PHENOBARBITAL AND METHYLCHOLANTHRENE STIMULATION OF RAT LIVER CHROMATIN TEMPLATE ACTIVITY

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Both phenobarbital and 3-methylcholanthrene increase the activity of liver microsomal drug metabolizing enzymes, as discussed by Conney (1967). This increased enzyme activity is due to de novo protein synthesis, as indicated by the studies of Von der Decken and Hultin (1960), Gelboin and Sokoloff (1961), and Conney and Gilman (1963). Madix and Bresnick (1967) have shown that chromatin isolated at 7 and 16 hours after administration of the carcinogen 3-methylcholanthrene is a more effective template for in vitro RNA synthesis than chromatin from control animals. The results presented in the present report indicate that chromatin isolated from rat liver 12 hours after administration of phenobarbital or 3-methylcholanthrene is a more effective template for RNA synthesis when compared to controls, and that differences in template activity are abolished when basic proteins are removed from the chromatin preparations.

Methods:

Male Holtzman rats (150-200g) were given saline (0.9%; i.p.), phenobarbital (100 mg/kg; i.p. in saline) or 3-methylcholanthrene (30 mg/kg; i.p. in corn oil), and were sacrificed by cervical dislocation 12 hours after injection. Livers were perfused in situ with ice-cold saline, removed, and frozen on dry ice. Chromatin was isolated from liver by the procedure of Marushige and Bonner (1966). Chromatin was washed 3 times with 0.01 M tris, pH 8, prior to dialysis to insure removal of sucrose.

Basic proteins were removed from chromatin by a procedure similar to that of Marushige and Bonner (1966). Samples of chromatin containing 5 mg of DNA

were treated with 50 ml of ice-cold 0.2 N HCl for 20 minutes in an ice bath. Acid insoluble material was collected by centrifugation of the mixture at 16,000 x g_{av}[1] for 20 minutes. Chromatin was then suspended in 25 ml of 0.01 M tris, pH 8, with a Teflon hand homogenizer, and was centrifuged at 78,000 x g_{av} for 2 hours. The gelatinous pellet was resuspended in 6 ml of 0.01 M tris, pH 8. Aggregates were centrifuged at 2200 x g_{av} for 15 minutes. The supernatant was aspirated, assayed for DNA and protein content, and subsequently referred to as acid-treated chromatin.

Chromatin was assayed for template activity in an RNA polymerase system similar to that of Nakamato, et al., (1964).

The assay mixture consisted of 50 μ moles tris buffer, pH 7.5, 1.25 μ moles $MnCl_2$, 0.2 μ moles each of ATP, CTP, GTP, and UTP,[2] 0.4 μC C^{14}-ATP, (specific activity 20 mC/mM), 2.5 units RNA polymerase from Micrococcus lysodeikticus (Miles Laboratories, Elkhart, Indiana), and chromatin as DNA template in a volume of 0.5 ml.

Reaction mixtures were incubated at 30°C for 10 minutes, and were stopped by addition of 2 ml of ice-cold 10% TCA. Precipitates were washed twice with 2 ml of ice-cold 5% TCA, and dissolved in 1.0 ml of 0.1 N NaOH. An 0.8 ml aliquot was added to 15 ml XDC scintillation solution (Bruno and Christian, 1961), and counted for radioactivity in a Beckman LS-100 liquid scintillation spectrometer.

DNA was determined by the method of Dische (1955), and protein by the method of Lowry, et al., (1951).

Results:

Both phenobarbital and 3-methylcholanthrene treatment increase the template activity for RNA synthesis, as shown in Figure 1.

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[1] Spinco #30 rotor.

[2] The following abbreviations were used: AMP, adenosine-5'-monophosphate; ATP, CTP, GTP, UTP, the three 5' triphosphates of adenosine, cytosine, guanosine and uridine; TCA, trichloroacetic acid.

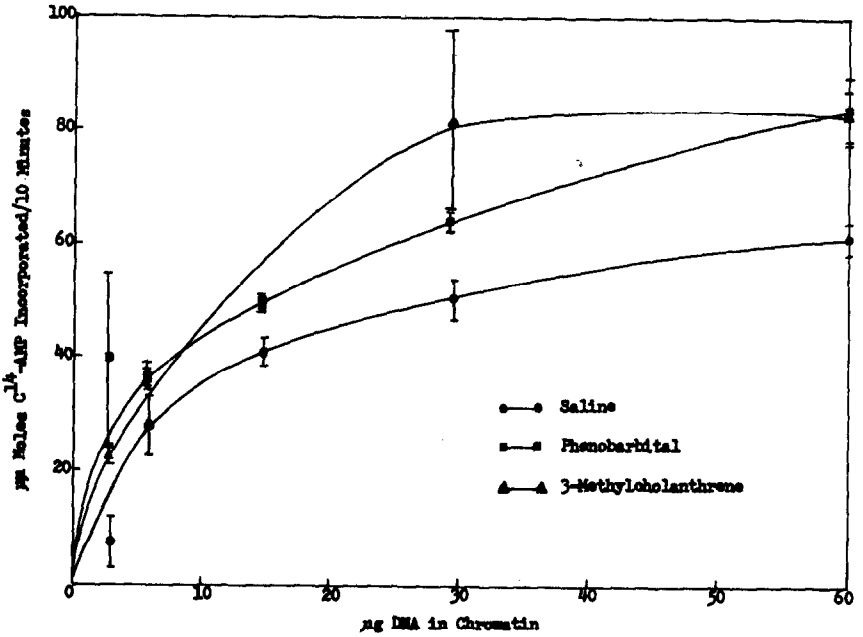

Fig. 1. Template activity of intact rat liver chromatin for RNA synthesis. Each point represents an in vitro assay conducted in triplicate.

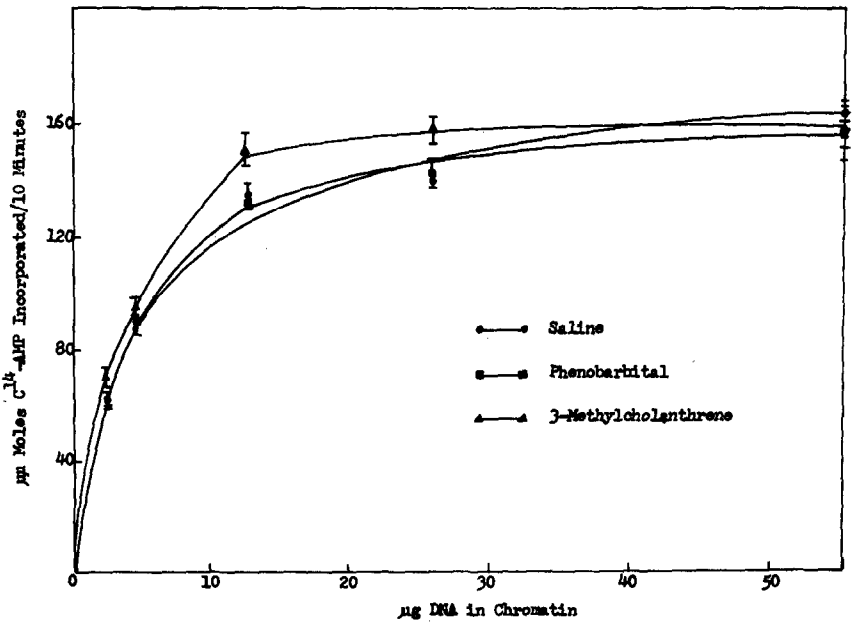

Fig. 2. Template activity of acid-treated rat liver chromatin for RNA synthesis. Each point represents an in vitro assay conducted in triplicate.

Differences in template activity were abolished when basic protein was removed, as shown in Figure 2.

Both phenobarbital and 3-methylcholanthrene modify chromatin, increasing its ability to function as a template for RNA synthesis. The data thus indicate that basic protein is involved in the differences of template activity.

Experiments are currently being conducted to determine the nature of the alteration of the template produced by phenobarbital and 3-methylcholanthrene.

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