Hormonal Control of Transcription in the Rat Uterus

STIMULATION OF DEOXYRIBONUCLEIC ACID-DEPENDENT RNA POLYMERASE III BY ESTRADIOL*

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DNA-dependent RNA polymerases were extracted from rat uterine tissue, partially purified and resolved by DEAE-Sephadex chromatography. RNA polymerases I, II, IIIa, and IIIb eluted at the characteristic ammonium sulfate concentrations of 0.15, 0.28, 0.34, and 0.42 M, respectively. The sensitivity of each peak of polymerase to α-amanitin was examined and was shown to be essentially identical to the three classes of RNA polymerases in other mammalian systems. RNA polymerase I was insensitive to high levels of α-amanitin, RNA polymerase II was sensitive to low concentrations of α-amanitin (50% inhibition at 0.005 µg/mI) and RNA polymerases IIIa and IIIb were sensitive to high concentrations of α-amanitin (50% inhibition at 0.025 µg/ml). The α-amanitin sensitivity curve of total RNA synthesis measured in isolated nuclei demonstrated that the activity of each class of RNA polymerase could be quantitated in uterine nuclei. Thus the initial decrease in activity at low concentrations of α-amanitin (50% inhibition at 0.005 µg/ml) was attributed to the inhibition of RNA polymerase II activity; the second decrease in activity at higher concentrations of α-amanitin (50% inhibition at 0.025 µg/ml) was attributed to the inhibition of RNA polymerase III activity; and the activity which was resistant to the highest α-amanitin concentration tested was attributed to RNA polymerase I activity. When estradiol was given to immature rats 6 h before killing both RNA polymerases I and III levels in nuclei were increased significantly over the control values. The time course of these changes demonstrated that the increases in RNA polymerases I and III were first evident between 1.5 and 3 h following hormone treatment. Significantly, these increases in polymerase I and III in nuclei parallel the published increases for rRNA and tRNA synthesis following hormone treatment. However, the amount of RNA polymerase I and III was not altered upon extraction, suggesting that these changes are due to the alteration in chromatin template activity. Both estradiol and estriol produced identical increases in uterine RNA polymerase I and III 6 h after treatment.

The existence of multiple DNA-dependent RNA polymerases (I, II, and III) in eukaryotic cells is well established (1-3). Each class of RNA polymerase exhibits a distinct structure and each performs a distinct biological function. RNA polymerase I is localized in the nucleolus (2) and is responsible for the synthesis of 45 S rRNA (4-6). RNA polymerase II is found in the nucleoplasm (2) and catalyzes the synthesis of heterogeneous nuclear RNA (4-6). More recent studies have indicated that RNA polymerase III is responsible for the synthesis of transfer RNA and 5 S ribosomal RNA (7-9). The existence of distinct RNA polymerases which carry out different biological functions thus provides one means by which the transcription of different classes of eukaryotic genes can be regulated. While many studies have focused on the regulation of RNA polymerase I and II activities (4, 10), there have been few studies to date on the physiological control of RNA polymerase III activity.

The transcription of various classes of genes in many mammalian tissues is regulated by steroid hormones, and one system which has been extensively studied is the stimulation of uterine RNA polymerase activity by estrogenic hormones. Following the pioneering studies of Gorski (11) it has since been shown that the in vivo administration of estradiol produces increases in the activity of RNA polymerase I (4, 10) and RNA polymerase II (12-15), and that the absolute amounts of various classes of uterine RNA are increased following hormonal stimulation (15-17). While it is known that the uterine content of ribosomal RNA (17, 18) and transfer RNA (17, 19, 20) is increased as a result of estrogen treatment, it is not known whether these increases are partially or wholly brought about by increases in uterine RNA polymerase III activity.

In this work we have determined that uteri from mature rats, immature rats, and estrogen-treated immature rats contain two forms of RNA polymerase III, IIIa and IIIb. These enzymes can be distinguished from uterine RNA polymerases I and II on the basis of elution from DEAE-Sephadex and on the basis of sensitivity to the toxin α-amanitin. RNA polymerase I is eluted from DEAE-Sephadex at 0.15 M (NH₄)₂SO₄, and...
is insensitive to a-amanitin; RNA polymerase II is eluted at 0.28 M salt and is inhibited by low concentrations of a-amanitin; RNA polymerases IIIA and IIIB elute at 0.34 and 0.42 M salt, respectively, and are both inhibited by high concentrations of a-amanitin. The activities of uterine RNA polymerases I, II, and III can also be measured in isolated nuclei on the basis of their differential sensitivities to a-amanitin.

Within 6 h after the administration of estrogenic compounds to immature female rats, the activities of uterine RNA polymerases I and III, measured in isolated nuclei, are greatly increased and the time courses of the increases are similar for these two enzymes. If, however, uterine RNA polymerases from saline (0.9% NaCl)-treated and estrogen-treated animals are extracted and separated on DEAE-Sephadex columns, no differences are observed in the total number of units of RNA polymerases I, II, and III in the two groups.

**MATERIALS AND METHODS**

**Animals and Reagents** – Female rats were obtained from Texas Inbred Mouse Co., Houston, Texas. The mature animals used in this study weighed 200 g, and the immature animals weighed approximately 40 g and were 20 to 21 days of age. Steroids were purchased from Schwarz/Mann, unlabeled nucleoside triphosphates from P.L. Biochemicals, a-amanitin and actinomycin D from Sigma, 1H1UTP from New England Nuclear, and poly[d(A-T)] from Miles Laboratories. All other reagents were the highest grade commercially available.

Animals were treated as indicated in individual experiments with either 2 µg of estradiol (estradiol-1,3,5(10)-triene-3,17β-diol), 2 µg of oestradiol (estradiol-1,3,5(10)-triene-3,16β,17α-triol) or saline at the indicated times before killing. Estradiol and estradiol were injected in a volume of 0.5 ml of 95% saline, 5% ethanol. All animals were killed by decapitation and the uteri were quickly stripped of adhering fat and mesentery, weighed, and placed in cold saline until processed. Unless otherwise stated all the following procedures were performed at 0°C.

**Isolation of Nuclei** – Nuclei were isolated as follows using a procedure similar to those described by Wray et al. (21) and Conn and O'Malley (22). The tissue was finely minced and homogenized in Buffer A (0.01 M 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid) (Hepes) buffer, 1 M hexylene glycol, and 10 µM CaCl, pH 7.0. The tissue was homogenized very gently by three strokes in a Polytron PT-10 homogenizer at a setting of 2 to 5. This was followed by several gentle strokes in a Teflon-glass homogenizer. Groups of 10 to 20 immature uteri were routinely homogenized in 8 to 10 ml of buffer. The remaining homogenate was filtered through four layers of cheesecloth, and a first filtrate was further filtered through nylon bolting cloth. The nuclei were pelleted by low speed centrifugation (800 × g) for 15 min, and washed twice with the Buffer A. The resultant low speed nuclear pellet was suspended in 2 to 3 ml of the buffer and layered over a 10-ml cushion of 2.1 M sucrose containing 0.05 M Tris (pH 7.5), 1 M MgCl, and 0.1 M dithiothreitol. The nuclei were pelleted by centrifugation in a Beckman SW 41 rotor for 60 min at 34,000 rpm. The resulting nuclear pellet was suspended in a small volume (0.5 to 1.0 ml) for a preparation from 10 to 20 immature uteri) of Buffer B (0.05 M Tris, 25% glycerol, 1 mM MgCl, 0.1 mM EDTA, and 0.1 mM dithiothreitol, pH 7.0), quick-frozen in ethanol/dry ice, and stored at −70°C until use. Protein content of the samples was measured by the method of Lowry et al. (20) after precipitation with trichloroacetic acid.

The samples were applied to DEAE-Sephadex columns at 1.25 to 1.50 mg of protein/ml of bed volume, and the columns were washed with 1 column volumes of Buffer B (pH 7.0) followed by 3 column volumes of Buffer C (pH 7.0). The columns were eluted with gradients of 50 to 600 mM (NH4)2SO, in Buffer C (4.5 column volumes) at a flow rate of 2 ml/h/ml of bed volume. Fractions of 5 to 10 ml were collected and assayed immediately for RNA polymerase activity using either native calf thymus DNA or poly[d(A-T)] as template. Assays were performed at 37°C for 20 min in a reaction mixture identical to that used for measurement of enzyme activity in nuclei (see above) except that 1 µCi instead of 2 µCi of [3H]UTP was used. One unit of activity is defined as the amount of enzyme which catalyzes the incorporation of 1 pmol of [3H]UMP under the above conditions.

**RESULTS**

Fig. 1 illustrates the profile observed when uterine RNA polymerases from mature animals are chromatographed on a DEAE-Sephadex column. For our initial studies we sought to determine the number of chromatographically distinct RNA polymerases present in uterine tissue and their characteristic sensitivities to inhibition by the toxin a-amanitin. Uteri from immature rats were thus used for these initial studies in order to obtain relatively large amounts of starting material.

The upper panel in Fig. 1 illustrates the profile observed when assays are performed with native calf thymus DNA as template. Three distinct peaks of activity (I, II, and III) are observed in the absence of a-amanitin (dashed line). In the presence of 0.4 µg/ml of a-amanitin (solid line) a fourth peak of activity (polymerase IIIA) is observed. As expected the activity of RNA polymerase II is essentially abolished by this concentration of a-amanitin while the activities of polymerases I and IIIB are essentially unchanged. RNA polymerases I, II, IIIA, and IIIB are eluted at salt concentrations of 0.15, 0.28, 0.34, and 0.42 M, respectively.

The lower panel of Fig. 1 represents the profile observed when the assays are performed with poly[d(A-T)] as a template. The profiles are essentially the same as in the upper panel, but the activities due to polymerases IIIA and IIIB are more easily visualized with the synthetic template, since the incorporation of [3H]UMP is increased. This increase in polymerase IIIA and IIIB activities with poly[d(A-T)] as template...
plate has also been noted in several other systems (24) if the poly[d(A-T)] template used is larger than 13 S.

It was of interest next to determine the sensitivity of each class of RNA polymerase to inhibition by the toxin a-amanitin. These experiments were performed by assaying the peak column fractions (I, II, IIIA, and IIIB) shown in Fig. 1 in the presence of varying concentrations of the toxin. The results of these studies are illustrated in Fig. 2.

The data in Fig. 2 indicate that the various polymerases have widely different sensitivities to the inhibitor. Polymerase II activity was clearly the most sensitive to inhibition; an a-amanitin concentration of 0.006 pg/ml produced a 50% inhibition of activity. Polymerases IIIA and IIIB showed identical sensitivities to the a-amanitin, and a 50% inhibition of activity was produced by a concentration of 18 pg/ml. Polymerase I activity was essentially unaffected by the highest concentrations of a-amanitin used.

The differential sensitivities of the solubilized, partially purified uterine RNA polymerases to a-amanitin suggested that polymerase I, II, and III activities could be measured in isolated uterine nuclei by the appropriate manipulation of a-amanitin concentrations in the assay system. To test this possibility nuclei were isolated from the uteri of immature female rats and [3H]UMP incorporation was measured as a function of a-amanitin concentration. To ensure that hormone treatment did not alter the sensitivity of the enzymes to a-amanitin, similar studies were performed with nuclei isolated from rats treated with estradiol (2 µg of 17β-estradiol administered 6 h prior to killing). The results of these studies are illustrated in Fig. 3.

It is seen in Fig. 3 that the sensitivities of the uterine polymerases measured in isolated nuclei are essentially identical in nuclei from both hormone-treated and saline-treated animals. In both cases there is an initial decrease in the activity as the a-amanitin concentration is raised to 0.03 pg/ml, and no further inhibition is noted until concentrations greater than 1 µg/ml are used. At higher concentrations there is a further decrease in activity and a second plateau is reached at concentrations greater than 100 µg/ml. As indicated in Fig. 3 (bottom curve) we attribute the initial loss of activity to the inhibition of RNA polymerase II, and the loss of activity at higher concentrations of a-amanitin to inhibition of RNA polymerase III. Since RNA polymerases IIIA and IIIB appear to have identical sensitivities to a-amanitin (Fig. 2), only the sum of these two activities can be measured in isolated nuclei. Activity which is resistant to the highest concentrations of a-amanitin used is attributed to RNA polymerase I.

Based upon the average of three separate titration curves similar to that shown in Fig. 3, it was determined that an a-amanitin concentration of 0.005 pg/ml produced a 50% inhibition of RNA polymerase II activity in isolated nuclei and a concentration of 15 µg/ml produced a 50% inhibition of RNA polymerase III activity. These values are in good agreement with the respective values of 0.006 and 18 µg/ml observed with the solubilized enzymes assayed with an exogenous template (Fig. 2).

Since the two sets of nuclei (SALINE and ESTRADIOL) used for these studies contained similar amounts of DNA, it appeared that estradiol treatment 6 h prior to killing had produced large increases in the activities of RNA polymerases I and III measured in the isolated nuclei. It was therefore

![Fig. 1. DEAE-Sephadex chromatography of uterine RNA polymerases. A sample (73 mg of protein) obtained by processing 4.1 g of uterine tissue was applied to a pre-equilibrated DEAE-Sephadex column (2.5 x 83 cm). The column was washed with 2 column volumes of Buffer C, containing 50 mM (NH4)2SO4, and eluted with 4 column volumes (160 ml) of a 50 to 600 mM (NH4)2SO4 gradient in Buffer C. The flow rate was 80 ml/h. Fractions of 5 ml were collected into tubes containing 2.6 mg of bovine serum albumin in 200 µl to make a final concentration of bovine serum albumin of 0.5 mg/ml in each fraction. Aliquots (25 µl) were assayed as described in the text at final (NH4)2SO4 concentrations in the assay which were 1/2 that depicted in the figure. The recovery from the column was greater than 90% for each class of RNA polymerase (I, II, III). The total units of RNA polymerases I, II, and III recovered were 3,110, 2,914, and 730, respectively, with native calf thymus (C.T) DNA as template, and 3,770, 10,245, and 4,630, respectively, with poly[d(A-T)] as template. The upper panel represents the activity profile with native calf thymus DNA as template, whereas the lower panel depicts the activity profile with poly[d(A-T)] as template. O - - O (-aA), without a-amanitin; •••• (+aA) with a-amanitin (0.5 µg/ml).

![Fig. 2. a-Amanitin sensitivities of solubilized rat uterine RNA polymerases. Peak fractions of RNA polymerases I, II, IIIA, and IIIB from a DEAE-Sephadex column were each assayed at 60 mM (NH4)2SO4 with native calf thymus DNA in the presence of increasing concentrations of a-amanitin: ▲, I; ●, aA, II; ■, ■, IIIA; and □, IIIB a-amanitin curves. The 100% levels were 3,200, 8,666, 2,231, and 3,013 cpm, respectively, for RNA polymerases I, II, IIIA, and IIIB.](http://www.jbc.org/)
of interest to study the time course of stimulation of these two enzymes following estradiol administration. These studies were of interest to determine the exact time course of enzyme stimulation and also to determine whether the time courses of polymerases I and III activation were similar or different. The results of these experiments are illustrated in Fig. 4.

For these studies, animals were treated with saline or estradiol (2 μg) and killed at the various times indicated in Fig. 4. Uterine nuclei were then prepared and assayed for RNA polymerases I, II, and III. The increases in uterine wet weight following hormone treatment are also included for comparative purposes. Increases in the activities of RNA polymerases I and III are noted between 1.5 and 3 h following hormone treatment, and further increases are noted between 3 and 6 h. Even though there are some differences in the magnitude of the increases in polymerases I and III and uterine wet weight (Fig. 4) the general profiles of the increases appear quite similar for all three parameters. We did not observe any increases in RNA polymerase II activity as a result of estradiol treatment during the period of time used in this work.

Since the activity of RNA polymerase measured in isolated nuclei depends on many variables, e.g. the number and properties of RNA polymerase molecules, chromatin template activity, stimulatory and inhibitory factors, the increases observed in polymerase I and III activities as a result of estradiol treatment could be due to a number of different factors. To gain further insights into the possible mechanisms responsible for the observed increases, we therefore sought to determine whether hormone treatment altered the chromatographic properties of uterine RNA polymerases and to determine whether changes in the amounts of solubilized, partially purified enzymes were observed after estradiol administration.

For these experiments groups of 50 immature female rats were treated with saline or estradiol (2 μg) and killed 6 h later. Uterine RNA polymerases were then solubilized by homogenizing uterine tissue directly in high salt (0.3 M (NH₄)₂SO₄) and sonicating the resultant homogenate. The solubilized enzymes were then chromatographed on DEAE-Sephadex. The results of such an experiment are shown in Fig. 5, the top panel illustrating the profile of polymerases from estradiol-treated animals and the bottom panel from saline-treated controls.

RNA polymerases I, II, IIIA, and IIIIB are observed in both profiles, and the enzymes elute at the same salt concentrations in both preparations. It is difficult to determine the effect of hormone treatment on polymerase levels directly from the data shown in Fig. 5 since estradiol treatment produced an increase in uterine weight and protein content which necessitated the use of different size columns and the collection of different size fractions for the two samples. When these factors are taken into account, the total units of each of the polymerases present in the two preparations can be determined. This data is presented in Table I.

The data in Table I illustrate that estradiol treatment does not produce any significant change in the total units of RNA polymerases I or II. This data suggests that the large increase...
The possible significance of these observations is discussed with a concomitant 50% reduction in RNA polymerase IIIA. Enzyme molecules. After hormone treatment is not due to an increased number of polymerases IIIA and IIIB. Thus estradiol treatment produces a 2-fold increase in the total units of RNA polymerase IIIB, RNA polymerase III. Estradiol treatment, however, does not lead to an increase in the total units of solubilized RNA polymerase IIIB. It was therefore of interest to determine the relationship of these relatively early changes in polymerase activities to subsequent physiological responses of the uterus, e.g., long term uterine growth and cell division. To approach this question we examined the effects of estradiol, another compound with estrogenic activity, on the activities of RNA polymerases in uterine nuclei. Estradiol was used since its long term effects on the uterus, such as increases in uterine dry weight (27, 28), protein and RNA content (14) and DNA synthesis (29, 30), are quite different than those produced by estradiol. Thus estradiol does not produce these long term (24 h) responses in the uterus as well as estradiol. A comparative study of the two compounds would therefore be expected to help elucidate the relationship between increases in RNA polymerase activities (occurring within 6 h after treatment) and subsequent tissue responses occurring at longer times.

The results of a series of experiments designed to test the relative effects of estradiol and estriol on uterine RNA polymerases (in isolated nuclei) 6 h after treatment are illustrated in Table II. As expected estradiol treatment produces large increases in the activities of RNA polymerases I and III. It is also seen in Table II that estriol produces essentially the same changes in RNA polymerase activities as estradiol. It should also be noted that estradiol and estriol produce similar increases in uterine wet weight (27, 28) and carbohydrate metabolism (30, 31) 6 h after administration to immature animals.

**DISCUSSION**

We have demonstrated that uterine nuclei contain two forms of RNA polymerase III, forms IIIA and IIIB. Our studies have indicated that these uterine enzymes are similar to RNA polymerase III from other eukaryotic systems. Weil and Blatti (9), for example, reported that HeLa cells contain two forms of RNA polymerase III which exhibit chromatographic properties and α-amanitin sensitivities similar to those we observed for the uterine enzymes. Several studies from Roeder's group (26, 32) have also described two forms of RNA polymerase III from several systems which are again similar to the uterine enzymes. In the various systems studied to date, RNA polymerase III accounts for 10 to 15% of the total RNA polymerase activity present in both nuclear and solubilized preparations (9, 24). This is also true for uterine RNA polymerase III measured in isolated nuclei from saline-treated as well as

**TABLE I**

| Enzyme | Enzyme units of poly[d(A-T)] |
|--------|-----------------------------|
|        | Control | Estradiol |
| I      | 4,250   | 4,680     |
| II     | 12,300  | 12,520    |
| III (total) | 4,510 | 4,980     |
| IIIB   | 3,290   | 1,530     |
| IIIA   | 1,230   | 2,560     |

* Two micrograms, 6 h prior to killing.

**TABLE II**

| Enzyme | Estradiol | Estriol |
|--------|-----------|---------|
| RNA polymerase I | 459 ± 25 | 535 ± 29 |
|               | (437–556) | (487–610) |
| RNA polymerase II | 86 ± 9  | 91 ± 5  |
|                | (71–106) | (81–104) |
| RNA polymerase III | 264 ± 38 | 294 ± 59 |
|                  | (193–367) | (174–457) |
Estradiol-treated animals. It is more difficult to compare relative amounts of RNA polymerase III to the total polymerase activity present in solubilized preparations because the measured activity is markedly dependent on the template used in the assays (see Fig. 1). Nevertheless, it is clear that the relative amounts and properties of uterine RNA polymerase III are quite similar to those in the above mentioned eukaryotic systems.

Our studies have also indicated that estrogenic stimulation increases the activity of RNA polymerase III in isolated nuclei. It is interesting to note that the time course of this stimulation is similar to that seen for RNA polymerase I (Fig. 4). This may suggest that the regulation of the enzyme responsible for producing rRNA (polymerase I) is coordinated with the regulation of the enzymes catalyzing the formation of transfer RNA and 5 S ribosomal RNA. In particular, several groups (19, 20, 33) have shown that the synthesis and methylation of uterine rRNA and tRNA increase dramatically following estradiol administration, and that the time course of the increases is similar to that seen in this work for RNA polymerases I and III. It thus seems quite likely that the increases in the activity of these enzymes are at least partially responsible for the observed increases in uterine RNA levels.

There have been few other studies of the regulation of RNA polymerase III, but Fuhrman and Gill (34) observed that adrenocorticotropic hormone stimulates adrenal RNA polymerase III and Jaehning et al. (35) demonstrated that lymphocyte RNA polymerase III levels are increased following mitogen stimulation. In the latter report, however, only solubilized polymerase III was measured. Webster and Hamilton (36) have also recently reported a stimulation of uterine RNA polymerase III in isolated uterine nuclei after estradiol treatment. In that report polymerase III activity was stimulated at 24 h, but not at 4 h after estradiol treatment. This apparent discrepancy with our results (3 to 6 h after hormone treatment) may be due to the fact that methods different than ours were used to measure RNA polymerase III in that work.

While our studies have indicated that uterine RNA polymerase III measured in isolated nuclei is stimulated by estradiol (Fig. 4 and Table II), we do not observe any increase in the total amount of solubilized polymerase III (Table I). This is similar to observations in other systems where RNA polymerase activity in isolated nuclei is stimulated, but the amount of solubilized enzyme activity is unaltered. For example, in rabbit uterine nuclei RNA polymerases I and II are stimulated by estrogen treatment without a concomitant increase in the amount of solubilized enzymes (13).

The lack of an increase in total units of solubilized RNA polymerase III relative to the observed increase in nuclear bound activity after hormone treatment could be accounted for in several ways. One likely explanation is that the template activity of uterine chromatin is increased following estradiol treatment. Such increases have in fact been observed by several workers (12, 37-39) who used Escherichia coli RNA polymerase to measure the template activity of uterine chromatin. The time course of increased template activity (12, 38) is quite similar to that observed in our work for increased activity of polymerases I and III in isolated nuclei. The presence of stimulatory factors in isolated nuclei which might be separated from the polymerases during the high salt extraction could also explain the increases in nuclear-bound RNA polymerases I and III without a concomitant increase in the amount of solubilized enzymes. Such a stimulatory factor for RNA polymerase I in the rabbit uterus has previously been reported (13).

With regard to RNA polymerase III another possible explanation for the increase in only the nuclear-bound enzyme might be that RNA polymerases IIIA and IIIB have different catalytic properties. Thus RNA polymerase IIIB, which is increased in solubilized preparations (see Table I), may be able to initiate transcription more efficiently or may elongate more rapidly than RNA polymerase IIIA. Since RNA polymerase IIIA is decreased in solubilized preparations, this possibility would then explain the lack of a major change in the total units of solubilized RNA polymerase III. Further studies are in progress to distinguish between the above possibilities, and to determine whether enzymes IIIA and IIIB are interconvertible.

At present there seems to be unanimous agreement that nuclear-bound RNA polymerase I activity is increased during the first 6 h following estradiol treatment (10, 12-14, 36) but there are conflicting reports concerning increases in RNA polymerase II activity. In both the rat uterus (12, 14) and rabbit uterus (13) an initial increase (within 30 min) in RNA polymerase II activity which subsequently declined to control levels (at 60 min) before a second increase (3 to 6 h) has been reported. Several other groups, however, have not observed increases in RNA polymerase II activity between 3 and 6 h after estradiol treatment (4, 10, 36). Similarly, we have not observed any increases in RNA polymerase II activity 3 to 6 h after estradiol treatment, the time period most extensively studied in this work. Since this work was aimed primarily at studies of uterine RNA polymerase III, we can offer no definitive explanation for these different observations. It is hoped, however, that further studies on the characteristics and properties of both solubilized (Figs. 1 and 5) and nuclear-bound RNA polymerase II may help to resolve this controversy.

The studies on the relative effects of estradiol and estriol (Table II) are of interest when considering the relationship between increases in RNA polymerases I and III activities (occurring within 6 h) and subsequent uterine responses occurring at later times. It is clear that both compounds produce essentially the same increases in enzyme activities (Table II) and yet it is known that the long term effects of these two compounds, occurring, for example, 24 h after treatment, are quite different (27-31). The studies of Clark and his colleagues (40) have shown that these differences can be explained by differences in the uterine retention of the two compounds and have emphasized that the overall uterine response to estrogens is more appropriately considered as a biphasic process rather than a simple cascade of events. Our results lend further support to this hypothesis since both compounds produce identical "early" effects on RNA polymerases I and III, and emphasize that increases in these enzyme activities may be necessary, but are not sufficient to ensure that a long term uterine response will occur.

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Stimulation of Uterine RNA Polymerase III by Estradiol

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