EFFECT OF ESTRADIOL ON THE RNA CONTENT AND THE ACTIVITY OF NUCLEOLAR RNA POLYMERASE FROM ROOSTER LIVER.

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SUMMARY

Estradiol administration to roosters results in changes in the macromolecular composition of the liver. Besides a gradual increase in liver protein and a sudden enhancement of liver DNA after 24 hours, the most pronounced change occurs in the RNA content, viz. an increase up to 190% of controls starting 26 hours after estradiol administration.

The activity of nucleolar RNA polymerase -solubilized and chromatographed on DEAE-Sephadex- is increased four-fold 26 hours after estradiol treatment. This increase was found to be due to an increased initiation frequency. Concurrently, the initiation characteristics of nucleolar RNA polymerase are changed.

INTRODUCTION

In birds the liver synthesizes the specific yolk proteins (1). Normally this synthesis only starts at the onset of laying, but administration of 17-β-estradiol induces yolk protein synthesis also in immature pullets and in cocks (2). With high doses of the hormone a synthetic rate can be obtained which is of the same order of magnitude as that of laying hens (3). It can be estimated that the amount of secretory proteins synthesized by the liver of estradiol-induced cocks is increased about three times as compared with non-induced cocks (4). This massive synthesis of induced proteins may have an impact on the synthetic machinery i.e. on the amount and hence on the formation of ribosomal RNA. We therefore studied the effect of estradiol administration on the RNA content of liver. Since we found an increase in RNA (4) we also studied the effect of estradiol administration in vivo on the activity of solubilized and DEAE-Sephadex purified DNA-dependent RNA polymerase in vitro. We found an increased activity of nucleolar RNA polymerase and analyzed this in terms of initiation frequency and elongation rate.

MATERIALS AND METHODS

Roosters were 8-12 weeks old hybrids of White Leghorn roosters and Rhode Island Red hens obtained from Van der Stern, Venray, The Netherlands. Estradiol, a gift from Organon, Oss, The Netherlands, was dissolved in propanediol-1,2 and injected subcutaneously (25 mg per kg body weight). 5-^{3}H -UTP (1Ci/mmol) was bought from the Radiochemical Centre, Amersham, Great Britain. GTP, CTP and UTP were obtained from Sigma, St. Louis, U.S.A. ATP, phosphoenolpyruvate (PEP) and phosphoenolpyruvate kinase (PEP-kinase) were obtained from Boehringer, Mannheim, G.F.R. Calf thymus DNA was purchased from Miles-Seravac, Lausanne, Switzerland and rooster liver DNA was isolated according to Miura (5). Soluene-100 was obtained from Packard, Brussels, Belgium.

The protein-phosphorus content in plasma was determined as described by Beuving and Gruber (2).

RNA and DNA were extracted according to a modified Schmidt-Tannhauser procedure (6). RNA was determined by the orcinol method (7) and DNA by the diphenylamine method (8). Protein was estimated by the biuret method.

Isolation of nuclei from about 60 g of liver from control and estradiol-treated roosters and solubilisation of RNA polymerase was carried out essentially according to Roeder and Rutter (9). The RNA polymerase solution to be chromatographed was frozen and stored in liquid nitrogen.

The solubilized enzyme solution was loaded onto a 1 x 15 cm DEAE-Sephadex A-25 column that had been equilibrated with TGMED (50 mM Tris-HCl pH= 7.9, 25% glycerol, 5 mM MgCl, 0.1 mM EDTA, 0.5 mM dithiothreitol), containing 50 mM$(NH)_4)_2SO_4$.

The column was washed with 15 ml of TGMED containing 50 mM $(NH_4)_2SO_4$ and then with 15 ml of TGMED containing 100 mM $(NH_4)_2SO_4$. The polymerases were eluted with 60 ml of a linear gradient of 0.1 - 0.6 M $(NH_4)_2SO_4$ in TGMED. Fractions of one ml were collected in 50 µl of bovine serum albumin solution (5 mg/ml) and immediately frozen in liquid nitrogen. The fractions could be stored without loss of activity during 2-3 weeks at - 90°C

Assay of RNA polymerase activity:

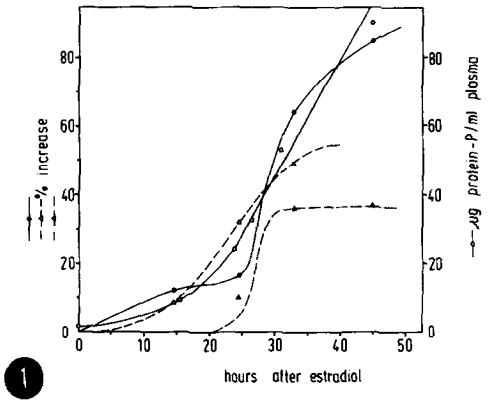
A standard reaction mixture of 0.5 ml contained 0.1 ml of divalent cation solution, 0.1 ml calf thymus DNA solution, 0.1 ml of TGMED solution, 0.1 ml of RNA polymerase solution and 0.1 ml of nucleosidetriphosphate solution. The final concentrations in the reaction mixture are: 142 µg calf thymus DNA per ml, 144 µg PEP per ml, 20 µg PEP-kinase per ml, 0.9 mM each of GTP, CTP and ATP, 0.2 mM 5-^{3}H-UTP (100Ci/mol), 0.4 mM K_2HPO_4, 20 mM Tris-HCl pH= 7.9,

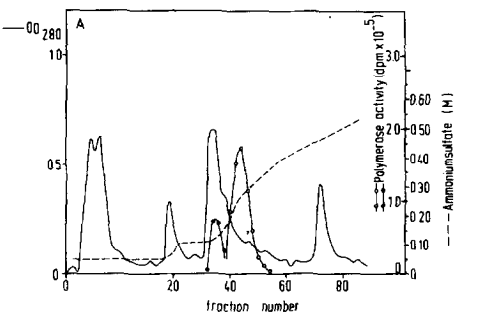
10% glycerol, 2 mM $MgCl_2$, 0.1 mM EDTA and 0.2 mM dithiothreitol. In order to assay polymerase I the solution was made 11 mM in $MgCl_2$, and for polymerase II the mixture contained 2.5 mM $MnCl_2$ and 110 mM $(NH_4)_2SO_4$. After 15 minutes incubation at 37°C the reaction was stopped by rapid cooling to 0°C. After addition of 1 ml of 0.1 M $Na_4P_2O_7$ containing 40 µg UTP and 200 µg of yeast RNA per ml the polynucleotides were precipitated with 1.5 ml of 10% trichloracetic acid containing 40 mM $Na_4P_2O_7$. The precipitate was collected on GF 83 glassfiberfilters and washed with about 75 ml of 5% trichloracetic acid containing 40 mM $Na_4P_2O_7$. The drained precipitates were solubilized in 1.0 ml of Soluene-100 at 50°C. Radioactivity was measured in a Philips liquid scintillation counter after addition of 10 ml of toluene containing 0.4% PPO and 0.008% bis-MSB. The counting efficiency was about 35%.

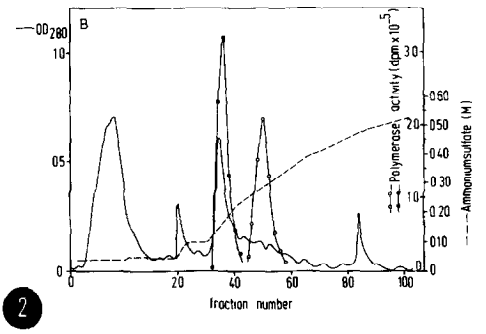
Determination of initiation frequency and chain elongation rate:
An RNA polymerase assay was carried out with the following modifications: the RNA polymerase was preincubated for 4 minutes at 37°C with 19 µg rooster liver DNA and 90 µM each of CTP, GTP and ATP. Then 5-^{3}H-UTP (1 Ci/mmol) was added to a concentration of 20 µM. At appropriate times, the reaction was stopped by adding 2.0 ml of ice-cold 40 mM $Na4P_2O_7$ containing 0.2 mg yeast RNA per ml, and 2.0 ml of 10% trichloracetic acid. The precipitate was washed four times with 5% trichloracetic acid containing 40 mM $Na_4P_2O_7$, and once with 95% ethanol and an ethanol-diethylether mixture (1:1). The precipitate was dried at 37°C. Then, 50 µg carrier uridine and 0.3 M KOH in a total volume of 1 ml were added and polyribonucleotides were hydrolyzed for 16 hours at 37°C. After neutralization with 10% $HClO_4$ the supernatant was concentrated by lyophylization. The solution was chromatographed on a polyethylene-imine-cellulose sheet with twice distilled water (10). Uridine and UMP were detected by UV absorption. The R_f values found -0.8 and 0.04 respectively- are in good agreement with published values viz. 0.81 and 0.03 respectively (10). The components were eluted with a concentrated ammonia solution for 45 minutes at room temperature. The ammonia was evaporated and 15 ml of scintillation fluid (600 ml toluene, 400 ml 2-methoxyethanol, 0.4% PPO, 0.008% bis-MSB) were added. Radioactivity was measured in a Philips liquid scintillation counter. The counting efficiency was about 25%.

RESULTS

We studied the macromolecular composition of roosters liver at different intervals after administration of estradiol (Fig. 1). A small







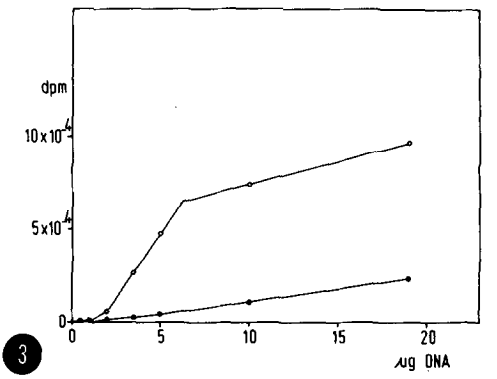


FIGURE 1: The effect of estradiol on the macromolecular composition of rooster liver.
At the times indicated livers of hormone-treated and control groups were assayed for RNA (●—●), DNA (▲---▲) and protein (Δ---Δ) as described in Materials and Methods. For each point six animals were assayed; the standard deviation of the mean was ≤5 %. The percentage increase was calculated by takingthe average value of the control roosters as 100 %. The induction of the phosphoproteins was followed by determination of the protein-phosphorus content (○—○) in the plasma.

FIGURE 2: DEAE-Sephadex A-25 chromatography of solubilized RNA polymerase from livers of control and estradiol-treated roosters.
RNA polymerase from about 60 g of liver was solubilized, chromatographed and assayed as described in Materials and Methods.
A: control ; B: estradiol treated for 26 hours.
——: E_{280}; activity of RNA polymerase I (●—●) and RNA polymerase II (○—○); – – –: $(NH_4)_2SO_4$ gradient.

FIGURE 3: Effect of estradiol on the activity of nucleolar RNA polymerase at different DNA concentrations.
0.1 ml of the DEAE-Sephadex fractions containing RNA polymerase I from control and estradiol treated roosters (Fig. 2) were preincubated with the indicated amount of rooster liver DNA without UTP. Then, 5-^{3}H-UTP was added and the reaction proceeded for 15 minutes. See Materials and Methods for further details.
●—●: control ; ○—○: estradiol treated for 26 hours.

but statistically significant increase occurs in the first 15 hours. The major increase in RNA content per total liver starts at about 26 hours. This increase in RNA content of the liver coincides with the increase of the induced yolk phosphoproteins in the blood.

The sharp increase in the amount of RNA 26 hours after estradiol administration strongly suggests a large increase in ribosomal RNA synthesis at that time. Therefore, we measured the activity of DNA-dependent RNA polymerase *in vitro* 26 hours after estradiol injection. Nuclei were isolated and RNA polymerase was solubilized as described by Roeder and Rutter (9). The elution profiles of a DEAE-Sephadex A-25 chromatography of RNA polymerase

TABLE I.

Some characteristics of DEAE-Sephadex purified DNA-dependent RNA polymerase from rooster liver.

	Polymerase I	Polymerase II
Eluted from DEAE-Sephadex at $(NH_4)_2SO_4$ concentration	0.11–0.14 M	0.25–0.30 M
Optimal $(NH_4)_2SO_4$ concentration	20 mM	110 mM
Divalent cation activation	Mg^{2+}	Mn^{2+}
Divalent cation optimal concentration	11 mM	2.5 mM
$\frac{\text{Activity with } Mn^{2+}(2.5 \text{ mM})}{\text{Activity with } Mg^{2+}(11 \text{ mM})}$	0.6 - 0.9	3.6 - 3.9

0.1 ml of appropriate fractions of a DEAE-Sephadex chromatography were assayed with the standard procedure as described in Materials and Methods.

TABLE II.

Effect of estradiol on the activity of DEAE-Sephadex purified DNA-dependent RNA polymerases from rooster liver.

	Control	Estradiol treated
Polymerase I activity	1.9	7.6
Polymerase II activity	5.9	6.4
Ratio I / II	0.3	1.2

0.05 ml of all DEAE-Sephadex fractions from Fig. 2 were assayed with the standard procedure. All activities (dpm x 10^{-5} incorporated per 15 minutes) of the appropriate peaks were summed.

TABLE III.

Effect of estradiol on the internal and 3' terminal incorporation of 5-^{3}H-UTP by DEAE-Sephadex purified DNA-dependent RNA polymerase I from rooster liver.

	Control (C)		Estradiol treated (E)			
Incubation time (min)	UMP dpm x 10^{-4}	Uridine dpm x 10^{-3}	UMP dpm x 10^{-4}	Uridine dpm x 10^{-3}	UMP/U (E) / UMP/U (C)	Ur (E) / Ur (C)
1	0.7	0.16	2.2	0.55	0.9	3.4
2	1.0	0.28	9.15	1.52	1.5	5.3
3	2.2	0.27	9.68	1.18	1.1	4.3
4	2.8	0.34	14.55	1.27	1.4	3.7
5	3.7	0.37	17.85	1.39	1.3	3.7
Mean value					1.2	4.1

0.1 ml of the DEAE-Sephadex fractions containing RNA polymerase I from control and estradiol treated roosters (Fig. 2) were incubated in a modified assay mixture (see Materials and Methods). The synthesized polyribonucleotides were hydrolyzed with alkali and the products separated on PEI-cellulose sheets. The radioactivity of UMP and Uridine (Ur) was determined after elution from the PEI-cellulose sheet.

both from control and estradiol-treated roosters are shown in Fig. 2. Two polymerase fractions can be separated, some properties of which are summarized in Table I. From these characteristics it can be concluded that polymerase I is probably of nucleolar origin whereas polymerase II is a nucleoplasmic enzyme (11). Administration of estradiol results in a four-fold increase in polymerase I activity (Fig. 2 and Table II). The activity of polymerase II is hardly changed. This increase in activity of polymerase I was consistent in all experiments.

The increase in polymerase activity may be due to an increased initiation frequency and/or an increased elongation rate. In order to assess their relative contributions we carried out the following experiment: the labeled RNA produced in vitro was hydrolyzed by alkali. This results in a conversion of the internal nucleotides into 2' and 3' ribonucleoside monophosphates while the 3' terminal ribonucleotide will be hydrolyzed to a nucleoside. The number of nucleosides reflects the number of chains started whereas the ratio of ribonucleotides over ribonucleosides per unit of time reflects the rate of chain elongation. The effect of estradiol treatment on the initiation frequency and the rate of chain elongation of polymerase I *in vitro* is shown in Table III. The rate of chain elongation is only somewhat increased after estradiol treatment whereas the number of chains initiated

is four times as high in estradiol treated roosters as in the controls. We can therefore conclude that the increased polymerase activity is mainly due to an increased initiation frequency. This increase might just reflect an increased number of polymerase molecules present, but might also be due to changes in the initiation characteristics of the polymerase molecules. The initiation characteristics were studied by testing the activity of the of the polymerase molecules as a function of DNA template concentrations (Fig. 3). At DNA concentrations below 7 µg per 0.5 ml the RNA polymerase I molecules of estradiol treated roosters behave differently from control roosters. The activity of RNA polymerase after estradiol treatment increases much more steeply at increasing DNA concentrations. But at concentrations above 7 µg DNA per 0.5 ml the ratio of activity after estradiol treatment overthat of the control levels off and becomes constant, about four at high DNA concentrations (at least up to 71 µg).
We conclude that estradiol induces altered initiation characteristics in the nucleolar RNA polymerase.

DISCUSSION

A change in the macromolecular composition of the liver from roosters after estradiol administration was observed. The amount of protein increases gradually in the first 30 hours. The DNA content does not change significantly during the first 24 hours. Then a sharp increase of about 35-40% is found. The amount of RNA increases biphasie-ally: within 15 hours a statistically significant increase of 15-20% and starting at 24 hours an increase up to 90% is found.

Jost et al (12) also examined the macromolecular composition of rooster liver after estradiol administration and found essentially the same results.

The increase in liver RNA content is reflected by a selective enhancement of the nucleolar RNA polymerase activity over that of nucleo-plasmic RNA polymerase activity. This relative increase in nucleolar RNA polymerase activity is also found in rat liver after (hydro-)cortisone treatment (13, 14, 15) and in mammary gland after estradiol administration (16).

We found that the increased RNA polymerase activity was the result of the larger number of chains initiated after estradiol treatment. Concurrent with this larger initiation frequency we found altered initiation charact-eristics.
We can explain our results in two ways:

a/ estradiol induces the synthesis of more RNA polymerase molecules with different initiation characteristics

b/ estradiol induces a change in the initiation characteristics in the already present RNA polymerase molecules as a result of which more chains can be initiated. This change may be either an allosteric change, a chemical modification or addition of some protein factor(s).

Sajdel and Jacob (13) also found changes in the initiation characteristics of nucleolar RNA polymerase after hydrocortisone treatment. They ascribed them to an allosteric change of the RNA polymerase. On the other hand, Yu and Feigelson (14) studied the effect of cortisone on the RNA synthesis in isolated nucleoli. Using poly dC as exogenous template and Actinomycin D to inhibit endogenous template activity, they conclude that the enhanced RNA synthesis is a result of an increase in the level of RNA polymerase. But they did not further analyze the initiation characteristics of RNA polymerase. We therefore conclude that at the present stage neither of the two explanations can be rejected.

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