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Effects of Polycyclic Hydrocarbons on Ribonucleic Acid Synthesis in Rat Liver Nuclei and Hamster Embryo Cells

L. R. YOUNGER, R. SALOMON, R. W. WILSON, A. C. PEACOCK, AND H. V. GELBOIN

Chemistry Branch, National Cancer Institute, National Institutes of Health, Bethesda, Maryland 20014

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SUMMARY

Polycyclic hydrocarbons induce the microsomal enzyme aryl hydrocarbon (benzo[*a*]pyrene) hydroxylase in the livers of intact rats and in hamster embryo cells. *In vivo*, methylcholanthrene causes an increased incorporation of precursors into nuclear RNA and an increased RNA polymerase activity in hepatic nuclei. Gel electrophoresis of RNA synthesized *in vitro* by isolated nuclei has shown that the synthesis of all sizes of RNA is enhanced by the methylcholanthrene treatment *in vivo*. Hypophysectomized or adrenalectomized rats previously treated with hydrocarbons fail to exhibit an increase in RNA polymerase activity, although they manifest an increase in the level of aryl hydrocarbon hydroxylase.

Double-labeling techniques and acrylamide gel electrophoresis showed no detectable change in the pattern of RNA synthesized during enzyme induction in cell culture. The latter studies and those carried out *in vivo* with adrenalectomized and hypophysectomized rats suggest that the methylcholanthrene-induced gross changes in liver nuclear RNA metabolism *in vivo* are not requirements for microsomal enzyme induction, and that the induction-specific RNA synthesis, indicated as a requirement by our other studies, is of small magnitude.

INTRODUCTION

A single dose of certain polycyclic hydrocarbons given parenterally to rats induces microsomal enzyme activity and causes a marked alteration in hepatic nuclear RNA metabolism. The latter includes increases in the nuclear RNA content, in incorporation of RNA precursors into RNA (1), and in RNA polymerase activity (2–4) measured *in vitro*. One of many hepatic microsomal enzyme systems induced by hydrocarbons is the aryl hydrocarbon (benzo[*a*]pyrene) hydroxylase (5–7). Benz[*a*]anthracene and 3-methylcholanthrene are both inducers of this enzyme system. Induction of aryl hydrocarbon hydroxylase by 3-methylcholanthrene *in vivo* is blocked by inhibitors of protein or RNA synthesis (7). Aryl hydrocarbon hydroxylase in cultured hamster embryo cells is also induced by benz[*a*]anthracene (8). As was the case with the liver enzyme studied in the intact rat, this induction in cultured hamster embryo cells was prevented by inhibitors of protein or RNA synthesis (9), indicating a requirement for both protein and RNA synthesis. Inhibition of RNA synthesis prevents induction of other enzymes in cell culture systems (10–15). The present study is concerned with the nature of changes in RNA metabolism both *in vivo* and in cell cultures during hydrocarbon-induced microsomal enzyme induction.

MATERIALS AND METHODS

Female hamsters pregnant for 10–12 days and male Sprague-Dawley rats weighing 50–

70 g were obtained from the National Institutes of Health animal supply; male Sprague-Dawley hypophysectomized rats and adrenalectomized rats were obtained from the Charles River Breeding Laboratories. [6-^{14}C]Orotic acid (3.8 mCi/mmole), [^{3}H]cytidine (generally labeled, 5.4 Ci/mmole), and [^{3}H]CTP (generally labeled, 5.4 Ci/mmole) were obtained from New England Nuclear Corporation. [^{14}C]Uridine (generally labeled, 490 μCi/μmole) and [5-^{3}H]uridine (29.8 mCi/mmole) were purchased from Nuclear-Chicago Corporation. 3-Methylcholanthrene and benzanthracene were obtained from Eastman Organic Chemicals; ATP, GTP, UTP, and CTP, from P-L Biochemicals; cycloheximide, from the Cancer Chemotherapy National Service Center; and Eagle's No. 2 medium and Dulbecco's phosphate-buffered NaCl solution, from the National Institutes of Health media supply (16).

Hepatic nuclear RNA synthesis in vivo following 3-methylcholanthrene treatment. Groups of three male rats weighing 50–70 g were fasted for 24 hr prior to death. At 18 or 3 hr prior to death they received 1 mg of MC[1] in 0.25 ml of corn oil intraperitoneally. Control animals received 0.25 ml of corn oil alone, 18 hr before death. Thirty minutes before death all rats were treated intraperitoneally with [^{3}H]cytidine (5 mCi/kg, 1 mCi/ml, 5.4 Ci/mmole). The animals were killed and exsanguinated by decapitation, and their livers were rapidly removed, pooled, chilled in an iced solution of 0.35 M sucrose–2 mM $MgCl_2$–1 mM potassium phosphate, pH 6.8 (solution A), and homogenized in 9 volumes of the same solution with five strokes of a loosely fitting pestle in a Dounce homogenizer. The nuclei were separated and purified by sedimentation through hypertonic sucrose according to Sporn and Dingman (17).

The nuclear pellet from approximately 4 g of liver was treated with 5 ml of 0.1% sodium lauryl sulfate and then stirred vigorously with an equal volume of water-saturated phenol for 30 min at room temperature. The phases were separated centrifugally, the aqueous phase was removed, and the phenol precipitate phase was re-extracted for 10 min with 4 ml of 0.1% sodium lauryl sulfate. The phases were separated, and the two aqueous phases were combined. This mixture was made 0.2 M in potassium acetate (pH 6.0) and re-extracted for 15 min with 0.5 volume of 90% phenol, and the aqueous phase was removed. The RNA was precipitated overnight at −20° by the addition of 2.5 volumes of ethanol. The precipitate was washed twice with an iced solution of 1 volume of 0.05 M NaCl–0.001 M EDTA (pH 6.8) and 2.5 volumes of ethanol, and then dissolved in 0.4 ml of 0.05 M NaCl–0.001 M EDTA for electrophoresis. Acrylamide gel mixed acrylamide–agarose gel electrophoresis and gel sectioning and counting were performed according to the methods of Dingman and Peacock (18). Gel strips (12 mm wide) were placed on Saran Wrap over a paraffin-coated tray, and cut using a Lucite block which contained 150 stainless-steel blades set 1 mm apart. Recovery of isotope was usually better than 90% for both ^{3}H and ^{14}C, and was achieved by incubating the gel fractions in 0.2 ml of 1 M NaOH overnight at room temperature, then adding 1 ml of "NCS" reagent (Nuclear-Chicago), mixing, allowing the mixture to stand with occasional agitation for 1 hr at room temperature, and adding 10 ml of toluene scintillator containing 5 g of 2,5-diphenyloxazole and 0.1 g of 1,4-bis[2-(5-phenyloxazolyl)]benzene per liter of toluene. Counting efficiencies for doubly labeled material (obtained in a Packard Tri-Carb scintillation spectrometer, model 4312) averaged 31% for ^{3}H and 56% for ^{14}C. All counts reported have been corrected for background.

RNA synthesis in vitro by isolated hepatic nuclei. Male Sprague-Dawley rats weighing 100–200 g were fasted for 20 hr before death. Three hours before death they were given 100 mg/kg of MC in corn oil intraperitoneally (8 mg/ml). Controls received only corn oil. One-half hour prior to death they received 0.5 mCi/kg of [6-^{14}C]orotic acid intraperitoneally (100 μCi/ml, 3.8 mCi/mmole). Preparation of the liver nuclei was performed as described above.

The nuclear pellet from the hypertonic

[1] The abbreviations used are: MC, 3-methylcholanthrene; BA, benz[*a*]anthracene.

sucrose centrifugation step was washed once with solution A and then suspended in a crude preparation of the rat liver cytoplasmic ribonuclease inhibitor described by Roth (19) and modified as follows. Eighteen-hour fasted male Sprague-Dawley rats weighing 100–200 g were killed by decapitation. The livers were rapidly removed, cooled in an iced solution of 0.35 M sucrose–5 mM $MgCl_2$–1 mM potassium phosphate (pH 6.8), and then homogenized in 2.5 volumes of the same solution with three strokes in a Dounce homogenizer. The homogenate was centrifuged in a Spinco preparative ultracentrifuge at 105,000 × *g* for 2 hr at 5°. The supernatant solution was passed through 10 volumes of coarse Sephadex G-25 equilibrated with 0.35 M sucrose–2 mM $MgCl_2$–1 mM potassium phosphate, pH 6.8. The midportion of the 280 nm elution peak was used as the inhibitor solution.

Synthesis in vitro of RNA analyzed by gel electrophoresis. The incubation mixture contained the following in a final volume of 2.5 ml: 500 μmoles of Tris buffer (pH 8), 15 μmoles of $MgCl_2$, 50 μmoles of cysteine, 2.5 μmoles each of ATP, GTP, and UTP, 0.21 μmole of [^{3}H]CTP (0.28 Ci/mmole), and nuclei derived from approximately 2.0 g of liver dissolved in 1.4 ml of ribonuclease inhibitor solution. Some incubations included 1.6 mmoles of $(NH_4)_2SO_4$. Incubations were carried out for 2 and 5 min at 37°. At the conclusion of the incubation period the reaction was terminated by the addition of 7.5 ml of a 0.13% sodium lauryl sulfate–0.21 M $(NH_4)_2SO_4$ solution. If the reaction mixture contained ammonium sulfate, 0.13% sodium lauryl sulfate alone was added. Then 10 ml of 90% phenol were added, and the mixture was stirred vigorously on a magnetic stirrer for 30 min at room temperature. The phenol and aqueous phases were separated by centrifugation, and the aqueous phase was reextracted for 15 min with 0.7 volume of water-saturated phenol. The aqueous phase was dialyzed overnight at 0° against 0.05 M NaCl–0.001 M EDTA (pH 6.8) and then made 0.07 M in $MgCl_2$. The RNA was precipitated overnight at −20° by the addition of 2.5 volumes of ethanol. Electrophoresis of the RNA and sectioning and counting of the gels were performed as described above.

System for assay of total nuclear RNA polymerase activity. The nuclei from 8 g of liver were prepared as described above from 20–24-hr fasted, 200-g rats that had previously been treated intraperitoneally at various times with 20 mg of MC in 2.5 ml of corn oil. The adrenalectomized animals were prepared 7–10 days earlier, and the hypophysectomized rats, 7–14 days prior to experimentation. The nuclear pellet from the hypertonic sucrose centrifugation was washed once with 0.35 M sucrose–2 mM $MgCl_2$–1 mM phosphate, pH 6.7, and then suspended in 2.0 ml of the same medium. The assay medium contained the following in a final volume of 0.5 ml: 100 μmoles of Tris (pH 8.0), 10 μmoles of cysteine, 3 μmoles of $MgCl_2$, 0.5 μmole each of ATP, UTP, and GTP, 0.04 μmole of [^{3}H]CTP (2×10^7 cpm/μmole), 120 μmoles of $(NH_4)_2SO_4$, and liver nuclei containing 50–100 μg of endogenous DNA. Incubations were carried out for 5 or 10 min at 37°. At the end of the incubation period, 0.4 ml of 0.2 M EDTA containing 2.0 mg of yeast RNA was added, followed immediately with 2.5 ml of 10% perchloric acid containing 40 μmoles of sodium pyrophosphate and 10 μmoles of EDTA. The tubes were chilled for 10 min and then centrifuged at 2000 × *g* for 10 min. The pellet was washed once with cold 5% perchloric acid–0.02 M pyrophosphate, twice with 5% perchloric acid, once with absolute ethanol, and once with ethanol–chloroform–ether (2:2:1), and then dried overnight. The nucleic acids were hydrolyzed with 1.0 ml of 5% perchloric acid at 90° for 15 min, and the tubes were cooled and centrifuged. Aliquots of 200 μl were taken for liquid scintillation counting and for DNA determination.

RNA synthesis in cultured hamster embryo cells following benz[a]anthracene treatment. The characteristics of the inducible hamster embryo cell culture system have been described (8, 9). Each experiment was performed with four groups of three 75-mm plates of secondary cultures, which had been plated 3 days earlier and were approaching confluent growth at the time of the experiment. Induction was accomplished by replacing the media with 5 ml of Eagle's No. 2

medium containing 3 μg/ml of BA. Control plates received Eagle's No. 2 medium alone. The occurrence of induction was checked by enzyme assay on a parallel set of plates. In the continuous labeling experiments the medium also contained either 1 μCi/ml of [^{14}C]uridine (490 μCi/μmole) or 2 μCi/ml of [^{3}H]uridine (980 μCi/μmole). In the pulse-labeling studies a similar amount of isotope was added at the indicated interval. At the conclusion of the labeling period, the plates were washed three times with cold Dulbecco's buffered NaCl solution and then scraped, and the cells were suspended in a small volume of the same solution. The plates were combined in the following fashion: the three induced plates which had been incubated with [^{14}C]uridine were combined with the three control plates incubated with [^{3}H]uridine, and, reciprocally, the three induced plates incubated with [^{3}H]uridine were combined with the three control plates incubated with [^{14}C]uridine. The cells were pelleted by centrifugation at 800 × *g* for 10 min at 4° and then homogenized in 3.5 ml of iced 0.25 M sucrose–0.01 M Tris (pH 8.0) with 20 strokes of a tightly fitting pestle in a ground glass homogenizer. The homogenate was made 0.1% in sodium lauryl sulfate and stirred vigorously at room temperature with an equal volume of water-saturated phenol for 30 min. The phases were separated, and the phenol precipitate phase was re-extracted with 3 ml of 0.1% sodium lauryl sulfate. The aqueous phase from the second extraction was combined with the first, made 0.2 M in potassium acetate (pH 6.0), and then extracted with 0.5 volume of 90% phenol for 15 min. The aqueous phase from this extraction was made 0.07 M in $MgCl_2$, and the RNA was precipitated with 2.5 volumes of absolute ethanol overnight at −20°. Electrophoresis of the RNA and sectioning and

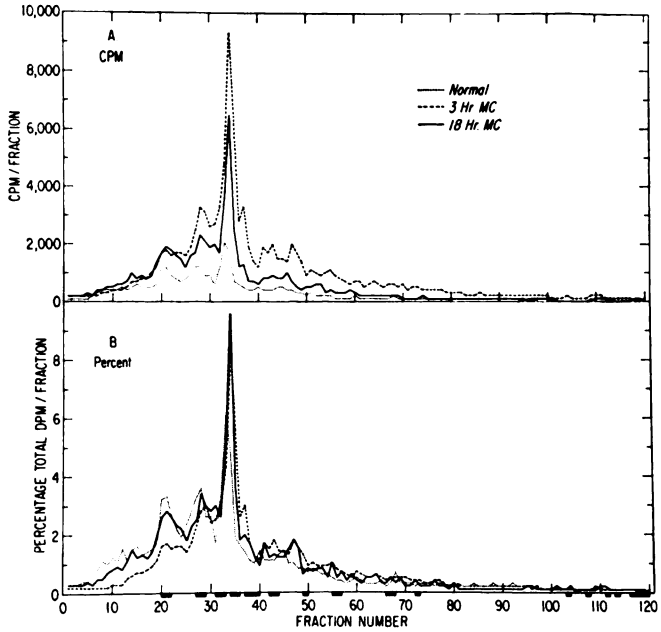

FIG. 1. *Polyacrylamide gel electrophoresis of 30-min pulse-labeled rat liver nuclear RNA following MC treatment*

Rats weighing 50 g were treated intraperitoneally with 1 mg of MC in 0.25 ml of corn oil at 18 or 3 hr prior to death. Controls received 0.25 ml of corn oil alone 18 hr before death. At 30 min before death all rats received intraperitoneally 250 μCi of [^{3}H]cytidine (5.4 Ci/mmole). Isolation of the nuclear RNA, gel electrophoresis, gel sectioning, and counting are described under MATERIALS AND METHODS. A 2% polyacrylamide–0.5% agarose gel was used. A. Total counts per fraction. B. Relative distribution of counts as a percentage of the total.

counting of the gels were accomplished as described earlier (18).

Aryl hydrocarbon hydroxylase was assayed by a modification (8) of the method of Wattenberg *et al.* (6).

RNA was determined by the method of Fleck and Munro (20), and DNA, by Burton's modification of the diphenylamine reaction (21).

RESULTS

Effects of MC treatment on rat liver nuclear RNA metabolism in vivo. Figure 1 shows the distribution of tritiated cytidine in RNA after a 30-min labeling period in normal rats and in rats treated with methylcholanthrene for 3 or 18 hr. The RNA from rats treated for 3 hours with MC exhibited almost a 2-fold increase in cytidine incorporation. At 18 hr after MC administration, the extent of incorporation was still greater than in the controls, but the pattern of incorporation was more similar to that of control rats. Figure 1A shows the separation and distribution of radioactivity in the 15–50 S regions of RNA. There are at least seven distinct bands, in addition to other RNA regions that exhibited staining, but not as distinct bands. These data show that the increase in isotope incorporation was observed in most of the different RNA species and was not confined to a unique species of RNA. There were some small variations in the pattern of labeling in the RNA from MC-treated rats, but these require further investigation to determine their significance. Thus, MC seems to stimulate the incorporation of radioactive precursors into a variety of different RNA species. Since this was a general increase in incorporation, it may have been due either to a higher specific activity of the precursor pool or to an increased synthesis of the RNA. Figure 1B shows the percentage distribution of the various RNA species depicted in Fig. 1A. Although many of the RNA species were labeled to a greater extent 3 and 18 hr after MC treatment, the percentage of total counts was considerably greater in the single peak which was more highly labeled in the RNA from MC-treated rats. Thus, the data suggest that MC causes a redistribution of the labeling pattern of the RNA species in the 15–50 S RNA region.

Effect of MC on liver RNA polymerase measured in vitro. Figure 2 shows the effect of time of methylcholanthrene treatment *in vivo*, varying from 1 to 20 hr, on rat liver RNA polymerase assayed *in vitro*. Maximal stimulation of the RNA polymerase was observed after 3–10 hr of methylcholanthrene treatment. At 20 hr the values for control and methylcholanthrene-treated rats were essentially the same. Prior treatment with MC for 3 hr was used in all other experiments reported here. These results are consistent with those of Loeb and Gelboin (1), who observed maximal stimulation of [^{14}C]orotic acid uptake into nuclear RNA *in vivo* 3 hr after injection of the hydrocarbon.

Figure 3A shows the kinetics of RNA synthesis in the presence and absence of $(NH_4)_2SO_4$ with preparations from control rats and rats treated with methylcholanthrene. $(NH_4)_2SO_4$ may stimulate polymerase activity either by activating certain polymerase forms or by removing certain proteins from DNA sites, which may then become available for RNA transcription. The $(NH_4)_2SO_4$ also strongly inhibited nuclease activity. The stimulation of RNA

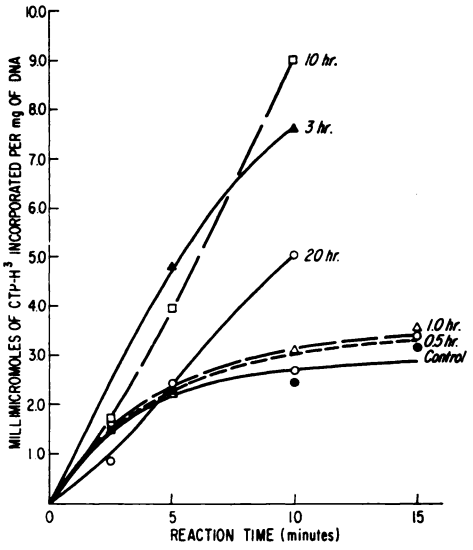

FIG. 2. *Effect of time of treatment with MC on rat liver RNA polymerase activity*

The complete assay system is described under MATERIALS AND METHODS. At 20, 10, 3, 1, or 0.5 hr before death, the animals received 20 mg of MC in 2.5 ml of corn oil. Control animals received 2.5 ml of corn oil alone 20 hr before death.

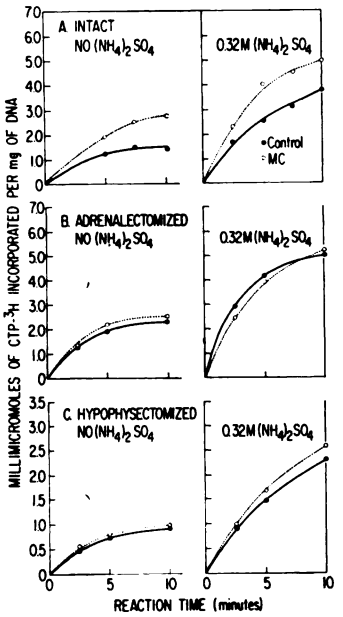

FIG. 3. *Effect of MC treatment on rat liver nuclear RNA polymerase activity*

Twenty-four-hour fasted, 200-g Sprague Dawley rats received 20 mg of MC in 2.5 ml of corn oil intraperitoneally 3 hr before death. Isolation of nuclei and the polymerase assay are described under MATERIALS AND METHODS.

polymerase activity by MC was observed in the absence as well as in the presence of levels of ammonium sulfate that had previously been found to yield optimal cytidine triphosphate incorporation. In the absence of $(NH_4)_2SO_4$ the reaction was almost complete by about 5–7 min in control preparations. In preparations from methylcholanthrene-treated rats there was an 80% increase in the amount of CTP incorporated. In the presence of optimal levels of ammonium sulfate the reaction proceeded for a longer period, about 10–12 min, and the preparation from methylcholanthrene-treated rats showed about a 45% increase in CTP incorporation. Others have reported that stimulation of RNA polymerase by estrogen (22) or growth hormone (23) was not observed in the presence of $(NH_4)_2SO_4$. On the other hand, Tata and Widnell (24) have reported that stimulation of RNA polymerase by thyroxine was observed in both the absence and presence of $(NH_4)_2SO_4$. Our results with methylcholanthrene are similar to the latter.

RNA polymerase in adrenalectomized and hypophysectomized rats. Methylcholanthrene treatment *in vivo* did not alter the polymerase activity (Fig. 3B) of liver nuclei from adrenalectomized rats. In other studies we reported that phenobarbital treatment stimulated RNA polymerase activity in adrenalectomized rats (25). These results with adrenalectomized rats are consistent with those of Jondorf *et al.* (26), who found that in adrenalectomized rats phenobarbital was effective in stimulating microsomal amino acid incorporation whereas methylcholanthrene was ineffective.

Figure 3C shows that methylcholanthrene was also ineffective as an inducer of RNA polymerase activity in the livers of hypophysectomized rats. The requirement for both intact adrenal and pituitary function for the methylcholanthrene stimulation of RNA polymerase suggests that if MC alters gene expression, as reflected by altered RNA synthesis, this action still requires a balance of appropriate hormonal factors. In the absence of this balance methylcholanthrene does not affect RNA polymerase. Another possibility is that the primary target of the hydrocarbon is the endocrine system. This is unlikely, since many of the effects of MC, such as microsomal enzyme induction, occur in rats that have been either hypophysectomized or adrenalectomized.

Aryl hydrocarbon hydroxylase induction in adrenalectomized and hypophysectomized rats. Table 1 shows microsomal aryl hydrocarbon hydroxylase activity in normal, adrenalectomized, and hypophysectomized rats treated with either corn oil or MC. The basal levels of enzyme were lower in adrenalectomized or hypophysectomized animals than in normal controls. With MC treatment there was a marked increase in the enzyme level in adrenalectomized and hypophysectomized rats. The relative increase was in fact greater than in the intact rat. Thus, enzyme induction occurs to at least the same extent in rats that have been either hypophysectomized or adrenalectomized. In these rats MC has no effect on polymerase and yet shows strong inducing activity. These experiments clearly

TABLE 1

Effect of administration of MC in vivo on aryl hydrocarbon (benzo[a]pyrene) hydroxylase in livers of adrenalectomized and hypophysectomized rats

The treatment of adrenalectomized and hypophysectomized rats is described under MATERIALS AND METHODS. The liver homogenates from these rats were assayed for aryl hydrocarbon (benzo[a]pyrene) hydroxylase activity by a modification (8) of the method of Wattenberg *et al.* (6). Each value below represents the range of values obtained from individual determinations of three rats. The MC was given as described in the text 16 hr prior to death.

Rats	Aryl hydrocarbon hydroxylase[a]	
	Control	MC-treated
Normal	2,430–4,950	9,165–10,440
Adrenalectomized	438–1,071	5,610–6,180
Hypophysectomized	1,080–1,650	6,135–6,615

[a] Picomoles of product equivalent to 3-hydroxybenzo[a]pyrene per 30-min incubation per milligram of protein.

indicate the independence of the gross changes in the polymerase from the induction of the microsomal hydroxylase. This, however, does not eliminate the possibility that subtle, undetectable changes in RNA polymerase activity or RNA synthesis are part of the induction process.

Analysis of RNA synthesized in vitro in nuclei from MC-treated rats. Figure 4 shows an electrophoretic analysis of the RNA synthesized *in vitro* by rat liver nuclei isolated 3 hr following the administration of MC. The nuclear RNA was labeled with ^{14}C by injection of [6-^{14}C]orotic acid 30 min before the animals were killed. The incubations *in vitro* were performed in the presence of a ribonuclease inhibitor derived from rat liver cytoplasm, in an effort to minimize degradation of the newly synthesized RNA by nuclear ribonuclease during the incubation. In the absence of ammonium sulfate, however, degradation was still observed. The degradation of the RNA thus labeled was shown during a 2- and 5-min incubation *in vitro*. The degradation of this RNA labeled *in vivo* was roughly similar in the nuclei from control and MC-treated rats, but at 5 min it might have been slightly greater in the latter. When [^{3}H]CTP was added during the incubation, however, incorporation into RNAs in the 18–45 S region was observed. The nuclei from MC-treated rats were considerably more active in this synthesis than control nuclei. At 5 min there was a large increase of isotope content in the low molecular weight (4 S region) RNA. Similar results were obtained in the presence of $(NH_4)_2SO_4$, in which degradation of RNA was largely absent, as shown in Fig. 4b. The results show that in incubations performed in both the presence and absence of $(NH_4)_2SO_4$, relative to the 30-min labeling with [6-^{14}C]orotic acid, there was increased incorporation of [^{3}H]CTP into RNA by nuclei from MC-treated animals. This increase was general and roughly proportionate throughout the RNA size range. This is similar to the effects of MC on nuclear RNA synthesis *in vivo* described in Fig. 1. In the presence of ammonium sulfate there was almost complete prevention of the degradation of RNA that had been observed in its absence. In the presence of ammonium sulfate, and hence in the absence of significant RNA degradation, the MC significantly increased the incorporation of precursor into RNA. These observations presumably were due to enhanced synthesis rather than to an altered degradation pattern.

Effects of BA treatment on RNA metabolism of cultured hamster embryo cells. In cell culture no changes in gross RNA metabolism during enzyme induction were detected. Thus the RNA content of the cells, and the incorporation of uridine into RNA during a short, 30-min and a long, 12-hr labeling period, were identical in control and BA-induced cells. Previous studies have shown no difference between induced and control cells in the rate of cell multiplication or the incorporation of labeled amino acid into protein during this initial 12 hr of induction (7). In contrast to the major changes observed *in vivo*, these studies show the absence of detectable over-all alterations in cellular RNA metabolism during induction of aryl hydrocarbon hydroxylase by BA.

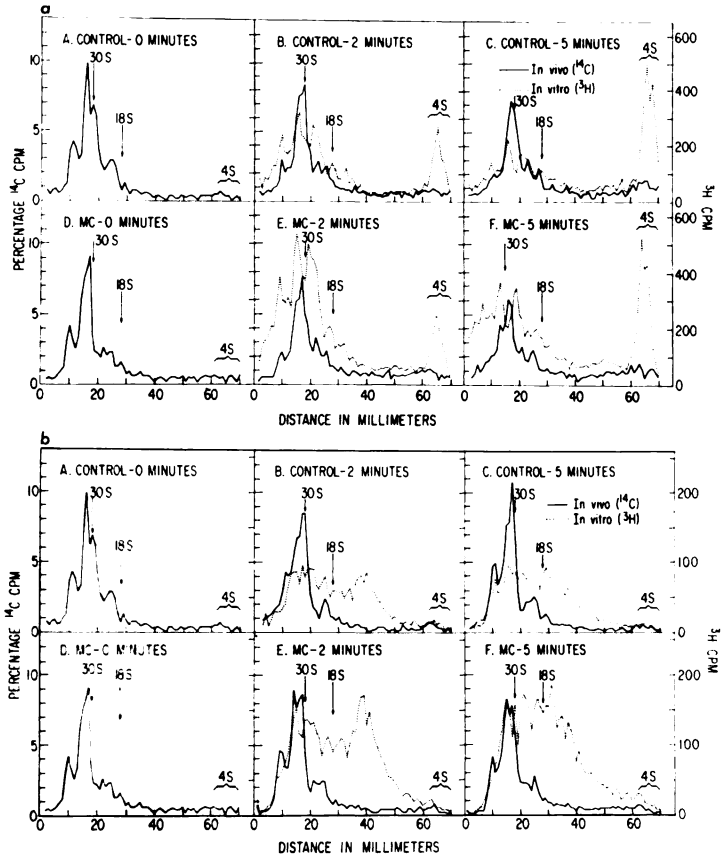

FIG. 4. *Gel electrophoresis of rat liver nuclear RNA synthesized in vitro following MC treatment*

Eighteen-hour fasted rats weighing 100–230 g were treated intraperitoneally with 100 mg/kg of MC 3 hr before death. At 30 min before death they received 500 μCi/kg of [^{14}C]orotic acid (3.8 mCi/mmole) intraperitoneally. Isolation of the liver nuclei, nuclear incubations, and isolation and electrophoresis of the nuclear RNA are described under MATERIALS AND METHODS. The ^{3}H counts were adjusted to comparable levels by correcting them to the same number of ^{14}C 30-min counts per minute. As the labels in the control and MC-treated groups were not identical because of the stimulatory effect of the MC, this method of reporting the data somewhat underestimates the extent of increase of incorporation into RNA *in vitro* in the MC-treated group. a. Incubations performed without $(NH_4)_2SO_4$. b. Incubations performed in 0.64 M $(NH_4)_2SO_4$. A. Control, no incubation. B. Control, 2-min incubation. C. Control, 5-min incubation. D. MC-treated, no incubation. E. MC-treated, 2-min incubation. F. MC-treated, 5-min incubation.

Electrophoretic analyses of RNA from control and BA-treated cells. The methodology employed in this study was a double-label technique. Control and induced cells were incubated with [^{3}H]uridine and [^{14}C]uridine, respectively. The cells were combined immediately following the incubation and prior to the RNA extraction and electrophoresis. An alteration in RNA metabolism in the induced cells would have appeared as a change in the ^{14}C:^{3}H ratio in the RNA species involved. Each experiment was done simultaneously with the isotopes reversed. Thus, any significant change would be indicated by an increase in the ^{14}C:^{3}H ratio in one set and a decrease in the corresponding ratio of the second set. The results were plotted as the ratio of the percentage of ^{3}H counts in a gel slice (relative to the total ^{3}H counts in the gel) to the percentage of ^{14}C counts in that slice. If the uptake, distribution, and kinetics of activation of the labeled

uridine were identical in the induced and control cells, a ratio of 1.0 for a slice would indicate that the species of RNA in that slice had been synthesized and/or metabolized at the same rate during the incubation period. A ratio differing from 1.0 could result from differences in uridine uptake or metabolism or from changes in RNA synthesis or metabolism. Significant deviations from a ratio of 1.0 would be reciprocal when the isotopes had been reversed when the deviation was a consequence of induction. The extent of variation inherent in the methodology is shown in Fig. 5. Two groups of cells were incubated with control media containing either [^{14}C]uridine or [^{3}H]uridine for 75 min. They were then combined, and the RNA was extracted and subjected to electrophoresis. As the labeled RNA in each combined pair should be identical, the ^{14}C:^{3}H percentage ratios should theoretically be 1.0 for each slice throughout the gel. As actually observed, the ratios in the 2% acrylamide–0.5% agarose gel (containing RNA species between approximately 50 and 16 S) fell in the range of 0.9–1.0, with few values outside this range. In the 10% acrylamide gel (containing RNA species between 4 S and approximately 12 S), the majority of values were between 0.9 and 1.15, except toward the top of the gel, where relatively few counts were found. As would be expected in this control experiment, when a slice showed a substantial deviation from a ratio of 1.0, the parallel experiment did not show corresponding reciprocal changes.

The kinetics of induction of aryl hydro-

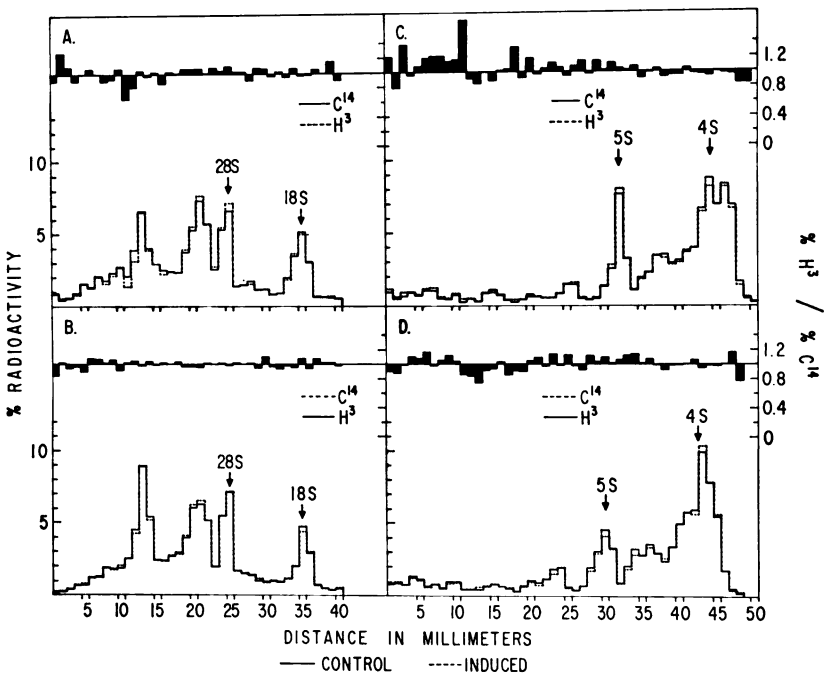

FIG. 5. *Gel electrophoresis of cultured hamster embryo cell RNA*

Calibration of the double-label technique for comparative evaluation of RNA metabolism during enzyme induction. Three 75-mm plates of secondary cultures of hamster embryo cells were used in each group. At zero time the media in two groups were replaced with 6 ml of fresh medium containing either [5-^{3}H]uridine (2 μCi/ml, 980 μCi/mmole) or [^{14}C]uridine (1 μCi/ml, 490 μCi/μmole). Two other groups were similarly treated, except that the media also contained BA, 3 μg/ml. After 75 min the cells were collected, combining the two control groups together and the two induced groups together. Extraction and electrophoresis of the RNA, sectioning, and counting of the gels are described under MATERIALS AND METHODS. A. 2% acrylamide–0.5% agarose gel electrophoresis of RNA from control cells. B. 2% acrylamide–0.5% agarose gel electrophoresis of induced cells. C. 10% acrylamide gel electrophoresis of RNA from control cells. D. 10% acrylamide gel electrophoresis of induced cells.

carbon hydroxylase shows a lag period of approximately 35 min, followed by a linear increase in enzyme activity for more than 12 hr (9). At this time enzyme activity was 10–20 times the basal level. Possible alterations in cellular metabolism of RNA were examined at two intervals during induction: at the initiation of induction for 1¼ hr (Fig. 6), and for 6 hr (Fig. 7). The pattern of isotope incorporation into the RNA of the induced cells appeared identical with that of the controls according to the criterion of significant deviation from a ratio of 1.0 with a reciprocal change of similar degree in the paired experiment. Thus, careful analysis failed to detect changes in the profile of RNA synthesis during the induction of aryl hydrocarbon hydroxylase. Similar results, not shown, were obtained when the cells were exposed to isotope from 2 to 2¾ hr after addition of the inducer.

Cells incubated with inducer in the presence of 1 mg/ml of cycloheximide failed to show an increase in enzyme activity until the cycloheximide had been removed. At that time, induction began without the lag phase that ordinarily occurs at the onset of induction (27). These data suggest that during cycloheximide suppression of induction the RNA species involved in induction may still be synthesized and may accumulate because of the block in translation. Therefore the RNA labeled in the presence of 1 μg/ml of cycloheximide for 3 hr after addition of inducer was examined. Here also, no significant differences were observed in the patterns of RNA labeling in the control and induced cells.

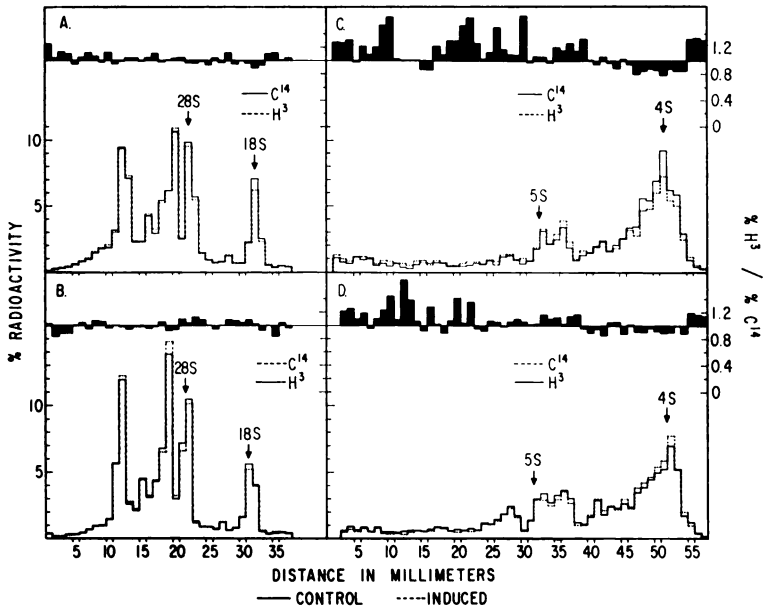

FIG. 6. *Gel electrophoresis of cultured hamster embryo cell RNA labeled during the 75-min period immediately following addition of inducer*

Four groups of three plates, each of secondary hamster embryo cell cultures, were used. At zero time the medium on each plate was replaced with 6 ml of fresh medium with the following additions: group 1, no BA, 12 μCi of [^{3}H]uridine (960 μCi/μmole); group 2, no BA, 6 μCi of [^{14}C]uridine (480 μCi/μmole); group 3, BA, 3 μg/mmole, 12 μCi of [^{3}H]uridine (960 μCi/μmole); group 4, 3 μg/ml of BA, 6 μCi of [^{14}C]-uridine (480 μCi/μmole). After 75 min the cells were collected, combining groups 1 and 4 and groups 2 and 3. Extraction and electrophoresis of the RNA and sectioning and counting of the gels are described under MATERIALS AND METHODS (17). A. 2% acrylamide–0.5% agarose gel, groups 2 and 3. B. 2% acrylamide–0.5% agarose gel, groups 1 and 4. C. 10% acrylamide gel, groups 2 and 3. D. 10% acrylamide gel, groups 1 and 4.

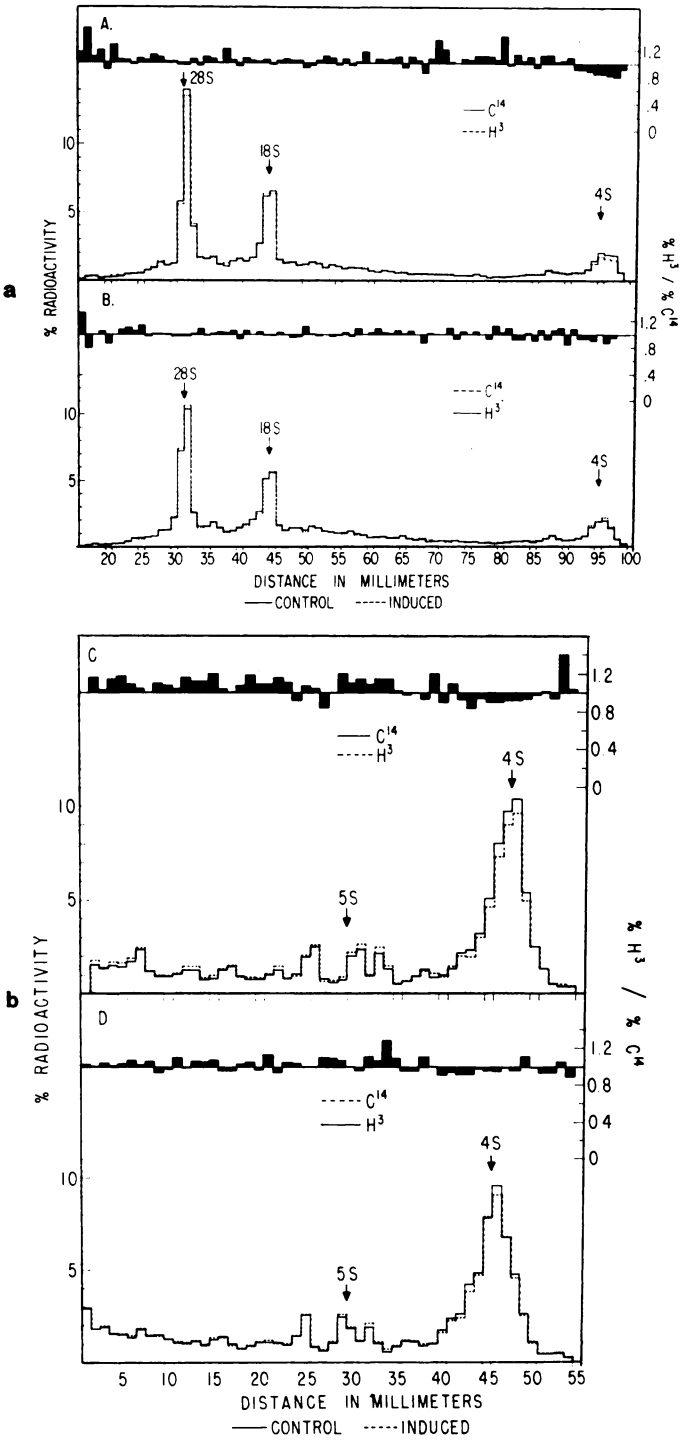

FIG. 7. *Gel electrophoresis of cultured hamster embryo cell RNA labeled during the 6-hr period following administration of BA*

Experimental details were the same as in Fig. 6, except for the length of the labeling period. a. 2% acrylamide–0.5% agarose gel: A, groups 2 and 3; B, groups 1 and 4. b. 10% acrylamide gel: C, groups 2 and 3; D, groups 1 and 4.

DISCUSSION

These studies show a disparity in the effects of certain polycyclic hydrocarbon inducers of microsomal enzymes on RNA metabolism in the intact animal and on cells grown in tissue culture. In the former and in isolated nuclei from MC-treated rats, there is a proportional over-all acceleration of many regions of the larger RNA synthesis (15–45 S). In cell cultures undergoing enzyme induction, no alterations of RNA metabolism were detected by a sensitive double-isotope technique. In both systems, aryl hydrocarbon hydroxylase was markedly increased by the hydrocarbons. MC treatment also did not increase the hepatic nuclear RNA polymerase activity of adrenalectomized or hypophysectomized animals, although induction of microsomal aryl hydrocarbon hydroxylase occurred. In this respect, adrenalectomized or hypophysectomized animals are similar to cultured hamster embryo cells. These findings establish that the generalized increase in RNA synthesis following MC administration to an intact rat is not a requirement for enzyme induction. These large effects *in vivo* may be relatively nonspecific and related to generalized systemic response to a toxic agent, perhaps mediated by the endocrine system. The MC-induced increase in RNA polymerase activity requires an intact pituitary-adrenal system.

Other studies on the effects of inhibitors of RNA synthesis on aryl hydroxylase induction in cultured hamster embryo cells have shown that administration of actinomycin or mercapto-β-pyridethylbenzimidazole simultaneously with inducer totally blocks enzyme induction (9, 27). Actinomycin, when applied 2 hr after induction, fails to prevent the subsequent rise in enzyme activity for 6–8 hr (28). Induction of the enzyme *in vivo* is also sensitive to actinomycin D (7). This is the basis for the conclusion that the synthesis of some RNA species is a requirement for the induction process. In our studies in cell culture, an altered pattern of RNA synthesis was not observed during BA induction of aryl hydrocarbon hydroxylase. Two explanations may be considered. One is that an alteration in the cellular metabolism of RNA is not a concomitant of induction. If this is so, an alternative and unorthodox explanation would need be devised to interpret the results with the inhibitors of RNA synthesis (9, 27). A more probable explanation is that RNA changes of small magnitude do occur during induction, but that the resolution of the technique employed was insufficient to detect them. It has been estimated that in HeLa cells the cellular fraction of RNA that has messenger function is only 3% (29). Also, if the turnover of the RNA species involved in induction were rapid, it is unlikely that they could accumulate in sufficient amount to be easily detected. This would be particularly so if the molecular sizes were such as to migrate with the rapidly labeled nuclear heterodisperse or ribosomal precursor RNA.

If the induction process is specific to a relatively small proportion of the enzymes in the cell, i.e. the microsomal drug-metabolizing enzymes, the relative number of RNA molecules involved in this process should be low compared to the RNA metabolism of the whole cell. Thus, if RNA changes are indeed a requisite for induction, these would be small. Thus, the large changes observed *in vivo* after MC administration may indeed partly comprise the changes relevant to microsomal enzyme induction, but the large generalized changes are not specific or requisite for microsomal enzyme induction.

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