Hormonal modulation of α -fetoprotein gene expression in newborn rat livers

Jen-Fu Chiu, Ronald J. Massari, Charles E. Schwartz, Natalie T. Meisler and John W. Thanassi

Department of Biochemistry, University of Vermont, College of Medicine, Burlington, VT 05405, USA

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ABSTRACT

Suppression of serum α -fetoprotein (AFP) levels in glucocorticoid treated newborn rats was investigated. Daily intraperitoneal injection of 2 μ g/g body weight of dexamethasone into newborn rats greatly reduced the concentration of AFP in the serum and liver cytosol. In contrast, this treatment stimulated liver ornithine decarboxylase activity. The reduction in AFP levels is not due to a change of distribution of AFP molecular variants, inhibition of secretion of synthesized AFP by the liver or disruption of liver polysomes. Glucocorticoids decrease the AFP levels in hormone-treated rats by suppressing the synthesis of AFP. The size of AFP polysomes isolated from the livers of dexamethasone-treated rats were as large as those from normal rats. However, the amount of AFP-producing polysomes in hormone-treated rat liver is only 14% of the controls. By hybridization assays, it was found that dexamethasone treated livers contained decreased amounts of AFP mRNA sequences in liver cytoplasmic and nuclear RNAs. The decreased amounts of AFP mRNA sequences in hormone-treated liver are caused by both a decrease in the rate of AFP mRNA transcription and in AFP mRNA stability.

INTRODUCTION

α -Fetoprotein (AFP) is a glycoprotein synthesized mainly by fetal liver and yolk sac, and to a small extent by the gastrointestinal tract during fetal life. AFP is present in very small amounts (0.06 μ g/ml) in the serum of normal adult rats and humans but at a very high concentrations (as much as 10,000 μ g/ml) in the serum of fetal and hepatoma-bearing animals (for reviews see ref. 1-3). A useful application of these findings has been the development of a serological test for the diagnosis of primary liver cancer in humans (4). Serum AFP has also been found to be elevated in a teratoblastoma (5), ataxia telangiectasia (6) and in fetal neural tube defects (7,8). In addition to the clinical applications, AFP may provide a unique opportunity for the understanding of the relationship between embryonic development and malignant transformation.

There is evidence that the absence of AFP protein synthesis in adult

normal liver is the result of a lack of AFP mRNA (9-14). The process of transformation of normal liver cells to hepatoma cells often coincides with an increased ability to synthesize AFP. Several laboratories have found that hepatoma cells contain specific AFP mRNA (9-11, 14). However, at the present time, the basic mechanisms for the synthesis of AFP and its regulation are essentially unknown.

It has been reported that serum AFP level is prematurely reduced in newborn rats by the administration of glucocorticoids (15,16). The magnitude of the response was so great that within 3-4 days the AFP level in serum dropped to 2.5% of the control level. This system therefore allows the study of glucocorticoid mediation of gene expression. Glucocorticoids have been shown to induce the synthesis of specific proteins (e.g., tryptophan oxygenase, tyrosine aminotransferase, ornithine decarboxylase, and α_{2u} -globulin) by increasing the levels of the mRNA molecules that code for these proteins (For reviews, see ref. 17 and 18). However, the inhibitory effect of glucocorticoids are not as well understood. Recent reports (19,20) demonstrated that the mechanism of glucocorticoid-mediated suppression of a specific protein can operate by decreasing a specific mRNA. We have provided evidence for this mechanism by showing that glucocorticoids reduce the level of AFP mRNA in dexamethasone treated newborn mouse liver (19). However, it is not clear that the observed decrease in AFP mRNA concentration completely accounts for the observed decrease in serum AFP levels. In this paper we investigated further whether the reduction of serum AFP following long-term treatment with dexamethasone is regulated at the secretory, translational, post-transcriptional or transcriptional level. We also investigated other hormones to see if AFP synthesis is altered by any hormones besides glucocorticoids.

We found that dexamethasone selectively inhibits AFP synthesis in newborn rat liver. The inhibition of dexamethasone on AFP synthesis is due to inhibition of AFP mRNA synthesis and enhancement of AFP mRNA turnover rate.

MATERIALS AND METHODS

Animals and Treatments: Three day old rats were used in all experiments with 5-10 rats per experimental time point; all experiments were repeated 2-3 times. Rats were injected daily with the following hormones: dexamethasone (Hypho-med) 2 μ g/g body weight; progesterone (Ruger) 2 μ g/g; testosterone (Upjohn) 5 μ g/g; estradiol (Squibb) 2 μ g/g; glucagon (Sigma) 2.5 μ g/g; insulin (Sigma) 1.6 μ /100 g; dibutyryl cyclic AMP (Sigma) 30 μ g/g; theophylline (Sigma) 30 μ g/g. All hormones and chemicals were injected intraperitoneally,

except insulin which was administered subcutaneously. Three day old rats were given hormones or chemicals daily for four days, except as noted otherwise.

Antigens and Antibodies: AFP was purified by antibody affinity column chromatography (21). The purity of the antigen preparations was assessed by polyacrylamide gel electrophoresis and by double diffusion against anti-normal adult rat serum antisera. High titer anti-AFP sera were raised in rabbits. Their specificity was confirmed by double diffusion and cross-immuno-electrophoresis against normal adult rat serum. Rabbit anti-rat albumin antiserum was obtained by immunization with a preparation of albumin purified from normal adult rat serum as described by Taylor and Schimke (22). The IgG fraction of normal rabbit serum was purified to electrophoretic homogeneity by 33% ammonium sulfate precipitation and anion exchange chromatography on QAE Sephadex A-50 (11). Anti-rabbit IgG sera were raised in sheep.

AFP and Albumin Measurement: AFP was measured by double antibody radioimmunoassay as previously described (21). AFP was radioiodinated by the lactoperoxidase technique (23). Total serum albumin was measured by electroimmunodiffusion with the use of goat antiserum, against standards of normal adult rat serum with known albumin content. Dilutions of test and standard samples were made in phosphate buffered saline (PBS, 0.01 M sodium phosphate, 0.15 M NaCl, pH 7.2). The assay sensitivity was 10 µg/ml.

Ornithine Decarboxylase (ODC) Assay: High speed supernatants from rat liver homogenates (1 part liver and 9 parts 0.25 M sucrose) were used as the source of enzyme. ODC activity was determined by measuring $^{14}\text{CO}_2$ liberated from L-(1- ^{14}C)ornithine obtained from Amersham (24). A heated enzyme preparation (100°/5 min) was carried through the procedure and was used to correct the observed experimental values. The $^{14}\text{CO}_2$ that was evolved was collected in 50 µl of Protosol (New England Nuclear) on folded filter paper in a plastic well mounted in the septum stopper. The cocktail was Econofluor (New England Nuclear) containing 10% methanol.

Polyacrylamide Gel Electrophoresis: Ten percent polyacrylamide gel electrophoresis was performed according to Ornstein (25). Serum AFP isolated by immunoabsorption, was determined in samples containing 3-10 µg of protein. Coomassie Blue stained gels were scanned at 650 nm on a Gilford spectrophotometer.

Binding of Anti-AFP to Polysomes: Polysomes were prepared by the "cushion" method as described previously by Palacios *et al* (23). Anti-AFP globulin was iodinated by the lactoperoxidase method essentially as described (23). Iodinated antibody was made ribonuclease-free as described in previous

papers (10,11). Sedimentation profiles and anti-AFP antibody-binding activity of polysomes were determined as described (10).

Extraction of Liver Cytoplasmic RNA: Livers were homogenized in 5 volumes of 0.25 M sucrose, 5 mM magnesium chloride, 25 mM NaCl, and centrifuged at 10,000 x g for 10 min. The supernatant was then brought to 25 mM sodium acetate, pH 5.0, 10 mM EDTA, 0.5% SDS, and the solution was extracted once with an equal volume of buffer-saturated phenol and 2-3 times with phenol/chloroform/isoamyl alcohol (49:49:2). The RNA in the aqueous phase was precipitated with ethanol overnight at -20°C.

Preparation of AFP cDNA and Molecular Hybridization: AFP mRNA purification and cDNA preparation were described previously (10). RNA excess hybridizations were performed in a final volume of 20 µl of 50% formamide, 0.5 M NaCl, 25 mM Tris-HCl buffer, pH 7.5, 10 mM EDTA containing 1500 cpm of cDNA and various amounts of RNAs. Hybridization was carried out at 41° for 72 hr. The formed hybrids were assayed utilizing nuclease S₁ from *Aspergillus Oryzae* (26). A C₀t analysis was carried out on each RNA sample.

In Vitro Transcription of Nuclei: Liver nuclei were prepared from 7 day old normal control and dexamethasone treated baby rats according to Commers et al (19). In vitro transcription was conducted as described (27) with some modifications. Briefly, fresh nuclei were incubated at 25°C at a DNA concentration of 1.5 mg/ml in a reaction mixture of 50 mM Tris-HCl pH 7.9, 5 mM magnesium acetate, 1 mM MnCl₂, 12 mM mercaptoethanol, 150 mM KCl, 10% glycerol, 0.4 mM each of ATP, GTP and CTP, 0.25 mM UTP plus 0.125 mM mercurated UTP (Hg-UTP). [³²P]ATP (final sp. act.: 80 mCi/mmol) was used to label the RNA to low specific activity. The mixture was gently agitated during incubation for 1 hour. After incubation, 50 µg/ml RNase-free DNase (Worthington) was added and further incubated for 20 min. The reaction mixture was made 1% SDS, 50 mM NaAc, pH 5.1, 10 mM EDTA and gently homogenized. After heating at 55°C for 3 min, an equal volume of water saturated phenol (pre-warmed to 55°C) was added and then shaken at 55°C for 5 min. The mixture was centrifuged at 10,000 rpm for 10 min in a Sorvall centrifuge. The aqueous phase was re-extracted twice with phenol:chloroform:isoamyl alcohol (49:49:2) and finally precipitated with 2.5 volumes of ice cold ethanol overnight at -20°C.

The RNA pellet was collected by centrifugation and dissolved in a buffer containing 1% SDS, 10 mM Tris HCl, pH 7.9, 2 mM EDTA. The RNA solution was heated to 107°C for 3 min and then applied in a large volume (10 times bed volume) to an SH-agarose column equilibrated in the same buffer (but 0.1% SDS) at 55°C. The column was washed with 0.1% SDS-buffer, and then water. Then

the column was returned to room temperature, washed with 50 mM Tris-HCl, 0.1% SDS, 5 mM EDTA, and the Hg-RNA eluted in the same buffer containing 0.25 M 2-mercaptoethanol. To the final eluted RNA fraction, 10 µg/ml of yeast tRNA, 0.4 M NaCl and 1.5 M 2-mercaptoethanol were added. The mixture was left at room temperature for 3 hrs to allow demercuration, and was then ethanol precipitated and dissolved in H₂O.

Dot Hybridization: The dot hybridization procedure was followed by the method of Kafatos *et al* (28). Nuclear RNA samples were spotted onto nitrocellulose filters previously saturated with 20 x SSC (SSC = 0.15 M NaCl, 0.15 M sodium citrate). To obtain a uniform dot size the concentrations of the various RNA samples were identical. The dots were allowed to dry at room temperature and then baked at 80°C for 2 hours. Prehybridization, hybridization and washing procedures were identical to those of Thomas (29). Approximately 1×10^6 cpm/ml of the nick translated HAE II fragment of cloned AFP cDNA pA5 (sp. act. $1-3 \times 10^8$) were included in the hybridization media. The filters were exposed for approximately 20 hours at -70°C using a Dupont Cronex intensifying screen. *E. coli* containing recombinant plasmid AFP cDNA pA5 was kindly donated by Dr. David L. Miller from the Roche Institute of Molecular Biology, Nutley, New Jersey.

RESULTS

Belanger *et al* (15,16) described the suppressive effect of glucocorticoid hormone on serum AFP levels. However, they did not determine what effect the concentration of hormone employed had on the liver by examining enzymes known to be affected by dexamethasone. Therefore, we examined the activity of ornithine decarboxylase in liver cytosol which is known to be induced by glucocorticoids (30,31). We observed that ornithine decarboxylase activity was induced when rats were treated with 2 µg/g of dexamethasone while the serum and cytosol AFP levels were significantly depressed (Figure 1). These results indicate that glucocorticoids are selectively inhibiting AFP protein synthesis at the concentration of dexamethasone used in these experiments. It is evident that liver cytosol AFP concentration decreases more rapidly than serum AFP in dexamethasone treated rats (Figure 1). Since the reduction of liver AFP concentration occurs faster than the reduction of serum AFP concentration the possibility of a hormone effect on the secretion of AFP from hepatocyte to serum is ruled out. This suggests that the effect of glucocorticoid on the serum AFP level is due to reduction of AFP biosynthesis in liver.

Figure 1. The serum AFP level, cytosol AFP concentration and ornithine decarboxylase activity in livers of rats treated with dexamethasone. Four day old rats were injected intraperitoneally daily with 2 μ g/g body weight of dexamethasone for various numbers of days. At each time point, 5-6 rats from the control and treated groups were sacrificed. Sera were collected. Livers were homogenized in 5 vol. of 0.25 M sucrose and centrifuged for 100,000 $\times$ g for 30 min. Cytosol was used to determine AFP concentration and ornithine decarboxylase activity. The amount of AFP in serum and liver cytosol was determined by radioimmunoassay. The activity of ornithine decarboxylase was determined as described in the Methods. : serum AFP level, : cytosol AFP concentration and, : cytosol ornithine decarboxylase activity.

Several other known inducers of liver enzyme activities such as glucagon and dibutyryl cyclic AMP were also studied. As shown in Table I, glucagon and dibutyryl cAMP do not affect the levels of AFP in serum and liver cytosol. When dibutyryl cAMP was tested together with dexamethasone, it did not have an effect on the inhibitory activity of glucocorticoids on AFP production. To determine if the inhibitory effect of steroids on AFP production is glucocorticoid specific, we treated newborn rats with several different steroids as shown in Figure 2. It is evident from the figure that dexamethasone has the greatest inhibitory effect, while testosterone, estradiol and progesterone have little effect on serum AFP levels. With respect to albumin, dexamethasone and other steroid hormones cause either a slight increase or have no effect on albumin levels either in liver cytosol or in the serum (Figure 3).

From the data shown in Table I, it would appear that dexamethasone is reducing total AFP protein in the serum or liver cytosol. However, this conclusion may not be valid since there are two variants of AFP in the serum and liver cytoplasm (21). The antisera may react differently on a molar basis

Figure 2. The effect of various steroid hormones on serum AFP levels in newborn rats. Ten litters of four day old rats were divided into five groups. Each group was composed of 20-25 rats, and received daily either dexamethasone (■), testosterone (▲), progesterone (○), estradiol (△) or saline (●) for various days. At each time point, 5-6 rats of each group were sacrificed by decapitation. Sera were collected and AFP was measured in serum by radioimmunoassay.

Figure 3. The effect of various steroid hormones on serum albumin in new-born rats. The detailed experimental conditions were described in Figure 2.

Table I: EFFECT OF VARIOUS LIVER ENZYME INDUCERS ON AFP LEVEL

Treatment	Serum Level (mg/ml)	Cytosol Concentration (μ g/ml)
Control	3.9 (100%)	34.0 (100%)
Dexamethasone	1.1 (28%)	0.55 (1.6%)
Insulin	4.0 (102%)	37.5 (110%)
Glucagon	3.6 (92%)	35.0 (103%)
Dibutyl cAMP/theophyllin	3.6 (92%)	31.7 (93%)
Dibutyl cAMP/theophyllin plus dexamethasone	0.7 (18%)	0.31 (0.9%)

All inducers were administered daily for 4 days at the concentrations indicated under "Materials and Methods". At the end of experiments, the animals were sacrificed and AFP level in serum and liver cytosol were determined by radioimmunoassay.

with different forms of AFP in the radioimmunoassay. In other words, the apparent reduction of AFP by dexamethasone treatment could be achieved by shifting the distribution of the forms of AFP in favor of the ones with less radioimmunoactivity rather than by lowering the total amount of AFP. Therefore, it is necessary to know whether glucocorticoids reduce AFP level by altering the molecular distribution of the two AFP variants, or by inhibiting the overall synthesis of AFP in liver. To this end, we studied the distribution of two AFP variants in the serum of normal rats and rats treated with dexamethasone for 4 days. Serum AFP was isolated by anti-AFP antibody affinity column chromatography (21). Serum AFP was then analyzed on 10% polyacrylamide gel electrophoresis under nondenaturing conditions. Dexamethasone had little effect upon the distribution of two AFP variants (Figure 4). Both serum AFP from normal and dexamethasone treated rats were distinctly separated into two peaks with little difference in the ratio between the slow and fast variants. Therefore the major effect must be a lowering of AFP production.

A shift of preexisting AFP mRNA from polysomes into smaller size polysomes or subpolysomal particles could possibly cause a decrease in AFP synthesis in dexamethasone-treated animals. Therefore, we wanted to know if glucocorticoids affect the size of AFP-synthesizing polysomes. Polysomes prepared from normal control or dexamethasone-treated rats were incubated with a constant amount of [125 I]labeled anti-AFP and centrifuged on linear sucrose gradients. In Figure 5 the radioactive profiles show that the size of AFP-synthesizing polysomes from normal rats are as large as polysomes isolated from dexamethasone-treated rat liver. This figure also shows that the total [125 I]antibody bound in dexa-

Figure 4. Effect of dexamethasone on the distribution of AFP variants. Rats (4 days old) were treated with or without 2 $\mu\text{g/g}$ body weight of dexamethasone for 4 days. Serum AFP isolated by immunoadsorption column chromatography from control rats and rats treated with dexamethasone was analyzed on nondenaturing polyacrylamide gel electrophoresis as described in Methods. —, Control rat serum AFP; ----, dexamethasone-treated rat serum AFP.

Figure 5. The profile of $[^{125}\text{I}]$ anti-AFP bound polysomes. Twenty A_{260} units of polysomes were incubated with 50 μg of $[^{125}\text{I}]$ anti-AFP for 1 hour at 0° . The polysomes were then layered on linear (10 to 40%) sucrose gradients and centrifuged at $200,000 \times g$ for 2½ hours. Fractions (0.3 ml) were collected from the top and their absorbancies were determined at 260 nm with a 5 mm path length flow cell in a Perkin-Elmer spectrophotometer. The polysome bound anti-AFP was determined by $[^{125}\text{I}]$ radioactivity with a Packer gamma counter. A. Liver polysomes from normal controls. B. Liver polysomes from rats treated with dexamethasone for four days. —, absorbance at 260 nm; ----, anti-AFP radioactivity.

methasone-treated liver polysomes is much lower (approximately 14% of control) than antibody bound in the control liver polysomes. These results are in good agreement with the decreases observed in AFP production. These data suggest that the relative rate of AFP synthesis depends directly on the amount of AFP-synthesizing polysomes. Since the size of these polysomes does not change, the quantitative decrease is likely due to a reduction in the amount of AFP mRNA, or shifting of the existing AFP mRNA from polysomes into inactive mRNP particles. These can be distinguished by quantitation of AFP mRNA in total cytoplasmic RNA.

To demonstrate that the decrease in AFP synthesis during dexamethasone administration results from a decrease in the concentration of cytoplasmic AFP mRNA, the reduction of AFP mRNA concentration was determined by using total cellular RNA to drive RNA-excess hybridization reactions with [³H]labeled AFP cDNA. We chose to use total cellular RNA for these assays rather than poly(A)-containing RNA because some AFP mRNA is not completely retained by affinity matrices (i.e., oligo-dT-cellulose) currently in use and also because of the possibility that AFP mRNA may vary in its poly(A) content during various phases of the hormone effect. RNA was extracted from livers of control rats and rats treated with dexamethasone at various time points. RNA was extracted from the livers of 5-10 rats at each time point to reduce variation among individuals. AFP mRNA in these total cellular RNA samples was assayed by RNA-excess hybridization to cDNA AFP. The results are shown in Figure 6. These data indicate that the level of AFP mRNA decreased very rapidly after treatment with dexamethasone. The AFP mRNA is reduced to 50% of control after the first day of treatment. The concentration of AFP mRNA dramatically dropped to 5.6% and 2.6% of its control level on third and fourth day of treatment respectively. The decrease in AFP mRNA concentration during dexamethasone administration may result from a decrease in the transcription of the AFP gene. However, the observed decrease in AFP mRNA concentration could have also resulted from post-transcriptional changes, including a change in the stability of AFP mRNA. From the results shown in Figure 6, it seems as if dexamethasone acts not only to decrease the rate of AFP mRNA transcription but also to destabilize this mRNA.

Earlier data (19) demonstrated that the content of AFP mRNA sequences in dexamethasone-treated mouse liver nuclear RNA is much lower than that in control rat liver. However, due to the extremely low concentration of AFP mRNA in hormone-treated animal nuclear RNA, the hybridization kinetics could not go far enough to compare $\text{Rot}_{1/2}$ value. In order to determine how much less

Figure 6. Kinetics of [^3H]labeled AFP cDNA hybridization to cytoplasmic RNA, from rats treated with dexamethasone for 1 day ($\circ$), 3 days (Δ) or 4 days ($\square$) and control rats for 1 day ($\bullet$), 3 days ($\blacktriangle$) or 4 days treatment ($\blacksquare$). Molecular hybridization was carried out using different amounts of cytoplasmic RNAs at 42°C for various times up to 72 hours. Hybrid formation was detected by treatment of samples with single stranded specific nuclease as described in Methods.

AFP mRNA content there is in hormone-treated rat liver, we used a convenient and semi-quantitative dot hybridization procedure (28). Nuclear RNA (nRNA) was isolated from normal control and dexamethasone-treated rat livers. The extracted nRNA was baked onto a nitrocellulose filter and hybridized to the nick-translated [^{32}P]labeled AFP gene specific probes. Figure 7 demonstrates that both nRNAs hybridized to [^{32}P]AFP DNA. The data in Figure 7 indicate that dexamethasone-treated rat liver contains about 10 times less of AFP mRNA sequence in nuclear RNA than that in normal control rat liver. This procedure could easily detect AFP mRNA sequence in 500 ng of total nuclear RNA.

Figure 7. Autoradiograms of dot blot hybridization of [^{32}P]labeled AFP cDNA probe to nuclear RNA prepared from 7-day-old rat livers (A) and livers of same age rats treated with dexamethasone for 4 days (B). 1 (1), 5 (2), and 10 (3) μg of nuclear RNA were spotted on Millipore filter discs and hybridized to AFP cDNA probe as described in Methods.

To determine the true transcriptional activity of AFP genes, we adopted a transcriptional system of isolated nuclei with endogenous RNA polymerases. The incorporation of [^3H]CTP into an acid-precipitable fraction was linear up to 60 min. When Hg-UTP was used in place of UTP, the rate of transcription was reduced by approximately 20%. In order to isolate newly synthesized transcripts, Hg-UTP (1/3 of total UTP amount) was used in the reaction mixture, and the mercurated transcripts were purified on a SH-agarose column after being heated.

Hg-RNAs were synthesized with the same number of nuclei isolated from 7 day old control rats and rats treated with dexamethasone for 4 days. The Hg-RNAs were then extracted and isolated on SH-agarose as described in the Methods. The Hg-RNAs which contain the *in vitro* transcripts and are free of endogenous mRNA contamination, were hybridized to AFP [^3H]cDNA. As shown in Figure 8, AFP mRNA sequences were synthesized during the incubation of the nuclei. A much reduced amount of AFP mRNA was detectable in Hg-RNA sample prepared from dexamethasone-treated rat liver nuclei. Dexamethasone reduced the *in vitro* synthesis of AFP mRNA by about 70%.

The contamination by endogenous AFP mRNA was estimated by the parallel control experiments. Purified AFP mRNA (200 ng) was mixed with nuclei and transcribed *in vitro*. Hg-RNA was then purified by the identical method and hybridized to AFP cDNA. We found that the contamination by the endogenous AFP mRNA sequences contributed at most 0.03% of the purified transcripts.

Figure 8. Hybridization titration curve of AFP mRNA sequences in nuclear transcripts. Nuclei were transcribed in the presence of Hg-UTP. Various amounts of mercurated transcripts were hybridized with constant amounts of [^3H]AFP cDNA (3000 cpm/0.1 ng) as described in Methods. □—□, nuclei from 7-day-old rat livers; ■—■, nuclei from livers of same age rats treated with dexamethasone for 4 days.

DISCUSSION

The effect of glucocorticoids on AFP production in newborn rat liver was found to be specific in terms of steroid structure. These results are similar to the results reported by other laboratories (20,32) working with the ACTH system. We have found (Figure 1) that glucocorticoids can enhance the activity of specific enzymes in liver such as ornithine decarboxylase. These results are in agreement with literature reported (30,31), and suggest that the effect of glucocorticoids in newborn rat liver is to selectively inhibit AFP synthesis.

Dexamethasone does not affect the serum albumin level (Figure 3). This supports the argument that dexamethasone specifically inhibits AFP synthesis. Our results are in good agreement with those reported by Cain *et al* (33). Cain *et al* (33) did not find a significant change of albumin concentration in the plasma of patients with hepatocellular disease before and after a 13 day course of prednisolone treatment (1 mg/kg body weight/day). However, these results are contrary to a number of reports by other investigators who worked in hepatoma cultures (34,35). The apparent discrepancy may be due to the different experimental systems used. It is of interest to note that different hepatoma cell lines do not respond to the same hormone in a similar manner. Becker *et al* (36) have found that dexamethasone inhibits AFP synthesis in Morris hepatoma 7777 while dexamethasone strongly stimulates AFP synthesis in Morris hepatoma 8994.

The reduction of AFP level in glucocorticoid-treated animals is not due to the molecular modification of AFP proteins and shifting in the distribution of the forms of AFP which might interfere with the radioimmunoassay. From the results shown in Figure 4 it appears that the glucocorticoid induced a decrease in the level of all of the forms of AFP. Additional data on the amount of AFP-synthesizing polysomes (Figure 5) show that the content of AFP-synthesizing polysomes in dexamethasone-treated livers is much lower than in the control livers. These results indicate that glucocorticoids decrease the AFP levels in hormone-treated rats by suppressing synthesis of AFP and not by altering secretion or degradation of AFP.

To ensure that [¹²⁵I]labeled AFP antibody is bound specifically to AFP synthesizing polysomes (Figure 5) and that the binding is a valid measure of the quantity of these polysomes, we have performed separate experiments on immunoprecipitation of AFP synthesizing polysomes as described (11). Polysomes were isolated from normal control and dexamethasone-treated rat livers (11), and incubated at 0°C with rabbit IgG anti-AFP and then with sheep IgG

anti-rabbit IgG. The immunoprecipitated polysomes were collected and washed by centrifugation. RNA was isolated from immunoprecipitated polysomes and translated in a reticulocyte translational system. The translational product was AFP. The AFP translational activity of RNA which was isolated from dexamethasone treated liver polysomes was 3-5% of that isolated from normal liver. (The number of 14% in Figure 5 includes background). The experimental procedures described above are standard methods for isolation of AFP mRNA as described in an earlier paper (10,11).

Regulation of liver protein synthesis at the translational level has been demonstrated by several laboratories (37-40). Yap *et al* (40) demonstrated that the regulation of albumin synthesis in rat liver is associated with the shuttling of albumin mRNA between an actively translating form (in polysomes) and an inactive form (a non-ribosomal-ribonucleoprotein complex) depending upon the physiological state of the animal. Administration of glucocorticoid may alter translating activity by redistribution of AFP mRNA between a translating active compartment (in polysomes) and a translating inactive compartments (ribonucleoprotein particle). However, an examination of the profiles of total liver polysomes as well as the apparent size of AFP polysomes (Figure 5) shows that there are no detectable differences between normal and dexamethasone-treated rats. These observations suggest that glucocorticoid treatment does not selectively change the rates of peptide initiation, elongation, or termination. We have directly compared the amount of AFP mRNA in total cytoplasmic RNA isolated from dexamethasone-treated and untreated rat livers. If dexamethasone reduced the level of translatable AFP mRNA without causing a redistribution of the mRNA between an active and an inactive form, one would expect the glucocorticoid to reduce the AFP mRNA concentration in the cytoplasmic RNA to the same extent. This is the result that we found (Figure 6).

The amount of AFP mRNA in liver of control rats and rats treated with dexamethasone was determined by an AFP cDNA-mRNA hybridization assay. The hybridization data (Figure 6) indicates that cytoplasmic AFP mRNA sequences dramatically decrease with exposure of rats to glucocorticoid. More importantly, the decrease of AFP mRNA concentration parallels the observed decrease in serum AFP level in both control and hormone-treated rats (fig 2 and 6). The concentration of serum AFP in normal rats fell about 40% within 4 days of the experimental period (fig 2). The reduction of rat serum AFP in the first week of postnatal life is not so drastic as in the third and fourth weeks. These results are in agreement with the data of Sell *et al* (41). The kinetics of disappearance of AFP mRNA however, did not follow those expected for a

molecule with a half-life of 40 hours (42). If 40 hours is the correct figure for the half-life of AFP mRNA in baby rat liver, the half-life of AFP mRNA in liver of rats treated with dexamethasone can be estimated by assuming that hormone completely suppressed AFP gene transcriptional activity on the very first day. The concentration of AFP mRNA should still be around 28% and 18% in liver of rats on third and fourth day's dexamethasone treatment. However, from the results shown in Figure 6 it appears that AFP mRNA is degraded more rapidly in rats treated with dexamethasone. It seems as if dexamethasone acts not only to decrease the rate of AFP mRNA transcription but also to de-stabilize this mRNA. Hormone effects on the stability of mRNA are well known. Several other observations indicate that estrogen and progesterone have significant effects on mRNA stability as well as on transcription in other systems (43,44). By using sensitive semi-quantitative dot hybridization procedure, we also found that AFP mRNA content in dexamethasone-treated rat liver nuclear RNA was about ten times less than that in control rat liver (Figure 7).

There is a possibility that the reduced AFP mRNA concentration in dexamethasone-treated rat liver may be caused by a fast turnover rate (Figure 6). However, it is not the only means by which dexamethasone can suppress AFP synthesis. In order to know if dexamethasone inhibited AFP gene transcription in the present studies, we examined the transcriptional activity of the AFP gene in control and dexamethasone-treated newborn rat livers by measuring transcription in isolated nuclei. When the nuclei were isolated from control rat liver, significant amounts of AFP mRNA specific sequences were found in the transcripts. On the other hand, the transcripts from dexamethasone-treated liver nuclei were only 30% of control rat liver nuclei. The amounts of AFP mRNA sequences found in the nuclear transcripts from dexamethasone-treated rat liver nuclei were higher than the values obtained in the cytoplasmic RNA (Figure 6) and in the nuclear RNA (Figure 7). The reason for this difference is probably due to the higher post-transcriptional turnover rate of AFP mRNA in dexamethasone-treated rat liver nuclei and cytoplasm.

Zasloff and Felsenfeld (45) have reported that RNA-dependent RNA synthesis could cause endogenous mRNA contamination to persist on an SH-agarose column. However this can be eliminated by heating samples to 107°C before SH-agarose chromatography (46). We therefore heated the sample at 107°C before applying the sample on SH-agarose column. RNA synthesis in isolated nuclei by endogenous RNA polymerase II is reported to be due mainly to chain elongation, rather than initiation (4). Even though the reaction is chain elongation, the results in Figure 8 can be regarded as a reflection of the transcriptional

activity of AFP genes in liver nuclei at the particular times of isolation. The results also suggest the AFP gene transcriptional activity was suppressed in dexamethasone-treated liver nuclei.

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