Effect of Estradiol on Rat Uterus DNA-dependent RNA Polymerases

STUDIES ON SOLUBILIZED ENZYMES

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DNA-dependent RNA polymerases of immature and castrated rat uteri were studied after estradiol administration. The enzymes were solubilized from either whole uterus homogenate or nuclei and their activities were measured on an exogenous DNA template. α-Amanitin was used to distinguish α-amanitin-resistant from α-amanitin-sensitive forms of the enzyme. The number of α-amanitin-sensitive RNA polymerase molecules was measured by a binding assay using labeled amanitin.

In the first series of experiments RNA polymerases were solubilized from whole uterus homogenate. α-Amanitin-sensitive and -resistant activities were constant during the first 6 hours after estradiol treatment, followed by a late and moderate increase in their activities (50% at 12 hours for the resistant form and 40% at 24 hours for the sensitive form). The number of sensitive polymerase molecules evolved in an identical manner to its activity (+40% at 24 hours), suggesting that the increase in activity is due to the synthesis of new enzyme molecules. For both forms, no diffusible stimulatory factor was detected in the uterus of hormone-treated animals.

In the second series of experiments, disrupted nuclei were washed with 0.15 M (NH₄)₂SO₄ in order to release only enzyme molecules which were not firmly bound to DNA in a transcription complex. The amount of the sensitive form of polymerase which remains firmly bound to chromatin, was constant for 6 hours after estradiol administration and was doubled by 24 hours. The firmly bound α-amanitin-resistant activity was solubilized and was measured in the presence of an exogenous template. There was a progressive increase in activity first detectable in 1 to 2 hours, amounting to 50% at 6 hours and 100% at 24 hours.

The reported results show that during the first 6 hours of hormone treatment: (a) the total content of RNA polymerases remains unchanged in the uterus; (b) the number of α-amanitin resistant molecules tightly bound to DNA increases progressively while the α-amanitin sensitive remains constant. At a later time (24 hours), an increase is observed both for the total amount of enzymes and for their fraction engaged in a transcription complex.

Increase of RNA polymerase activities measured in vitro have been reported in isolated nuclei of uterus of ovariectomized or immature rats in the first hours following estradiol treatment (1–6). Due to the complexity of the system in which RNA synthesis is dependent on the endogenous template, interpretation of the data is difficult. In fact, the observed modifications can be the result of several phenomena which may eventually act simultaneously. (a) A change in the availability of the chromatin template; this was already investigated using bacterial RNA polymerase and a 25 to 50% increased RNA synthesis was observed as early as 1 hour after hormone treatment (6–10). (b) A change in the number of active RNA polymerase molecules; this could be related to a change in the actual number of enzyme molecules or to a change in the activities of preexisting enzyme molecules (by covalent modification or interaction with a control "factor").

In an attempt to distinguish between these possibilities, the variations in RNA polymerase activities solubilized from

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ovariectomized or immature rat uterus were studied after estradiol administration. RNA polymerase activities were measured using a nonspecific template (Calf thymus DNA) under conditions where the amount of RNA synthesized is most likely reflecting the number of active RNA polymerase molecules. α-Amanitin was used to differentiate between RNA polymerases A (I) and B (II) (11), and the actual number of RNA polymerase B molecules was measured by O- [3H]methyl- 

**Materials and Methods**

Adult and immature female Wistar rats were obtained from Iffa Credo (France). Ovariectomy was performed on adults weighing 200 g and the animals were used in the experiments 3 weeks after castration. Immature animals (21 to 23 days) weighed 50 g. The animals received a single intraperitoneal injection of 17β-estradiol dissolved in 1 or 0.5 ml of 5% ethanol in 0.15 M NaCl, 10 μg for the castrated and 1 or 5 μg for the immature. Controls were injected with 5% ethanol in 0.15 M NaCl. The animals were killed by decapitation and the uteri were removed and frozen immediately in liquid nitrogen.

All chemicals were reagent grade. 17β-Estradiol was a gift of Roussel Uclaf (Romainville, France). Polyethylene glycol 6000 was purchased from Fawberke Höchst (6320 Francfort/M 80), unlabeled ribonucleo-

**Protein and DNA Measurements**—Protein was determined by a

**RNA Polymerase Solubilization**—All steps were carried out at 0°C as rapidly as possible. Frozen uteri (0.5 to 1 g) were pulverized in liquid nitrogen with the help of a mortar and homogenized with a glass-glass homogenizer in 14 ml of MS(25) buffer and aliquots were taken for the measurement of DNA and [3H]amanitin binding to RNA polymerase B. The remainder of the homogenate was diluted to 20 ml with MS(30) buffer containing 0.8 M ammonium sulfate. The viscous mixture, maintained below 0°C in a dry ice-acetone bath, was sonicated four or five times at the lowest energy for 10 s periods. The temperature was controlled between each period and was at all times below 5°C. The sonicate was centrifuged at 20,000 × g for 10 min in a Sorvall centrifuge and the supernatant was precipitated by ammonium sulfate (50% saturation). After 30 to 45 min the precipitate was collected by centrifugation at 200,000 × g for 30 min in a Spinco SW 41 rotor. The pellet was suspended in 5 ml of MS(50) buffer (PSO fraction) and the ammonium sulfate concentration was determined by conductivity measurement after a 1,000-fold dilution. This extract can be stored in liquid nitrogen for several months without any loss of activity.

**Preparation of Nuclear Fraction**—Uteri were homogenized in 6 volumes of 0.32 M sucrose/1 mM MgCl2 by 10 strokes in a glass-glass homogenizer. The homogenate was centrifuged at 600 × g for 10 min and the nuclear pellet washed twice with the same medium. The nuclei were disrupted in MS(25) buffer containing 0.15 M (NaH)2SO4 and centrifuged at 20,000 × g for 30 min. The final pellet, taken up in MS(25) buffer, was used for measuring enzyme B by [3H]amanitin binding and enzyme A activity after solubilization by sonication in the high ionic strength medium.

**RESULTS**

**Determination of RNA Polymerases A and B from Whole Tissue Homogenate**

**Determination of RNA Polymerase B by [3H]Amanitin Binding**

**Method**—It was previously demonstrated that amatoxins bind to RNA polymerase B with a high affinity and with I1 stoichiometry (13). Three methods can be used in crude homogenates for determining the cellular number of RNA polymerase B molecules by measuring the binding of [3H]amanitin (12, 13). These methods involve either membrane filtration or ammonium sulfate or polyethylene glycol precipitation. In the case of uterus, the three methods give similar results. However the nitriloacellose membrane filtration method was not used because of a higher background and a limited protein capacity of the filters. The two precipitation techniques were equally valid and the polyethylene glycol assay was preferred because the precipitate was easier to collect.

As previously described (12), the [3H]amanitin/RNA polymerase B complex is precipitated by 10% polyethylene glycol. As shown in Fig. 1, complete precipitation of the complex is achieved with this concentration, whereas nonspecific binding is constant and independent of polyethylene glycol concentration. Since the precipitation of the complex by polyethylene glycol is affected by the concentration of ammonium sulfate, binding experiments were always carried out at an optimal ammonium sulfate concentration (150 mM).

In order to establish that under these experimental conditions, RNA polymerases B were the only components binding labeled amanitin, the equilibrium dissociation constant (Kd), of the reaction was determined at 0°C. Fig. 2 shows the Scatchard representation of the data. The single slope indicates that in uterine only one cellular component binds labeled amanitin with high affinity. Therefore if there were several isoenzymes B in the uterus, as described for calf thymus (18), they have the same affinity for [3H]amanitin. The calculated dissociation constant is 0.51 nM, a value which is similar to that of 0.52 nM.

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Effects of Estradiol—The amount of enzyme B in the uterus was measured on the uterine homogenate at different times after injection of estradiol to immature and castrated rats. In order to express the results on a cellular basis, the amount of enzyme B was related to the DNA content of the organ. The base-line values for the immature rat (4 pmol/mg of DNA) is 50% higher than that of the castrated animal (2.6 pmol/mg of DNA) (Fig. 3). The calculated number of polymerase B molecules per haploid genome is of the order of $8 \times 10^4$ for the immature and $5 \times 10^4$ for the castrated animal. This value is 4 to 5 times lower than that found for the uterus and other tissues of the normal adult rat (12). The variation in the amount of enzyme B as a function of time after a single estradiol injection is shown in Fig. 3. The amount of enzyme per cell remains constant over the first 12 hours and then increases by 40% in both the immature and castrated animals at 24 hours. Since at this time the protein content of the uterus is also increased, the amount of enzyme B molecules per mg of protein is increased by only 10 to 15%.

**Determination of RNA Polymerase A and B by Enzymatic Assay after Solubilization**

**Method**—In order to assess the effect of estradiol treatment on the activities of solubilized RNA polymerases, the solubilization procedure should not result in any significant loss of activity or possible factors affecting enzymatic activity. Therefore the recovery of enzyme B was studied by $[^3H]$amanitin binding at each step of the procedure. The results shown in Table I indicate an almost total recovery after sonication followed by centrifugation. After precipitation, at and above 50% ammonium sulfate, the recovery of enzyme B was more than 80%. For each series of experiments, the recovery of enzyme B was checked by measuring the amount present in the initial homogenate and in the P50 fraction. The recovery varied between different series of animals (65 to 90%) but within a given series, it is fairly constant. As seen in Table II, the solubilized enzyme activity exhibits all of the characteristics of DNA-dependent RNA polymerases. The effect of $\alpha$-amanitin concentration on the activity of solubilized RNA polymerase is shown in Fig. 4. This inhibitor distinguishes enzyme A activity $\alpha$-amanitin-resistant, from enzyme B activity, $\alpha$-amanitin-sensitive. At the concentration of inhibitor (1 to 10 $\mu$M) used in these experiments, enzyme C activity, is measured along with enzyme A activity.

### Table I

**Recovery of solubilized RNA polymerase B from rat uterus**

| Fraction               | Bound $[^3H]$amanitin | Recovery pmol/mg DNA |
|------------------------|-----------------------|----------------------|
| Homogenate             | 40                    | 100                  |
| Sonicate               | 38.9                  | 97                   |
| Sonicate supernatant   | 38.6                  | 96.5                 |
| P40                    | 18.4                  | 46                   |
| P50                    | 22.8                  | 82                   |
| P60                    | 34.7                  | 87                   |
| P70                    | 32.8                  | 82                   |

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![Fig. 1 (left). Effect of polyethylene glycol concentration on the solubility of $[^3H]$amanitin and $[^3H]$amanitin-RNA polymerase B complex—Experiments were carried out as described under “Materials and Methods.” The enzyme was from a crude lysate of normal adult rat uterus. DNA, 70 $\mu$g, was used for each experiment point. $\bullet$—$\bullet$, binding of $[^3H]$amanitin; $\circ$—$\circ$, binding of $[^3H]$amanitin diluted by a large excess of cold $\alpha$-amanitin (nonspecific binding).](image1)  

![Fig. 2 (center). Determination of the equilibrium dissociation constant of the reaction RNA polymerase B $[^3H]$amanitin—Bound $[^3H]$amanitin was measured by the polyethylene glycol precipitation assay as described under “Materials and Methods.” Increasing concentrations of $[^3H]$amanitin (4.7 $\times$ 10$^{-11}$ to 1.8 $\times$ 10$^{-4}$ $\mu$M) were incubated with 0.1 ml of a crude rat uterus homogenate for 18 hours at 0°. Data are represented as a Scatchard plot (17).](image2)  

![Fig. 3 (right). Amount of RNA polymerase B in the immature and castrated rat uterus as a function of time after estradiol treatment—Enzyme B was measured by the $[^3H]$amanitin binding assay as indicated under “Materials and Methods.” $\circ$—$\circ$, castrated; $\bullet$—$\bullet$, immature. Each point (±S.D.) is the average of three independent experiments.](image3)
Table II

Characteristics of uterine solubilized RNA polymerase activity

Standard enzyme assays (see "Material and Methods") were carried out with the indicated modifications. Enzyme source was a P50 fraction (4.2 mg of protein/ml) prepared from 24-hour estradiol-treated adult rat uteri.

| Incubation medium | [3H]UMP incorporated (pmol x min⁻¹) |
|-------------------|-----------------------------------|
| Complete, 37°C     | 1.31                              |
| Complete, 37°C + 8.8 μM a-amanitin | 0.74                              |
| Complete, 0°C      | 0                                 |
| Complete + 12 μg of actinomycin D | 0.13                              |
| -ATP              | 0                                 |
| -GTP              | 0                                 |
| -CTP              | 0.01                              |
| DNA               | 0.01                              |

Fig. 4. Effect of α-amanitin on the activity of solubilized RNA polymerase B—The activity of solubilized enzymes (P50 fraction) prepared from estradiol-treated (24 hours) immature rat uterus was measured under standard conditions (see "Materials and Methods") except that α-amanitin was present in the incubation medium at the indicated concentration.

The effects of divalent cation concentration and ionic strength on the solubilized enzymatic activities are presented in Fig. 5. In the presence of 4 mM Mn²⁺, the optimum concentration of ammonium sulfate was 40 mM for enzyme A and 100 mM for enzyme B. With 40 mM ammonium sulfate, Mg²⁺ and Mn²⁺ at their optimum concentration were equally effective for enzyme A. At this concentration of ammonium sulfate, Mn²⁺ was a better activator (2-fold) than Mg²⁺ for enzyme B. These differences between enzymes A and B are in agreement with those reported for purified enzymes of calf thymus (19) and rat liver (20).

The Kₐ for UTP of the total enzyme (A + B) was 0.05 mM, a value similar to that obtained for purified calf thymus enzymes (11). Based on this value and those reported for the other three nucleotides with pure enzymes (11), concentrations of 0.3 mM UTP and 2 mM for the other nucleotides were used in our assay. The kinetics of the reaction are shown in Fig. 6. The incorporation of [3H]UMP is linear for the first 6 min. Very often after this time, there was a decrease in the rate of RNA synthesis and the nucleotide incorporation was no longer linear. Consequently, only short incubation times (6 min) were used for these assays, in order to measure a maximum initial velocity. When the protein concentration in the incubation mixture was lower than 2 mg/ml, a linear relationship was observed between initial velocity and the amount of enzyme incubated (not shown). The presence of RNase activities was checked in the enzyme preparations in the following way. At the end of [3H]UMP incorporation, 20 μg of actinomycin D was added to the reaction mixture and the incubation continued for an additional 10 min at 37°C. As seen in Fig. 6 no RNase activity was detected in the P50 fraction. Occasionally a small decrease in the amount of synthesized RNA was observed, but it was always less than 10%.

Effects of Estradiol—The variation of solubilized RNA polymerase A and B activities were studied in three series of immature and three series of castrated animals for time periods up to 24 hours, after a single injection of estradiol. As seen in Fig. 7, the basal activities of the immature animals were about 60% higher than those of the castrated group. In the control animals of both groups, enzyme B activity was greater than that of enzyme A. Under the influence of estradiol, enzyme A activity remains unchanged up to 6 hours. The first increase (50%) was observed after 12 hours and reached 100% by 24 hours. In the case of enzyme B, the increase in activity occurred later (24 hours) and was less pronounced (40%) than that observed for enzyme A at this time.

It was not possible to measure the recovery of enzyme A during the solubilization procedure. However, it should be pointed out that at a given time after hormone injection, the ratio of A to B activity was always the same in different experiments.

After 24 hours, the increase in enzyme B activity is the same (40%) as the increase in the amount of enzyme B measured by [3H]aminitin binding (Fig. 3), indicating that the enhanced B activity corresponds to a higher number of enzyme molecules. In the case of enzyme A, it is not possible to know whether the change in activity observed after 12 hours is due to an increase in the number of active molecules or to a higher enzyme specific activity. That this increased activity is not due to the stimulation of preexisting molecules by a diffusible factor was suggested by the following experiment. The enzymes were solubilized from uteri of control and 24-hour-treated immature animals. Enzyme A activity was measured in each preparation and in mixtures of both extracts. Table III shows that the activity of a mixture of the two enzyme preparations is equal to the sum of the activities of each fraction. This strict additivity excludes the presence of an available diffusible stimulatory factor(s) in the extract prepared from uteri of estradiol-treated animals. During precipitation by ammonium sulfate, two fractions are discarded: the dialyzable components and the proteins which do not precipitate at 50% ammonium sulfate. These two fractions were prepared from three batches of castrated uteri (control, 6 hour, and 24-hour-treated), in the following way. Part of the uteri was processed as usual and the ammonium sulfate supernatant was dialyzed and concentrated against MS(0) buffer to obtain Fraction I. Another part was homogenized and dialyzed against distilled water. The concentrated dialysate is designated as Fraction II. RNA polymerase activities of the three enzyme preparations were then measured as usual and in the presence of an aliquot of the homologous fractions I, II, and I + II at concentrations corresponding to their respective tissue proportions. For the three batches of uteri, a similar nonspecific 30% decrease in activity was observed, regardless of the fraction added (result not shown). These experiments suggest that no component, which could affect RNA polym.
Estradiol Effect on Solubilized Uterine RNA Polymerases

A.S. [mM] Mn++ or Mg++ [mM]

Y

A.S. 40 mM

Mn++ or Mg++ [mM]

iOZOdO60 60100 125150 175 0246610 15 20 0146610 15 20 25 30

FIG. 6 (left). Incorporation of [3H]UMP by uterine soluble RNA polymerases—The enzymes were assayed in a P50 fraction (1.4 mg of protein/ml) of untreated immature rats, as described under "Materials and Methods." O---O, in absence of α-amanitin; 04, in presence of α-amanitin; A, actinomycin D (20 μg) added at the end of the reaction (see text). RNA polymerase B activity was determined by subtracting the activity obtained in the absence of α-amanitin from that found in the presence of the toxin.

Determination of Fraction of RNA Polymerase A and B Tightly Bound to Chromatin

 Previous studies (21) have shown that nuclear RNA polymerases are either readily extracted or tightly bound to the chromatin. The tightly bound enzyme was solubilized only by sonication in a high ionic strength buffer (22).

Nuclei prepared from immature rat uteri were lysed and extracted with different concentrations of ammonium sulfate. Enzyme B which remains bound to the chromatin, was measured by [3H]amanitin binding. Enzyme A tightly bound to chromatin was measured by enzymatic assay after the sonication-solubilization procedure. The results are shown in

Fig. 7 (right). Variation of solubilized total uterine RNA polymerase activities, after estradiol administration—Enzymatic activities of P50 fraction were measured as described under "Materials and Methods." Panel A, enzyme A: ▲—▲, castrated; O—O, immature. Panel B, enzyme B; ▲—▲, castrated; O—O, immature. Each point (±S.D.) is the average of three independent experiments (15 animals for each point).
TABLE III
Enzyme A activity in co-incubation of enzyme preparations from treated and control animals.

| Enzyme | Incubated volume | Measured activity | Anticipated activity |
|--------|------------------|-------------------|---------------------|
| C      | 50 µl            | 0.61 ± 0.02       |                     |
| 24 hours | 50 µl          | 1.17 ± 0.07       |                     |
| C + 24 hours | 35 µl + 17 µl   | 0.76 ± 0.04       | 0.79                |
| C + 24 hours | 17 µl + 33 µl   | 1.01 ± 0.04       | 0.98                |

Fig. 8. With 0.1 M ammonium sulfate, all of the readily extractable enzyme is removed, no additional enzyme being extracted above this concentration (up to 0.25 M). Higher concentrations of ammonium sulfate resulted in the formation of a gel and consequently, concentrations greater than 0.25 M were not investigated. In control immature uterus, the amount of enzyme B and enzyme A activity which remain bound to the chromatin after 0.15 M ammonium sulfate extraction, were 42 and 30%, respectively, of the activity found in the total tissue (Fig. 8). Fig. 9 shows the variation of tightly bound enzyme A and B, after administration of estradiol. The number of enzyme B molecules bound to the chromatin remains constant during the first 5 hours and shows a 100% increase at 24 hours. The results are different for enzyme A. There is a progressive increase in bound enzyme A activity, already measurable at 1 hour (20%) and which reaches 80% at 24 hours.

DISCUSSION

RNA polymerases A (I) and B (II) were studied in estradiol-treated rat uteri, either in whole tissue homogenate or in nuclei.

In whole tissue homogenate, similar results were obtained for castrated and immature rats. The amount of enzyme B molecules measured by [3H]amanitin binding in crude extracts, as well as the enzyme B activity measured after solubilization, remained constant over the first 12 hours after hormonal treatment and then showed a 40% increase at 24 hours. This strict correlation between the number of enzyme B molecules determined by amanitin binding and the enzymatic activity clearly shows that the delayed enhancement of activity is related to the synthesis of new B enzyme molecules. As previously discussed (12), this strict correlation makes also very unlikely the possibility that amanitin binding assays would measure the binding of the labeled toxin to free enzyme subunit(s) or enzymatic inactive B molecules which would be present in the cellular homogenates. Enzyme A activity solubilized from whole tissue did not change within the first 6 hours and then rose progressively (50% at 12 hours, 100% at 24 hours). However, the experiments did not differentiate between an actual increase in the number of enzyme A molecules and an activation of pre-existing enzyme molecules via nondiffusible macromolecular factor(s) present in limiting amount or a covalent modification of the enzyme. In this respect, it is worth mentioning that, for both enzymes, no dialyzable or macro-

molecular stimulatory "factor(s)" was detected.

In a second series of experiments, isolated nuclei were washed with 0.15 M ammonium sulfate in order to eliminate any readily extractible RNA polymerases, while keeping enzyme molecules tightly bound to the chromatin. Since increasing the ionic strength prevents initiation of RNA synthesis and results in the release from the DNA template of RNA polymerase molecules which are not engaged in a ternary transcription complex (23-26), it is very likely that the enzyme activity which remains tightly bound to the chromatin corresponds to the fraction which is actively engaged in transcription in the form of enzyme-DNA-RNA ternary complexes.

The number of enzyme B molecules tightly bound to the chromatin measured by [3H]amanitin binding was constant over the first 6 hours following estradiol administration. This suggests a constant number of enzyme B molecules engaged in the process of transcription, but does not preclude any changes in the elongation rate of RNA chains or any qualitative changes in the nature of the template being transcribed. In contrast, there is a progressive increase in the amount of enzyme A tightly bound to the chromatin. This increase is already evident (20%) at 1 hour, reaching 45% at 6 hours. During this period, the enzyme A activity which can be extracted from total tissue and which represents the sum of readily extractible and tightly bound enzyme activities, remained constant. It therefore seems that, under the influence of hormone treatment, a portion of the enzyme A, which was originally present in a free form, becomes engaged in RNA synthesis. It remains to be seen whether this phenomenon is due to an increased availability of the nucleolar chromatin or to the effect of a factor which would be required for initiation of RNA synthesis.

It is interesting to compare the results obtained with solubilized RNA polymerases to those obtained when measuring the same enzyme activities in whole nuclei, under conditions where the enzymes are transcribing their own template. Such studies are reported elsewhere. They led to the conclusion, which differs to some extent from that reached by other author(s) (6), that there was an early increase in the number of transcribing A enzyme molecules, while the number of transcribing B molecules was unchanged. Our present results fully support this interpretation.

In vivo RNA metabolism has been extensively studied in the mouse and rat uterus. After administration of estradiol, an increase in total uterine RNA has been observed after 6 hours of hormone treatment (27-30). The results are however conflicting when early quantitative and qualitative changes in RNA are studied by the incorporation of radioactive RNA precursors. After hormone treatment, some reports showed an early (2 min to 1 hour) increase of RNA specific activity (28, 29, 31, 32) and in others, this increase was not seen before 6 hours (30, 39). In addition it is not clear if this is related to a general increase of incorporation of precursors into all RNA species (33-37) or to an increase limited to either pre-ribosomal (38) or heterogenous high molecular weight RNA (38, 39). Although no clear conclusion can be drawn at the present time from these in vivo studies, it is interesting to note that the progressive increase in the tightly bound polymerase A activity which we have observed in the first hours after hormone injection agrees with that reported for ribosomal RNA synthesis (33, 40). In addition, the delayed enhancement in the amount of enzyme B molecules tightly bound to chromatin may be corre-

1. M. M. Bouton, J. C.Couvain, and E. E. Baulieu, manuscript in preparation.
FIG. 8 (left). Removal of readily extractible nuclear RNA polymerases by increasing concentration of ammonium sulfate—Nuclei prepared from immature rat uteri were washed with increasing concentrations of ammonium sulfate. The remaining nuclear enzyme fractions were measured by [H]amanitin binding (enzyme B) and enzymatic assay (enzyme A) after solubilization (see "Materials and Methods"). T, total tissue; N, nuclei; 0.05, 0.1, 0.15, and 0.25 indicate the ammonium sulfate concentration. Panel A, enzyme A activity; Panel B, enzyme B amount.

FIG. 9 (right). Chromatin-bound RNA polymerases A and B as a function of time after estradiol treatment—Nuclei prepared from immature rat uteri were lysed and extracted with 0.15 M ammonium sulfate and enzyme A and B were measured as described in the legend of Fig. 8. Panel A, enzyme A activity; Panel B, enzyme B amount.

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ammonium sulfate concentration. Panel A, enzyme A activity; Panel B, enzyme B amount.

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