A Comparative Study of the Early Effects of Phenobarbital and 3-Methylcholanthrene on the Synthesis and Transport of Ribonucleic Acid in Rat Liver

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At 2–3 h after phenobarbital administration, the drug has no effect on nucleoplasmic RNA synthesis and decreases nucleolar RNA synthesis. However, at this time there is an increase in the labelling of cytoplasmic poly(A)-containing RNA, even though there is decreased labelling of total polyribosomal RNA. The decrease in labelling of nucleolar and total polyribosomal RNA owing to phenobarbital is a transient phenomenon. Under similar conditions, 3-methylcholanthrene has no effect on nucleolar RNA synthesis, but leads to an increase in synthesis of nucleoplasmic and cytoplasmic poly(A)-containing RNA. Cytosol isolated from phenobarbital-treated, but not from 3-methylcholanthrene-treated, animals facilitates an enhanced transport of RNA from nuclei. At the time points investigated, 3-methylcholanthrene or its metabolite shows a 10–15-fold higher concentration in the chromatin than that of phenobarbital or its metabolite. It is suggested that the primary effect of phenobarbital is at the cytoplasmic level, promoting the transport of RNA from the nuclei, which can act as a trigger for enhanced transcription at later periods. 3-Methylcholanthrene or its metabolite directly binds to the chromatin and evokes a selective transcriptional response.

The sedative, phenobarbital, and the pro-carcinogen, 3-methylcholanthrene, represent two prototype inducers of the mixed-function oxidase system of the liver endoplasmic reticulum. Phenobarbital induces almost all the cytochrome *P*-450 species and the associated drug-metabolizing complexes. 3-Methylcholanthrene is a specific inducer of a particular species of the cytochrome *P*-450 group of proteins, cytochrome *P*-448, and the dependent hydroxylase reactions (Alvares *et al.*, 1967; Orrenius *et al.*, 1969; Gielen & Nebert, 1971). The inducing effect of phenobarbital is not confined to the mixed-function oxidase system, and the drug is a hypertrophic agent. 3-Methylcholanthrene has very little effect on the proliferation of the liver endoplasmic reticulum and brings about the induction of a limited number of proteins (Fouts & Rogers, 1965).

In general, phenobarbital and 3-methylcholanthrene are thought to bring about gene activation and are often considered together when an explanation is sought to understand their molecular mechanism of action. Thus the two drugs have been reported to enhance nuclear RNA polymerase (Bresnick, 1966; Wilson *et al.*, 1967) and chromatin template activities (Bresnick & Mosse, 1969; Piper & Bousquet, 1968). The drugs have been reported to

alter the phosphorylation and acetylation states of nuclear proteins and bring about enhanced transcription (Blackenship & Bresnick, 1974; Procaccini & Bresnick, 1975). Phenobarbital and 3-methylcholanthrene have also been reported to bring about an increase in cyclic AMP concentration leading to enhanced protein kinase, ornithine decarboxylase and RNA polymerase I activities (Bycus *et al.*, 1976; Costa *et al.*, 1977).

However, these results do not explain the basic difference between the molecular responses evoked by the two drugs, with phenobarbital behaving as a general inducer and 3-methylcholanthrene eliciting the synthesis of a limited number of proteins. The involvement of transcription is perhaps warranted in view of the results described above and the observations that the effects of the two drugs are sensitive to actinomycin D and α -amanitin (Marver, 1969; Nebert & Gielen, 1971; Jacob *et al.*, 1974). However, the earlier results (e.g. Wilson *et al.*, 1967) reporting the increase in RNA polymerase activities in response to phenobarbital and 3-methylcholanthrene have been questioned. It has been reported that the two drugs have no effect on nucleolar RNA polymerase activities and do not bring about an enhanced synthesis of 45S RNA, the rRNA precursor. The increased synthesis of the mature RNA species brought about by phenobarbital has been attributed to

Abbreviation used: SDS, sodium dodecyl sulphate.

stabilization of the 45S RNA precursor rather than to its enhanced synthesis (Cohen & Ruddon, 1970; Smith *et al.*, 1972, 1977). A more recent report states that no significant alteration in the activity of any of the RNA polymerases is observed by comparison of phenobarbital-treated rats with saline-treated controls, and decreased turnover or stabilization of RNA in drug-treated animals has been favoured (Lindell *et al.*, 1978).

In the present study, the early effects of phenobarbital and 3-methylcholanthrene on synthesis of nuclear and cytoplasmic RNA and RNA transport from nucleus to cytoplasm have been studied. The localization of the two drugs in the chromatin fraction has been investigated and a model is proposed to explain the difference between the respective molecular effects elicited.

Materials and Methods

Materials

[G-³H]3-Methylcholanthrene (10 Ci/mmol), phenobarbital (20 mCi/mmol) and [5-³H]uridine (27 Ci/mmol) were purchased from The Radiochemical Centre, Amersham, Bucks., U.K. [³²P]P_i (carrier free) and [6-¹⁴C]orotic acid (11 mCi/mmol) were purchased from BARC, Bombay, India. Biochemicals were purchased from Sigma Chemical Co., St. Louis, MO, U.S.A.

Treatment of animals

Male rats (60–70 g) of the Indian Institute of Science strain received a single intraperitoneal injection of phenobarbital (80 mg/kg) or 3-methylcholanthrene (40 mg/kg). The schedule of the injection of labelled compounds is described below.

Measurement of nuclear RNA synthesis

For this purpose, the animals received [³H]uridine (200 μ Ci/rat) or ³²P (500 μ Ci/rat) at different time intervals after the injection of phenobarbital or 3-methylcholanthrene. The animals were killed 30 min after the injection of the label and the livers processed for the isolation of nuclei and nuclear subfractions.

Nuclei were isolated essentially by the procedure of Blobel & Potter (1966). Nucleolar and nucleoplasmic subfractions were isolated by the method of Muramatsu & Fujisawa (1968). Briefly, the nuclear pellet from 1 g wet wt. of liver was suspended in 1 ml of 0.34 M-sucrose in TKM buffer (0.01 M-Tris/HCl, pH 7.6, 0.05 M-KCl and 0.005 M-MgCl₂) containing 20 μ g of polyvinyl sulphate/ml. The nuclear suspension was sonicated for 4 $\times$ 15 s in a Branson sonifier at a setting of 3 A. The lysate was layered over 0.88 M-sucrose in TKM buffer and centrifuged at 2000g for 20 min to obtain the nucleolar pellet. The supernatant was spun at 5000g for 5 min, and the supernatant

from this step, representing the nucleoplasmic fraction, was recovered.

RNA was extracted from the nucleus and nuclear sub-fractions by the method of Abe *et al.* (1972). The standard extraction medium contained 0.1 % SDS, 0.14 M-NaCl and 0.025 M-sodium acetate, pH 5.1. The suspension was shaken with an equal volume of phenol/*m*-cresol/water (8:1:1, by vol.) containing 0.1 % 8-hydroxyquinoline and centrifuged (10000g, 10 min). The aqueous phase was collected and the phenol interphase was successively shaken with the standard extraction medium containing progressively increasing concentrations of SDS (0.5 % and 1 %). RNA was precipitated with ethanol and washed thoroughly. The method carried out at room temperature has been reported to be as efficient as those carried out at higher temperatures, at the same time yielding intact RNA.

Measurement of cytoplasmic synthesis of poly(A)-containing RNA

For this purpose, 1 or 6 h after drug administration, ³²P (carrier-free, 2 mCi/rat) was injected intraperitoneally and the animals were killed 2 h after the injection. Polyribosomes were isolated from the post-mitochondrial supernatant by the magnesium precipitation procedure of Palmiter (1974). RNA was isolated from the polyribosomes by the phenol/chloroform extraction procedure as described by Penman (1966). Poly(A)-containing RNA was isolated from polyribosomal RNA by using poly(U)-Sephadex chromatography as described by Padmanaban *et al.* (1975).

Measurement of nucleotide-pool specific radioactivity

In experiments with ³²P as the label, the total free nucleotide pool was isolated from liver homogenates by using HClO₄ to precipitate proteins and nucleic acids, followed by adsorption of the nucleotides with charcoal. The charcoal pellet was washed thoroughly with water and the nucleotides were eluted with 50 % (v/v) ethanol containing 0.3 M-NH₃. Portions of the eluate were used for radioactivity and A₂₆₀ measurements (Sardana *et al.*, 1975).

Transport of RNA from nucleus to cytoplasm

This was studied as in the procedures described by Webb and co-workers (Yu *et al.*, 1972; Schumm & Webb, 1974). The nuclei were labelled *in vivo* by injecting [¹⁴C]orotic acid 30 min before death. Nuclei were isolated from the livers as described above. Cytosol was prepared separately from normal and drug-treated animals. The animals were killed 2 h after the drug injection and the livers were homogenized with 3 vol. of 0.25 M-sucrose in TKM buffer. The 100000g supernatant was dialysed against water for 18 h at 4 °C with two changes. The labelled nuclei,

freshly prepared, were incubated (5×10^6 nuclei/ml) at 37°C or 0°C in 5 ml of a complete medium containing 0.6 vol. of cytosol, 50 mM-Tris/HCl buffer, pH 7.6, 25 mM-KCl, 2.5 mM-MgCl₂, 0.5 mM-CaCl₂, 2.5 mM-Na₂HPO₄, 5 mM-NaCl, 5 mM-spermidine, 2 mM-dithiothreitol, 2 mM-ATP, 25 mM-phosphoenolpyruvate, 35 units of pyruvate kinase and 500 µg of yeast RNA/ml. Additional phosphoenolpyruvate (1 mM) was added at 10 min intervals. Portions of the reaction mixture were removed at different intervals of time, centrifuged to remove the nuclei and the supernatant was precipitated with HClO₄ (0.3 M final concn.). The pellet was washed and the alkali-labile radioactivity was measured. A portion of the nuclear suspension was similarly processed to measure the radioactivity present in the RNA fraction of the nuclear input.

Subnuclear localization of pheno[2-¹⁴C]barbital and [G-³H]3-methylcholanthrene or their derivatives

Rats (60 g body wt.) were injected with 15 µCi of pheno[2-¹⁴C]barbital containing 5.3 mg of the drug (80 mg/100 g body wt.) or 100 µCi of [G-³H]3-methylcholanthrene containing 2.7 mg of the drug (40 mg/kg body wt.). The animals were killed 2½ or 6 h after the drug administration and nuclei were isolated from the livers as described above. Chromatin fraction was isolated from the nuclei by the method of Huang & Huang (1969). Briefly, the nuclei were first washed with 0.34 M-sucrose in 0.01 M-Tris/HCl buffer (pH 7.6)/0.01 M-MgCl₂ containing 0.5% (v/v) Triton X-100 to remove the outer membrane. The Triton-washed nuclei were suspended in 10 vol. of 0.075 M-NaCl/0.025 M-EDTA/0.01 M-Tris/HCl buffer, pH 8.0, and incubated at 0°C for 10 min. The suspension was centrifuged at 10000g for 10 min and the pellet was collected. The extraction/incubation steps were repeated twice. The final pellet was extracted with 3 × 10 vol. of 0.01 M-Tris/HCl buffer, pH 8.0, with incubation at 0°C for 10 min each time, followed by centrifugation at 10000g for 10 min. The final jelly-like chromatin pellet was dispersed in a known volume of formic acid and portions were transferred to filter-paper discs for radioactivity measurements. DNA was measured in the different fractions by using diphenylamine (Burton, 1956).

Radioactivity measurements

These were done in a Beckman LS-100 liquid-scintillation counter. Filter discs were counted for radioactivity in 0.5% 2,5-diphenyloxazole (w/v) in toluene. Direct counting of radioactivity in aqueous samples was carried out by using 0.5% 2,5-diphenyloxazole (w/v) in toluene/Triton X-100 (2:1, v/v).

Results

The aim of the present study is to examine the early effects of phenobarbital and 3-methylchol-

anthrene on nuclear and cytoplasmic RNA synthesis. The results presented in Fig. 1 depict the effect of the two drugs on nuclear RNA synthesis. At different time intervals after the drug administration, the animals received a 30 min pulse of [³H]uridine before being killed. The initial effect of phenobarbital is actually to cause a decreased labelling of nuclear RNA, which returns to normal values around 4 h after drug administration and then reaches a plateau. The decreased labelling of nuclear RNA at 2 h after phenobarbital administration was further examined in detail by using ³²P as the precursor. The results presented in Table 1 indicate that the drug causes a decreased labelling of nuclear RNA by 20–25% at this time period. 3-Methylcholanthrene causes an increase by 10–15%. The effect of phenobarbital is essentially due to a significant decrease in the labelling of nucleolar RNA, whereas nucleoplasmic RNA shows no change. 3-Methylcholanthrene has no effect on the labelling of nucleolar RNA, but leads to a significant increase in the labelling of nucleoplasmic RNA. The specific radioactivity of the nucleotide pool does not show a significant change with administration of either phenobarbital or 3-methylcholanthrene.

Next, the effect of the two drugs on cytoplasmic polyribosomal-RNA synthesis was examined; the labelling of total polyribosomal RNA as well as poly(A)-containing RNA was examined. The results presented in Table 2 indicate that, with 1 h of drug

Fig. 1. Rates of nuclear RNA synthesis in rat liver at different time intervals after administration of phenobarbital or 3-methylcholanthrene

At different time intervals after administration of phenobarbital or 3-methylcholanthrene, the animals received 200 µCi of [³H]uridine and were killed 30 min after the administration of the label. Total nuclear RNA was isolated as described in the text. Δ, 3-Methylcholanthrene treatment; ○, phenobarbital treatment. The experiment has been repeated at least four times and livers from two animals are pooled for each treatment. The ranges of values obtained are indicated by bars.

Table 1. Rates of nuclear, nucleolar and nucleoplasmic RNA synthesis in rat liver after administration of phenobarbital or 3-methylcholanthrene

At 2 h after administration of phenobarbital or 3-methylcholanthrene, the animals received 500 μ Ci of ^{32} P and were killed 30 min after administration of label. Total nuclear, nucleolar and nucleoplasmic RNA were isolated and their specific radioactivities were determined as described in the text. The results obtained in two independent experiments are given.

	Radioactivity in RNA (c.p.m./ A_{260} unit)						Radioactivity in nucleotide pool (c.p.m./ A_{260} unit)	
	Nuclear		Nucleolar		Nucleoplasmic		I	II
	I	II	I	II	I	II		
Control	2150	2650	2546	2880	1987	2263	10116	12384
Phenobarbital	1828	2118	1360	1940	2048	2393	9650	12000
3-Methylcholanthrene	2358	3092	2684	2956	2536	3264	10146	14300

Table 2. Effect of phenobarbital and 3-methylcholanthrene on the incorporation of ^{32} P into polyribosomal and poly(A)-containing RNA in rat liver

The animals received 500 μ Ci of ^{32} P (carrier-free) 1 h or 6 h after drug administration and were killed 2 h after the tracer administration. Total polyribosomal and poly(A)-containing RNA were isolated from the postmitochondrial supernatant as described in the text. Polyribosomal RNA (30 A_{260} units) was loaded on poly(U)-Sephadex columns for the isolation of poly(A)-containing RNA. The results represent the averages of two independent experiments, the values of which are given in parentheses.

Treatment	Radioactivity in polyribosomal RNA (c.p.m./ A_{260} unit)	Radioactivity in poly(A)-containing RNA		Nucleotide pool (c.p.m./ A_{260} unit)
		(c.p.m./ A_{260} unit of polyribosomal RNA)	(% of polyribosomal RNA)	
Control				
1 h	2050 (1850, 2250)	123 (115, 130)	6.0 (6.2, 5.8)	10250 (10000, 10500)
6 h	2104 (1900, 2308)	125 (110, 140)	5.9 (5.8, 6.1)	11152 (10500, 11804)
Phenobarbital				
1 h	1552 (1280, 1824)	161 (135, 187)	10.4 (10.5, 10.3)	9823 (9200, 10446)
6 h	2555 (2431, 2679)	284 (261, 307)	11.1 (10.7, 11.5)	10155 (9662, 10647)
3-Methylcholanthrene				
1 h	2251 (2050, 2452)	239 (209, 269)	10.6 (10.2, 11.0)	11256 (10250, 12261)
6 h	2987 (2774, 3200)	457 (405, 509)	15.3 (14.6, 15.9)	12252 (12126, 12378)

administration followed by 2 h of labelling, the total cytoplasmic polyribosome RNA labelling is decreased by phenobarbital. However, the labelling of poly(A)-containing RNA, in the absolute sense or expressed as percentage of the total polyribosomal RNA, shows an increase. After 6 h of phenobarbital administration, labelling of both total polyribosomal RNA as well as of poly(A)-containing RNA increases. With 3-methylcholanthrene, the increased labelling of total cytoplasmic polyribosomal RNA as well as of poly(A)-containing RNA is evident at both 1 and 6 h after the drug administration. The increase at the latter time point is very pronounced.

The next question was to examine whether the drugs would have any specific effect on the transport of RNA from the nucleus to the cytoplasm. For this purpose, [^{14} C]orotate-labelled nuclei were isolated from normal and drug-treated animals and incubated with the cytosol isolated from another set of normal and drug-treated rats which had not received the radioactive label. For the isolation of liver nuclei or cytosol from drug-treated animals, the rats were treated with phenobarbital or 3-methylcholanthrene for 2½ h. The incubation was carried out under conditions which permit a temperature- and energy-dependent release of labelled RNA from the nucleus

to the cytoplasm (Yu *et al.*, 1972; Schumm & Webb, 1974). The results presented in Figs. 2 and 3 reveal that the nature of the cytosol has a pronounced effect on the release of RNA from the nuclei. Maximum

Fig. 2. Effect of phenobarbital treatment on the transport of RNA from the nucleus to cytoplasm

Nuclei were labelled by injecting [¹⁴C]orotic acid (30 μ Ci) to control or phenobarbital-treated animals. The label was given 2 h after phenobarbital administration and the animals were killed 30 min after the label administration. Cytosol was isolated from another set of control or phenobarbital-treated animals that had not received any radioactive label. Labelled nuclei were incubated with the cytosol, and the labelled RNA released into the cytoplasm was measured as described in the text. ■, Nuclei (phenobarbital-treated)+cytosol (phenobarbital-treated); □, nuclei (control)+cytosol (phenobarbital-treated); △, nuclei (phenobarbital-treated)+cytosol (control); ▲, nuclei (control)+cytosol (control).

release of RNA is observed when nuclei isolated from phenobarbital-treated animals are incubated with the homologous cytosol. The cytosol isolated from phenobarbital-treated animals facilitates a greater release of RNA from nuclei than does the cytosol from control rats. However, cytosol from 3-methylcholanthrene-treated animals has no significant effect on the release of RNA from nuclei, and in fact leads to a marginal inhibition of this release. Thus the two drugs phenobarbital and 3-methyl-

Fig. 3. Effect of 3-methylcholanthrene treatment on the transport of RNA from the nucleus to cytoplasm

The experimental details are as given in Fig. 2 except that 3-methylcholanthrene was used in place of phenobarbital. ■, Nuclei (3-methylcholanthrene-treated)+cytosol (control); □, nuclei (3-methylcholanthrene-treated)+cytosol (3-methylcholanthrene-treated); ▲, nuclei (control)+cytosol (control); △, nuclei (control)+cytosol (3-methylcholanthrene-treated).

Table 3. Localization of phenobarbital and 3-methylcholanthrene or their derivatives in chromatin
Pheno[2-¹⁴C]barbital (15 μ Ci, containing 5.3 mg of the drug) or [G-³H]3-methylcholanthrene (100 μ Ci, containing 2.7 mg of the drug) was injected into rats (66–67 g body wt.) intraperitoneally and the animals were killed 2½ or 6 h after the drug administration. Nuclei were isolated from liver and processed for the isolation of chromatin as described in the text. Under the experimental conditions used, ¹⁴C radioactivity was counted with an efficiency of 35% and ³H with an efficiency of 5%. The results given pertain to the radioactivity detected in the different fractions obtained from 2.5 g of liver. DNA was determined in the homogenate, nuclear and chromatin fractions. On this basis, the recovery of nuclei was 60 ± 2.5% of the homogenate and chromatin recovery was 55 ± 3% of that of the nuclei. The values in parentheses give the content in nmol/mg of DNA calculated by using the molecular weights of the parent compounds. The results given are the average values obtained from two independent experiments, in the second of which the absolute values obtained were 10–15% lower for all the fractions. The patterns were identical in both the experiments.

Labelling period ...	2½ h		6 h	
	Phenobarbital (c.p.m.)	3-Methylcholanthrene (c.p.m.)	Phenobarbital (c.p.m.)	3-Methylcholanthrene (c.p.m.)
Fraction				
Homogenate	691 500	632 500	4095 000	2311 500
Nuclei	1140	11 500	15 000	10 000
Triton X-100-washed nuclei	1020	10 600	7075	6200
Chromatin	44 (0.05)	986 (0.63)	237 (0.26)	5050 (3.80)

cholanthrene do show differences in their early action at the level of nuclear RNA synthesis, cytoplasmic poly(A)-containing RNA labelling and RNA transport from the nucleus to cytoplasm. It was decided to examine whether the two drugs would show differences in nuclear localization, particularly at early time periods. For this purpose pheno[2-¹⁴C]-barbital and [G-³H]3-methylcholanthrene were injected along with the usual dose of these drugs given to the rats. The animals were killed 2½ h or 6 h after the drug administration. The distribution of the label in nuclear subfractions was examined. The results presented in Table 3 indicate that, at 2½ h with pheno[2-¹⁴C]barbital, the nuclear fraction accounts for 0.16% of the homogenate radioactivity. With [G-³H]-3-methylcholanthrene, 1.8% of the homogenate radioactivity is found in the nuclear fraction. At 6 h after drug administration, the nuclear fraction accounts for around 0.4% of the homogenate radioactivity with either drug. At both time intervals, the radioactivity of phenobarbital localized in the chromatin is significantly lower than that of 3-methylcholanthrene. In absolute terms the concentration of 3-methylcholanthrene in the chromatin is 10–15 times that of phenobarbital at both time intervals.

Discussion

The premise of the present investigation is to suggest that the primary site of action of phenobarbital and 3-methylcholanthrene in liver at the molecular level may be different, although ultimately the effects of both the drugs involve an increase in transcription rates. Thus, at least up to 8 h after drug administration, phenobarbital has no stimulating effect on nuclear RNA synthesis: in fact, an early effect of the drug is to depress transiently the labelling of nucleolar RNA. The exact reason for this is not clear, although similar observations have been reported (Cohen & Ruddon, 1970; Gielen & Nebert, 1971). In the present study the specific radioactivity of the nucleotide pool does not show any appreciable change with administration of either phenobarbital or 3-methylcholanthrene. The drug has no effect on nucleoplasmic RNA synthesis, at least for 2½ h. 3-Methylcholanthrene has no effect on nucleolar RNA synthesis, but does have a stimulating effect on nucleoplasmic RNA synthesis.

The increase in cytoplasmic poly(A)-containing RNA synthesis, the presumed mRNA fraction, in response to 3-methylcholanthrene administration is associated with an increase in nucleoplasmic RNA synthesis. With phenobarbital administration, the increase in cytoplasmic poly(A)-containing RNA synthesis is not reflected in an increase in nucleoplasmic RNA synthesis. Although cytoplasmic poly(A)-containing RNA constitutes only a small fraction of the heterogeneous nuclear RNA, since

most of the primary transcript is degraded within the nucleus, a direct stimulation of functional mRNA synthesis is generally reflected by enhanced transcription contributed by enhanced RNA polymerase activity or template activity or both (Feigelson *et al.*, 1975; Means *et al.*, 1972).

The lack of such a reflection at the level of nucleoplasmic RNA synthesis, at least during early periods of phenobarbital action, has given rise to the possibility of whether the drug acts primarily at the level of transport of RNA from the nucleus to the cytoplasm. The results obtained in the present investigation establish that the cytosol isolated from the livers of rats treated with phenobarbital for 2 h is capable of eliciting enhanced transport of RNA from the nucleus. Under the conditions used for labelling and incubation of nuclei, essentially messenger-like RNA is released (Yu *et al.*, 1972). Interestingly, the cytosol isolated from 3-methylcholanthrene-treated rats does not show this effect. Previously, it has been shown that the cytosol isolated from the livers of rats treated with phenobarbital for 16 h elicits enhanced transport of RNA from the nucleus compared with the cytosol isolated from normal livers (Hazen & McCauley, 1976). Further, the increased transport of RNA from the nucleus brought about by the liver cytosol of phenobarbital-treated rats may not be due to alterations of ribonuclease activity, since the liver cytosol is known to contain an endogenous ribonuclease-inhibitor protein (Gribnau *et al.*, 1969). In addition, under the incubation conditions used here, the addition of excess of yeast RNA should saturate and thus eliminate the possibility of ribonuclease activity contributing to increase in RNA transport. It is noteworthy that it has been shown previously in this laboratory that administration of 2-allyl-2-isopropylacetamide, a potent inducer of the enzyme δ -amino-laevulinate synthase and a hypertrophic agent, also enhances the transport of RNA from the nucleus to cytoplasm (Sardana & Padmanaban, 1976).

Phenobarbital and 3-methylcholanthrene show a substantial difference in their extent of localization in the chromatin. At the concentrations that are widely used to elicit the variety of effects reported in the literature, namely 20.8 μ mol/100 g body wt. for phenobarbital and 10.1 μ mol/100 g body wt. for 3-methylcholanthrene, the latter drug shows 10–15-fold higher concentration in the chromatin than phenobarbital. The results in Table 2 indicate the actual radioactivity measured, and the total radioactivity handled at the homogenate level are comparable for both the drugs. It is also unlikely that C-2 is lost during the metabolism of phenobarbital and therefore it appears reasonable to conclude that a much smaller amount of phenobarbital or its derivative than of 3-methylcholanthrene or its derivative reaches the chromatin. The significance of this result is not clear, although it is tempting to relate the lower concentra-

tion of phenobarbital in the chromatin to its lack of direct effects on chromatin activity.

Transport of RNA from the nucleus to cytoplasm has been suggested to regulate the process of transcription in terms of both quantity and nature of the RNA species synthesized (Lichtenstein & Shapot, 1976; Bastian, 1978). On the basis of the results of the present investigation it is suggested that phenobarbital primarily exerts its effects at the cytoplasmic level by enhancing the transport of RNA from the nucleus to the cytoplasm, which in turn can enhance transcription at later time points. This sort of stimulus can perhaps be broad-based and therefore can elicit the activation of a large number of genes. On the other hand, 3-methylcholanthrene or its active metabolite binds directly to the chromatin at early time points and leads to specific gene activation enhancing the synthesis of a limited number of proteins.

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