 (1965).
41. H. V. Gelboin, in *Handbook of Experimental Pharmacology* (Eds. B. B. Brodie and J. R. Gillette), Part 2, p. 431. Spinger, New York (1971).
42. R. McCauley and D. Couri, *Biochim. biophys. Acta* **238,** 233 (1971).
43. W. Staubli, R. Hess and E. R. Weibel, *J. Cell Biol.* **42,** 92 (1969).
44. T. Hishizawa, H. Otsuka and H. Terayama, *J. Biochem. Tokyo* **56,** 97 (1964).
45. E. Bresnick, R. Brand and J. A. Knight, *Biochim. biophys. Acta* **114,** 227 (1966).
46. H. V. Gelboin and L. Sololoff, *Science, N.Y.* **134,** 611 (1961).
47. H. V. Gelboin and N. R. Blackburn, *Biochim. biophys. Acta* **72,** 657 (1963).
48. R. Kato, L. Loeb and H. V. Gelboin, *Biochem. Pharmac.* **14,** 1164 (1965).
49. A. Von der Decken and T. Hultin, *Archs Biochem. biophys.* **90,** 201 (1960).
50. L. W. Wattenberg, J. L. Leong and A. R. Galbraith, *Proc. Soc. exp. Biol. Med.* **127,** 467 (1968).
51. K. D. Lanclos and E. Bresnick, *Drug Metab. Dispos.* **1,** 239 (1973).
52. J. Hopkinson, P. M. Prichard and E. Bresnick, *Biochim. biophys. Acta* **374,** 375 (1974).
53. N. Venkatesan and W. J. Steele, *Chem Biol. Interact.* **11,** 41 (1975).
54. J. C. Madix and E. Bresnick, *Biochem. biophys. Res. Commun.* **28,** 445 (1967).
55. W. N. Piper and W. F. Bousquet, *Biochem. biophys. Res. Commun.* **33,** 602 (1968).
56. K. D. Lanclos and E. Bresnick, *Chem. Biol. Interact.* **12,** 341 (1976).

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