Changes in Liver Cytoplasmic RNA after Administration of 3-Methylcholanthrene

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(Received November 3, 1967)

SUMMARY

The administration of 3-methylcholanthrene to rats is accompanied by an increase in the incorporation of orotic acid-^{14}C into the 45 S cytoplasmic particle in the liver. The elevation reaches a maximum at 15 hr after the injection of the polycyclic hydrocarbon and diminishes to control values by 36 hr. This effect was also observed in adrenalectomized animals, eliminating any role of the adrenal corticosteroids in the phenomenon. In addition, the turnover of 18 and 28 S ribosomal RNA in liver cytoplasm was elevated after administration of the polycyclic hydrocarbon. These results suggest that the synthesis of ribosomal constituents, in particular, ribosomal RNA, may play an important role in the "induction" phenomenon observed in liver after administration of 3-methylcholanthrene.

INTRODUCTION

Previous studies from this laboratory (1, 2) have indicated that the administration of 3-methylcholanthrene to rats is attended by an elevation in the activity of the "aggregate" RNA polymerase of liver nuclei which appears to be the result of an enhanced efficiency of the genome rather than an elevation in actual enzyme. A similar enhancement of the RNA polymerase system after treatment of rats with 3-methylcholanthrene has recently been reported by Gelboin *et al.* (3). The activation of the genome occurred prior to the elaboration, i.e., "induction," of the drug-metabolizing enzymes of the smooth endoplasmic reticulum in 3-methylcholanthrene-treated rats. Attention was subsequently directed toward the end product of the RNA polymerase system. Since in earlier investigations, we had been unable to detect any marked increase in the turnover of nuclear RNA in methylcholanthrene-treated rats, the turnover of cytoplasmic RNA was followed and these results are reported in the present study.

In recent reports, a particle which is rapidly labeled after administration of orotic acid-^{14}C and which sediments at approximately 45 S has been demonstrated in a number of mammalian systems (4–7) although the function of this particle has not been completely elucidated. Some evidence has been presented which is in accordance with a role of the 45 S particle in the transport of messenger RNA from the nucleus to the cytoplasm (4). Hiatt and his colleagues (8) have demonstrated that following the administration of hydrocortisone to rats the isotopic labeling of the 45 S cytoplasmic particle in liver was markedly increased. They have suggested that the excess labeled RNA in the 45 S particle consists of a messenger RNA coding for the synthesis of hormone-induced enzymes. Accordingly, we have de-

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cided to investigate the turnover in liver of the 45 S cytoplasmic particle, following the administration of 3-methylcholanthrene. The results presented in this manuscript indicate that the labeling of the 45 S particle is markedly elevated within relatively short times after administration of the polycyclic hydrocarbon. Concurrently, an increase in the turnover of the 18 and 28 S RNA's of liver cytoplasm has been noted. These studies suggest that one of the effects of 3-methylcholanthrene may be upon the synthesis of ribosomal components and in particular, ribosomal RNA.

METHODS

Male 50–60 g rats were purchased from the Cheek-Jones Company, Houston, Texas, and were injected intraperitoneally with 0.5 ml of a solution of 3-methylcholanthrene in corn oil (20 mg/kg body weight). The control rats were injected with 0.5 ml of corn oil alone. In some experiments, adrenalectomized rats were employed 3–5 days postoperatively. The adrenalectomized rats were maintained with 1% saline in their drinking water. All animals were starved 12 hr before death.

The rats were lightly anesthetized under ether and the livers were perfused via the portal system with cold STKM (0.01 *M* Tris, pH 7.6, 0.01 *M* KCl, 0.25 *M* sucrose, 0.001 *M* magnesium acetate). The livers were removed, washed in cold STKM, and the connective tissue was discarded; the livers were minced with scissors. The minced liver was homogenized in 2 volumes of STKM at 4° in a grinding vessel with a Teflon pestle (2 strokes); the clearance between the pestle and the vessel was 0.006–0.009 inch. The homogenate was centrifuged at 17,000 *g* for 10 min in a SS 34 rotor. The sediment was discarded. The postmitochondrial supernatant fraction was diluted with an equal volume of STKM, and a sample was layered on a gradient of 10–30% sucrose containing 0.01 *M* Tris pH 7.6, 0.01 *M* KCl, and 1 m*M* magnesium acetate. The gradients were centrifuged at 63,600 *g* in a SW 25.1 rotor for 5.5 hr. The gradients were fractionated by means of an ISCO fractionator and absorbancy monitor which recorded the ultraviolet absorbancy at 254 mμ (A_{254}) automatically. One-milliliter fractions were collected, 2 drops of 5 *N* perchloric acid were added, and the samples were placed at 70° for 1 hr. The radioactivity of the hydrolyzed RNA was determined by liquid scintillation techniques after the addition of 10 ml of Bray's phosphor medium (9).

In several experiments, the turnover of the ribosomal subunits was determined. Orotic acid-6-^{14}C (34.7 μC/μmole), 2 μC, was injected intravenously into the rats via the tail vein and the rats were sacrificed 40 min later. The postmitochondrial supernatant fraction was obtained as described previously.

The synthesis of RNA of the postmitochondrial supernatant fraction was determined after adjusting the latter to 0.3% with respect to sodium dodecyl sulfate (SDS), 0.01 M with respect to acetate buffer, pH 5.0, and 0.1 M with respect to sodium chloride. Phenol–*m*-cresol–8 hydroxyquinoline–water (3 l:0.6 l:3.87 g:0.4 l) was added, the emulsion was shaken for 15 min at room temperature and was centrifuged at 15,000 *g* for 15 min. The aqueous layer was removed and extracted again with the phenol–*m*-cresol–8 hydroxyquinoline–water solution. The procedure was repeated a total of 5 times or until no sediment could be observed at the interphase. The RNA in the aqueous layer was precipitated upon the addition of 2.5 volumes of ethanol–2% potassium acetate. The sedimented RNA was dissolved in a minimum volume of distilled water and once again precipitated upon the addition of 2.5 volumes of ethanol–potassium acetate. The RNA was dissolved in distilled water and layered over 26.5 ml of a 10–40% linear sucrose gradient which was buffered to pH 5.1 with 0.01 *M* sodium acetate and contained 0.1 M NaCl and 1 mM EDTA (10). The tubes were centrifuged at 24,000 rpm in a Spinco SW 25.1 swinging-bucket rotor for 15 hr at 5°. The gradients were fractionated into 1-ml samples by means of an ISCO automatic fractionator and the samples were prepared for counting as described above.

RESULTS

The incorporation of orotic acid-^{14}C into the liver cytoplasmic particle is graphically depicted in Figures 1–3, and is tabulated in Table 1. The 45 S particle was rapidly labeled in these experiments and possessed the highest specific activity of the ribosomal fractions. As early as 3 hr after administration of 3-methylcholanthrene, the specific activity of the 45 S particle was augmented (Fig. 1). The total radioactivity present in this fraction was increased almost by a factor of 2 (Table 1). At 9 hr after administration of the polycyclic hydrocarbon, the total radioactivity incorporated into the 45 S particle was increased by a factor of approximately 3 (Fig. 2), while the specific activity of the peak tube in this fraction was augmented by 63% over control values (Table 1).

Additional values were obtained at 12, 15, 24, and 36 hr after administration of 3-methylcholanthrene; these results are presented in Table 1. The total radioactivity in the 45 S fraction reached a maximum at 15 hr after injection corresponding with the maximum specific activity of the peak tube in this fraction. At this time, the radioactivity in this fraction represented 39% of the total radioactivity placed on the gradient while the specific activity had been increased by 107% over control values. The normal pattern was restored by 36 hr after administration of the drug.

To eliminate any contribution of the adrenal corticosteroids to the overall pic-

TABLE 1

Incorporation of orotic acid-^{14}C into the 45 S ribosomal particle after administration of 3-MC

Intact rats, 60–80 g (A) were injected, i.p., with either corn oil (controls) or 3-MC in corn oil. The rats were sacrificed at the times indicated in table, and the 45 S profile was determined as described in the text. The 45 S particle was labeled after administration of 2 μC of orotic acid-^{14}C; the labeling period was 40 minutes. The specific activity was that of the peak tube; the values represent averages of 2–3 determinations. Similar experiments were performed with rats adrenalectomized 3–5 days prior (B).

Treatment	Cpm in 45 S fraction (%)	Specific activity of RNA (cpm/A_{260})	Percent increase
A. Intact rats, control	13	270	—
Intact rats, 3-MC, 3 hr	22	400	48
Intact rats, 3-MC, 9 hr	36	440	63
Intact rats, 3-MC, 12 hr	37	540	100
Intact rats, 3-MC, 15 hr	39	560	107
Intact rats, 3-MC, 24 hr	21	350	29
Intact rats, 3-MC, 36 hr	14	280	4
B. Adr-X, control	14	190	—
Adr-X, 3-MC, 12 hr	31	500	163

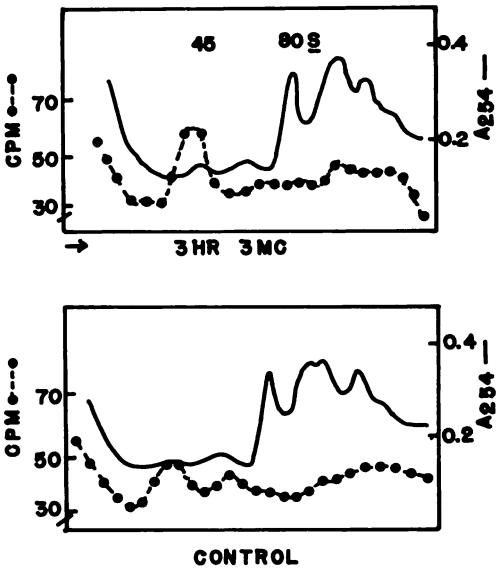

FIG. 1. *Labeling of the 45 S cytoplasmic particle 3 hours after administration of 3-methylcholanthrene*

Rats were injected with 3-methylcholanthrene (20 mg/kg) at −3 hr, with orotic acid-^{14}C, 2 μC, at −40 min and sacrificed at 0 time. The details of the experiments are presented in the text. These experiments have been repeated at least 3 times with essentially identical results. The direction of sedimentation is from left to right. The radioactivity, cpm/fraction, is presented on the left ordinate; the A_{254} on the right. The upper graph was obtained with RNA from 3-methylcholanthrene-treated rats (3-MC); the lower, from corn oil-treated controls. ————, A_{254}; - - - - - - - - -, cpm.

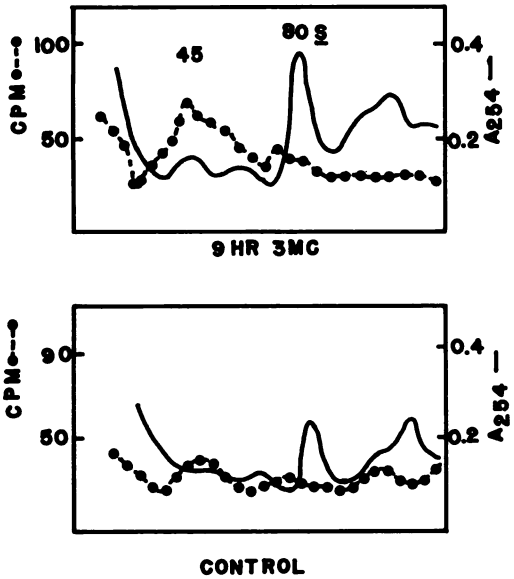

FIG. 2. *Labeling of the 45 S cytoplasmic particle 9 hours after administration of 3-methylcholanthrene*

See the legend of Fig. 1.

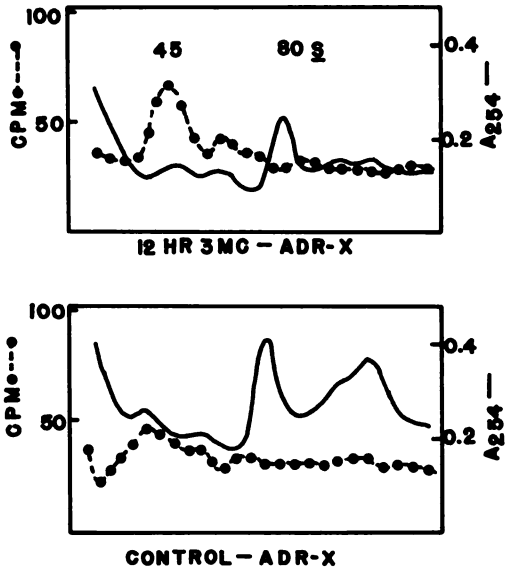

FIG. 3. *Labeling of the 45 S cytoplasmic particle of adrenalectomized rats 12 hours after administration of 3-methylcholanthrene*

Rats, adrenalectomized 3–5 days previously were injected with 3-methylcholanthrene as described in the text. See legend to Fig. 1.

ture, adrenalectomized rats were employed (Fig. 3). Although the total radioactivity in the 45 S fraction of livers from adrenalectomized rats was not significantly different from that of control rats, i.e., 14%, the specific activity of the peak tube was diminished in the former group from 270 to 190 cpm/A_{254} (Table 1). 3-Methylcholanthrene administration significantly elevated the total counts incorporated into the 45 S fraction as well as the specific activity of the peak tube by 12 hr after administration of the drug. At this time, the total radioactivity was increased by a factor of 2 and the specific activity was enhanced by 163%.

The biosynthesis of ribosomal RNA was investigated at 12 hr after administration of 3-methylcholanthrene, a time when the labeling of the 45 S fraction was markedly elevated. The results have been obtained at 40 min and 12 hr after injection of the labeled orotic acid and are presented in Figs. 4 and 5, respectively. Although the data of one experiment are presented in the figures, these results have been repeated three times. Even with a short pulse

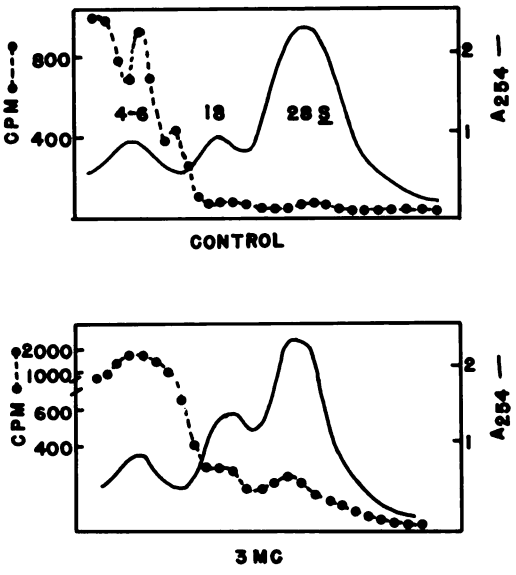

FIG. 4. *Labeling of cytoplasmic RNA after administration of 3-methylcholanthrene*

The details are presented in the text and in the legend to Fig. 1. The RNA was obtained 12 hr after administration of 3-methylcholanthrene.

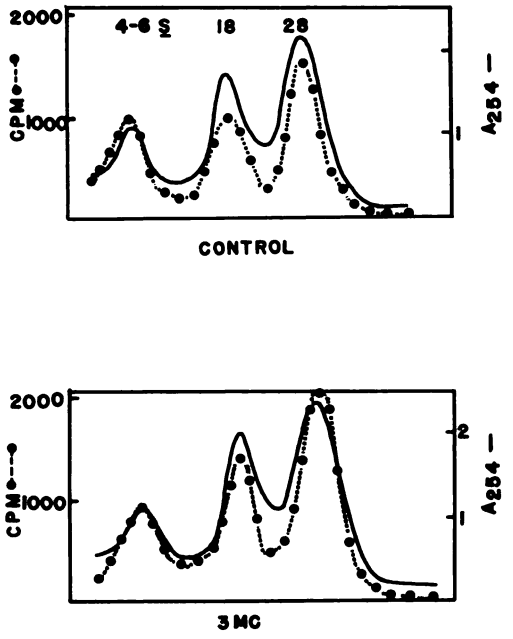

FIG. 5. *Labeling of cytoplasmic RNA after administration of 3-methylcholanthrene*

See legend to Fig. 4. The animals were injected with orotic acid-^{14}C and 3-methylcholanthrene 12 hr prior to sacrifice.

of 40 min, the specific activities of the 18 and 28 S RNA from the livers of drug-treated rats were substantially elevated over control values (Fig. 4).

After a labeling period of 12 hr, the percentage radioactivity in the 4–6, 18, and 28 S RNA of the control liver fraction was 32, 31, and 34% respectively (Fig. 5). The total radioactivity in these 3 fractions was 48,000, 40,000, and 52,000 cpm, respectively. The specific and total activities of the 18 and 28 S RNA from methylcholanthrene-treated rats were slightly augmented. The percentage distribution of radioactivity among the 4–6, 18, and 28 S RNA was 22, 32, and 46%, respectively; the total radioactivity was 42,000, 64,000, and 92,000 cpm, respectively. The increase in the labeling of the 18 and 28 S fractions was not as dramatic 12 hours after injection of the isotope.

DISCUSSION

These results clearly indicate that the administration of 3-methylcholanthrene to rats is attended by an increase in the incorporation of a precursor into the 45 S cytoplasmic particle of liver. The maximum increase occurs by 15 hr although a substantial elevation is noted as early as 3 hr after administration of the polycyclic hydrocarbon. By 36 hr, the effect had diminished to basal values. The effect of the polycyclic hydrocarbon was not mediated through adrenalcorticosteroids, i.e., was not the result of a chemical peritonitis, since a very pronounced elevation was observed in adrenalectomized rats.

Concomitantly with the elevation in the labeling of the 45 S cytoplasmic particle, occurred an increase in the incorporation of orotic acid into the 18 and 28 S fractions of RNA in liver cytoplasm suggesting an increased elaboration of ribosomal RNA after drug treatment.

Although messenger RNA has a relatively short half-life in bacteria, it is relatively stable in mammalian sytsems (11–13). Ribosomes in the latter, on the other hand, turn over more rapidly than had been suspected initially (14, 15). Furthermore, it has been shown that the synthesis of ribosomal RNA is stimulated by a variety of hormones, including thyroxine, hydrocortisone, and growth hormone (16–19). After the hormonally induced stimulation in ribosomal RNA synthesis, an increased rate of hepatic protein synthesis was observed. The results of the present investigations are in line with these studies and draw attention to the "pseudo-hormonal" action of 3-methylcholanthrene. The polycyclic hydrocarbon, presumably after activation by combination with an "apoprotein" (20) causes the elaboration of more ribosomes, perhaps for the purpose of increasing the efficiency of transport of the messenger RNA responsible for the synthesis of certain of the drug-metabolizing enzymes from the nucleus to the cytoplasm. It has not been unequivocally established if 3-methylcholanthrene also causes the enhanced formation of messenger RNA as well.

The administration of 3-methylcholanthrene appears to exert a profound effect upon protein synthesis. Thus, Gelboin and

Sokoloff (21, 22) have shown that the amino acid-incorporating systems from the liver of drug-treated rats when assayed *in vitro* are more active than suitably prepared control systems. Gelboin's work (23) suggests a greater amount of messenger RNA present in the microsomes from 3-methylcholanthrene-treated rats. This interpretation was strengthened by the finding that actinomycin D inhibited both the "induction" of benzpyrene hydroxylase and the elevation in microsomal protein synthesis as the result of administration of 3-methylcholanthrene (23).

Alterations in nuclear RNA metabolism after treatment of rats with 3-methylcholanthrene have been reported by Loeb and Gelboin (24). An increased orotic acid incorporation into nuclear RNA was not observed by Bresnick *et al.* (25). It is quite possible that the rapidly labeled nuclear RNA exits rapidly from the nucleus into the cytoplasm and thus only an extremely small pool of this RNA exists in the nucleus. The difference in the techniques for isolation of nuclei and nuclear RNA by the two laboratories may account for this apparent discrepancy.

Recently, we have reported the assay of template activity of nuclear RNA in a bacterial amino acid-incorporating system (26). The direction of our activities is in the utilization of this assay system for establishing the template efficacy of both nuclear and cytoplasmic RNA after administration of 3-methylcholanthrene.

ACKNOWLEDGMENT

This investigation was supported by a grant from the American Cancer Society, E-373.

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