Synthesis of Ribonucleic Acid during Early Estrogen Action*

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SUMMARY

The data reported indicate that rat uterus nuclei possess the capacity to synthesize RNA in vivo and in vitro by mechanisms similar to those previously reported for rat liver nuclei. Tritiated uridine administered to the intact adult rat is rapidly taken up by the uterus and incorporated into nuclear RNA. Maximum specific activity of the nuclear RNA is reached at 20 min following administration of the radioisotope and decreases thereafter, while that of cytoplasmic RNA increases to a maximum at 6 to 12 hours, which indicates a turnover of RNA from the nucleus to the cytoplasm.

RNA polymerase (EC 2.7.7.6) in intact nuclei isolated from the uterus was studied in the presence of Mg2+ ions at low ionic strength or in the presence of Mn2+ ions and 0.4 M ammonium sulfate. Both RNA polymerase reactions are inhibited by actinomycin D, DNase, or RNase. Analyses of base composition and nearest neighbor base frequencies indicate that the product of the Mg2+-activated RNA polymerase reaction is similar to ribosomal RNA, whereas that of the Mn2+(NH4)2SO4-activated reaction is more DNA-like. Sucrose density gradient sedimentation indicates extensive degradation of the RNA synthesized during incubation in both conditions.

During the 3-week period following ovariectomy of the adult rat, there is a progressive decline in the rate of uterine RNA synthesis. An initial decrease in the rate of synthesis of nuclear RNA rapidly labeled in vivo is followed sequentially by decreases in the activities of Mg2+-activated and Mn2+-(NH4)2SO4-activated RNA polymerase measured in isolated nuclei. The concentration of whole tissue RNA or protein per mg of DNA in the organ decreases following ovariectomy, but the ratio of nuclear RNA or protein per mg of DNA appears to be unaltered. A single injection of 10 μg of estradiol-17β to the adult rat ovariectomized 3 weeks previously stimulates uterine RNA synthesis. Nuclear RNA synthesis in vivo is accelerated prior to sequential stimulations of the two RNA polymerase activities. Concentrations of total RNA and protein are restored to normal levels as a result of hormone treatment. At 20 min following hormone treatment, the rate of synthesis of nuclear RNA rapidly labeled in vivo is accelerated 500 to 600%. This acceleration is accompanied by an increased uptake of 3H-uridine by the organ. Whether the increase in specific activity is a cause or a consequence of the increased uptake is unknown.

Ovariectomy or administration of estradiol-17β influences neither the principal characteristics and kinetics of the RNA polymerase reactions nor the base composition and nearest neighbor base frequencies of their products.

The stimulatory effect of estrogen in vivo on RNA synthesis in uterine nuclei is inhibited 80 to 100% by prior treatment of the ovariectomized rat with actinomycin D or cycloheximide. Treatment or prior treatment of the immature rat with histamine, the histaminase mephyramine, or the histamine releaser p-methoxy-n-methylphenethylamine ("48-80") has no effect on estrogen-stimulated RNA synthesis in uterine nuclei. No stimulation of RNA polymerase activity has yet been obtained by the incubation in vitro of uterine nuclei with estradiol-17β.

It is concluded that an acceleration of the synthesis of all types of RNA, but particularly of that of ribosomal RNA and of ribosomes, is an essential feature of the early action of estrogen in the uterus.

There is a considerable body of evidence that the physiological effects of estrogen on the uterus are mediated by increases in the rates of RNA and protein synthesis (1–8). More recently, it has also been shown that stimulation of synthesis of RNA in the organ precedes that of protein during the first 2 hours after the administration of estrogen to the ovariectomized rat (9, 10).

In this paper we present the results of a detailed study of the early effects of estrogen on RNA synthesis in the rat uterus. We have investigated nuclear RNA synthesis in vivo and the activity of RNA polymerase in intact nuclei assayed in vitro, as a function of time after ovariectomy or after a single injection of the hormone to the ovariectomized rat. In addition, we have studied the effects on the system of inhibitors of RNA and protein synthesis, and of histamine, histamine releaser, or antihistamine. We conclude that one of the earliest effects of estrogen in the uterus is the stimulation of nuclear RNA synthesis in vivo,
and that the stimulation of synthesis of ribosomal RNA and of ribosomes is an essential feature of the early action of the hormone. This conclusion is in accordance with our previous demonstration (11) that the rate of nuclear RNA synthesis and its transport to the cytoplasm in the uterus is more rapid during the estrus than the diestrous phase of the estrous cycle, when endogenous titers of ovarian hormones are at possibly the lowest level of the cycle. A preliminary account of some of the findings now reported has been presented elsewhere (12).

**MATERIALS AND METHODS**

**Animals and Their Treatments**—Intact and ovariectomized adult Sprague-Dawley rats were obtained from the Hormone Assay Laboratories in Chicago, Illinois. All adult animals used weighed 175 to 180 g. Ovariectomized animals were used not less than 3 weeks following operation, except in experiments concerned with the time course of the effect of ovariectomy. In experiments with immature females, Wistar rats weighing 48 to 50 g were used throughout. In all experiments animals were given food and water *ad libitum*. Single doses of steroid hormones were administered intraperitoneally in 0.1 ml of 1,2-propanediol. Control animals received 0.1 ml of 1,2-propanediol without hormone. Radioisotopes, inhibitors of RNA and protein synthesis, and amine compounds were dissolved in 0.1 ml of 0.15 N NaCl and administered intraperitoneally.

**Tissue Homogenization and Isolation of Nuclei and Other Subcellular Fractions**—Animals were killed by cervical dislocation, and the uteri of 5 to 10 animals were pooled in ice-cold 0.32 M sucrose containing 3 mM MgCl₂ (homogenizing medium). All subsequent procedures were at 0–2°C unless otherwise stated. Nuclei were isolated as described elsewhere (13). The recovery of homogenate DNA in the isolated nuclei was 45 to 65%. Mitochondria, microsomes, and cell sap were separated by differential centrifugation of the whole tissue homogenate that remained after sedimentation of the nuclei (14).

**Labeling of Nuclear and Cytoplasmic RNA in Vivo**—For assay of specific activity of rapidly labeled nuclear RNA, animals were given 100 μC of ³H-uridine 10 min before they were killed. For labeling of the RNA of cytoplasmic fractions, animals were killed at longer intervals of time following radioisotope administration. RNA from the subcellular fractions was extracted, determined, and measured for radioactivity, as previously described (14).

**Determination of Uterine Uptake of ³H-Uridine**—Uptake of ³H-uridine by the uterus was determined as the total tissue radioactivity and expressed as counts per min per mg of DNA. To 0.1-ml aliquots each of the homogenate, 0.4 ml of 20% trichloracetic acid was added. The suspension was heated to 95°C for 15 min, cooled, and diluted by the addition of 15 ml of Bray's scintillation solution (15) for assay of radioactivity as counts were as follows: for ¹⁴C, 66%, 20 cpm; for ³H, 50%, 15 cpm.

**Measurement of Radioactivity**—RNA labeled with ¹³C or ³²P and deposited on filter paper, as described elsewhere (16), was measured for radioactivity in 15 ml of ice-cold 0.5 N HClO₄. The RNA of the resulting precipitate was extracted and measured for radioactivity by procedures previously described (16). Activity of the RNA polymerase was expressed as counts of ¹³C-ATP incorporated into RNA per min per mg of DNA. Each value for activity cited represents the average of triplicate or duplicate determinations for one concentration of nuclei in the incubation medium with a variation of 5% or less.

**Determination of Base Composition and Nearest Neighbor Base Frequencies of RNA Products of Polymerase Reaction**—After incubation of nuclei, RNA was extracted as previously described (16), precipitated, and washed twice with 0.3 N HClO₄ and once with absolute ethanol. The RNA was then hydrolyzed by treatment with 0.4 N KOH for 16 hours at 37°C. The RNA mononucleotides were separated by the procedure of Katz and Cumb (17). Modifications of the conditions of incubation in these experiments are described in Table II.

**Measurement of Radioactivity**—RNA labeled with ¹³C or ³²P and deposited on filter paper, as described elsewhere (16), was measured for radioactivity in 15 ml of scintillation fluid containing 0.5% of 2,5-diphenyloxazole (PPO) and 0.5% of 1,4-bis[2-(4-methyl-5-phenyloxazolyl)]benzene (dimethyl-POP) in redistilled toluene. RNA labeled with ³H-uridine and acid-insoluble and acid-soluble material containing ³H-uridine were measured for radioactivity in Bray's solution as noted above. All measurements were performed by use of a Packard Tri-Carb scintillation spectrometer. Counting efficiencies and background counts were as follows: for ¹³C, 66%, 20 cpm; for ³²P, 35%, 36 cpm; for ³H, 50%, 15 cpm.

**Chemical Determinations**—DNA and RNA were determined by the procedures of Burton (18) and Ceriotti (19), respectively, after extraction of nucleic acids by the procedure of Widnell and Tata (16). Calf thymus DNA and yeast RNA were used as standards. Protein determination was by the procedure of Lowry et al. (20), with crystalline bovine serum albumin used for standard.

**Materials**—Estradiol-17β, estrone, estriol, ATP, GTP, CTP, UTP, and calf thymus DNA were obtained from Sigma Chemical Company. Yeast RNA, phosphoenolpyruvate, and pyruvate kinase were obtained from Boehringer und Soehne GmbH.
Mannheim, Germany. Cytoheximide was obtained from Nutritional Biochemical Corporation. Actinomycin D was a gift from Merck, Sharp, and Dohme Company. Tritiated uridine (1.0 C per mmole) was from New England Nuclear Corporation, and 8-3H-ATP (30 mCi per mmole), 8-32P-ATP (25 mCi per mmole), 2-3H-CTP (20.4 mCi per mmole), and 2-32P-UTP (28 mCi per mmole) were obtained from Schwarz BioResearch, Inc. α-32P-

ATP was obtained from the International Chemical and Nuclear Corporation, City of Industry, California. Histamine, the antihistamine mepyramine (pyrilamine maleate), and “48-80” (p-methoxy-n-methylphenethylamine) were generously supplied by Professor W. Feldberg, National Institute for Medical Research, London, England.

RESULTS

Characteristics of Uptake of 3H-Uridine by Uterus and its Incorporation into RNA in Vivo—The time course of the uptake of 3H-uridine by the uterus of the intact adult rat and its incorporation into the RNA of the subcellular fractions are shown in Fig. 1 and Table I. Although the uptake of 3H-uridine and the percentage of acid-insoluble radioactivity reached maximum values at about 1 hour (Table I), the incorporation into cytoplasmic RNA increased until 12 hours (Fig. 1). The specific activity of nuclear RNA exhibited a rapid increase, which reached a maximum at 20 min, after which the activity declined. Mitochondrial, microsomal, and soluble RNA were not labeled during the 1st hour, and their specific activities only increased thereafter. Microsomal and soluble RNA were labeled before mitochondrial RNA, and reached maximum specific activities at 6 and 12 hours, respectively, whereas mitochondrial RNA reached a maximum specific activity at 12 hours. At 24 hours, the uptake of 3H-uridine and the specific activities of the RNA of the subcellular fractions were reduced. These results are in agreement with the evidence for transport of RNA from the nucleus to the cytoplasm in mammalian cells (compare, e.g., References 11 and 21–23).

Characteristics of RNA Polymerase in Isolated Uterine Nuclei—RNA polymerase activity in isolated preparations of uterine nuclei was studied either in the presence of Mg2+ ions or in the presence of Mn2+ ions and 0.4 M ammonium sulfate. The principal characteristics of the incorporation of 8-3H-ATP into RNA by such preparations were as previously reported (12). The reaction was dependent on the presence of all four nucleoside triphosphates and was inhibited by actinomycin D, DNase, or RNase. The kinetics of the reaction at low and high ionic strengths is shown in Fig. 2. The incorporation of 8-32P-ATP, 8-32P-GTP, 8-32P-CTP, and 8-32P-UTP into the RNA formed, together with the nearest neighbor base frequencies utilizing incorporated 8-32P-ATP, are shown in Table II for both polymerase reactions. The ratio of AMP + UMP : GMP + CMP for the RNA product of the Mg2+-activated RNA polymerase reaction was consistently found to be smaller ( <0.8) than that (>1.0) for the product of the Mn2+- (NH4)2SO4-activated RNA polymerase reaction. Results of sucrose density gradient analysis of the products of the two polymerase reactions indicated extensive degradation of the RNA formed during incubation.

These results are in good agreement with the previous studies of Widnell and Tata (16, 24) and those of MacGregor and Mahler (25) on RNA polymerase in rat liver nuclei, and are consistent with our conclusion that the RNA product of the reaction in the presence of Mg2+ ions is similar to ribosomal RNA, whereas the product of the reaction in the presence of Mn2+ ions and ammonium sulfate is more DNA-like.

Effect of Ovariectomy on RNA Synthesis and RNA Polymerase

The sequence of changes in the capacity of the uterus to synthesize RNA in vivo and its RNA polymerase activities assayed in vitro following ovariectomy of the adult rat are shown in Fig. 3 and Table III. The most immediate effect was on the specific activity of nuclear RNA, measured after a 10-

![Fig. 1. Time course of incorporation of 3H uridine into uterine RNA in the intact adult rat. All animals received intraperitoneally 100 µCi of 3H-uridine at time zero. Animals were killed (three to five per group) at the times indicated, and the uteri were excised, weighed, and pooled. The whole tissue homogenate was prepared, the subcellular fractions were isolated, and the RNA was extracted for determination and for measurement of radioactivity, as described under "Materials and Methods." Aliquots of the homogenates were collected, quickly frozen, and stored for determination and for measurement of radioactivity, as described in the text. O-0, nuclear RNA; O-0, mitochondrial RNA; O-0, microsomal RNA; Δ-Δ, cell sap RNA.](http://www.jbc.org/content/243/2/410.full)
FIG. 2. Time course for the two RNA polymerase reactions in nuclei isolated from the uterus of the intact adult rat. Whole nuclei were incubated in the presence of Mg\(^{2+}\) ions and the absence of ammonium sulfate, or in the presence of Mn\(^{2+}\) ions with the salt at a concentration of 0.4 M. For assay of the activity at high ionic strength, the \(^{14}C\)-ATP was added to the reaction mixture 15 min after beginning of incubation. Activity was measured as counts per min of \(^{14}C\)-ATP incorporated into RNA per mg of DNA. The other details concerning constituents of the reaction mixtures and conditions of assay were as described under "Materials and Methods." O—O, Mg\(^{2+}\)-activated RNA polymerase; O—O, Mn\(^{2+}\)-(NH\(_4\))\(_2\)SO\(_4\)-activated RNA polymerase.

**TABLE II**
Analysis of base composition and nearest neighbor base frequency of product of RNA polymerase in isolated uterine nuclei

For each experiment, nuclei were isolated from uteri pooled from 10 intact adult rats. For analysis of base composition, all four nucleoside triphosphates were \(^{14}C\)-labeled and 0.3 \(\mu\)mole of each was added to the reaction mixture. The concentration of each nucleoside monophosphate in the RNA product was calculated (24) by dividing the radioactivity recovered in each by the specific activity (see "Materials and Methods") of the corresponding nucleoside triphosphate used. For the reaction in the presence of ammonium sulfate, the radioactive nucleoside triphosphates were added after the 15 min of prior incubation. For analysis of nearest neighbor base frequency, 0.06 \(\mu\)mole of \(^{32}P\)-labeled reaction was added to the reaction mixture containing 0.3 \(\mu\)mole each of the nonradioactive nucleoside triphosphates. For the Mn\(^{2+}\)-(NH\(_4\))\(_2\)SO\(_4\)-activated reaction, the \(^{32}P\)-ATP was added after 15 min of prior incubation of the reaction mixture containing 0.3 \(\mu\)mole each of the nonradioactive nucleoside triphosphates. Conditions of incubation and other experimental details were as already described.

**TABLE III**
Time course for effect of ovariectomy on uptake of \(^3H\)-uridine and its incorporation into acid-insoluble material by uterus of adult rat

As described in Fig. 3, adult rats ovariectomized for the number of days indicated were given 100 \(\mu\)Ci of \(^3H\)-uridine 10 min before death. Uteri from groups of animals were pooled and used for preparation of the whole tissue homogenate. Aliquots of the homogenate were used for determination of DNA and of total tissue, acid-insoluble, and acid-soluble radioactivity, as already described. Total tissue radioactivity was expressed as counts per min per mg of DNA in the homogenate. The values given here and in Tables V through VII for uterine weights are averages, with the range above and below the average being indicated.

<table>
<thead>
<tr>
<th>Time after ovariectomy</th>
<th>Radioactivity</th>
<th>Uterine weight</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total tissue</td>
<td>Acid-insoluble</td>
</tr>
<tr>
<td>0 (intact animals)</td>
<td>63,813</td>
<td>48</td>
</tr>
<tr>
<td>3</td>
<td>49,375</td>
<td>53</td>
</tr>
<tr>
<td>7</td>
<td>41,536</td>
<td>44</td>
</tr>
<tr>
<td>10</td>
<td>38,725</td>
<td>34</td>
</tr>
<tr>
<td>14</td>
<td>34,118</td>
<td>29</td>
</tr>
<tr>
<td>21</td>
<td>25,150</td>
<td>18</td>
</tr>
</tbody>
</table>

min pulse of \(^3H\)-uridine in vivo. One week after ovariectomy, this activity had decreased by more than 50%. The decline in the specific activity of the Mg\(^{2+}\)-activated RNA polymerase reaction was similar to the decline in uterine uptake of \(^3H\)-uridine.
in vivo, whereas the decrease in the activity of the Mn$^{2+}$-$(NH_4)_2$SO$_4$-activated RNA polymerase reaction was observed still later, and roughly paralleled the decrease in the wet weight of the uterus. At 21 days after ovariectomy, all these activities had reached their lowest values.

**Effect of Single Injection of Estradiol-17β on RNA Synthesis in Vivo**—The RNA:DNA and protein to DNA ratios of uteri from ovariectomized rats were found to be about 30 to 40% of the values observed for intact animals (Table IV). Following a single injection of 10 μg of estradiol-17β, no change was observed in the RNA:DNA or protein to DNA ratio of the tissue until after 2 hours. The maximum increase in the content of both these constituents was observed between 12 and 24 hours after hormone administration (Table IV). No marked change in the RNA:DNA or protein to DNA ratio was apparent in the nuclei isolated at any of the time intervals examined. The characteristics of incorporation of $^3$H-uridine into RNA of uterus from ovariectomized rats were essentially the same as described above for normal rats, except that the uptake of the radioisotope and the percentage of acid-insoluble radioactivity were both markedly decreased (Fig. 4 and Table IV). The general effect of the administration of estradiol-17β was to cause a pronounced increase in the specific activity of nuclear RNA. This activity decreased at longer intervals of time after injection of the hormone, but remained higher than the control at all time intervals examined. The results obtained for total tissue radioactivity and its acid-soluble fraction were consistent with the changes in specific activity of nuclear RNA. Estradiol and estrone were found to be less effective than estradiol-17β in stimulating RNA synthesis and uterine uptake of $^3$H-uridine in vivo. These experiments will be reported elsewhere.

**Effect of Single Injection of Estradiol-17β on RNA Polymerase in Isolated Uterine Nuclei**—The time course of the stimulation of RNA polymerase, in preparations of isolated nuclei following a single injection of estradiol-17β to ovariectomized rats, is shown.

**TABLE IV**

<table>
<thead>
<tr>
<th>Time after administration of estradiol-17β</th>
<th>DNA per uterus</th>
<th>Nuclei</th>
<th>Whole tissue homogenate</th>
</tr>
</thead>
<tbody>
<tr>
<td>mg</td>
<td>DNA</td>
<td>Protein: DNA</td>
<td>DNA</td>
</tr>
<tr>
<td>0 (controls)</td>
<td>0.93</td>
<td>0.26</td>
<td>2.12</td>
</tr>
<tr>
<td>20 min</td>
<td>0.91</td>
<td>0.30</td>
<td>2.00</td>
</tr>
<tr>
<td>40 min</td>
<td>0.96</td>
<td>0.23</td>
<td>2.28</td>
</tr>
<tr>
<td>2 hrs</td>
<td>0.05</td>
<td>0.29</td>
<td>2.28</td>
</tr>
<tr>
<td>12 hrs</td>
<td>0.05</td>
<td>0.30</td>
<td>2.64</td>
</tr>
<tr>
<td>24 hrs</td>
<td>1.14</td>
<td>0.32</td>
<td>2.79</td>
</tr>
<tr>
<td>0 (intact animals)</td>
<td>1.27</td>
<td>0.34</td>
<td>3.30</td>
</tr>
</tbody>
</table>

**FIG. 4.** Effect of a single dose of estradiol-17β on the incorporation of $^3$H-uridine into RNA in the uterus of the ovariectomized adult rat. All animals were given $100 \mu$g of $^3$H-uridine (A) or $100 \mu$g of the radioisotope and 10 μg of estradiol-17β (B) at time zero. At the times indicated, animals were killed (three to five per group), and the uteri were excised and pooled, and the subcellular fractions were isolated. Determination of radioactivity in the whole tissue homogenate (see Table V) and of specific activity of the RNA extracted from the subcellular fractions was as described under "Materials and Methods." •••, nuclear RNA; ○○○, mitochondrial RNA; □□□, microsomal RNA; △△△, cell sap RNA.
in Fig. 6. At 1 hour there was a reproducible increase in the activity of the reaction assayed in the presence of Mg\(^{2+}\) ions, as reported by Gorski (5). The peak stimulation, which essentially corresponded to the values obtained from the normal controls, was observed at 12 hours. No change was detected in the activity of the reaction assayed in the presence of Mn\(^{2+}\) ions and ammonium sulfate until about 12 hours, after which a small stimulation of 50 to 60% was observed until 24 hours. Essentially the same pattern of differential stimulation of the two polymerase reactions was also observed in uterine nuclei from immature rats treated with 5 \(\mu\)g of the hormone.

As was found for the actions of thyroid and growth hormone on the liver (14, 34), no change could be detected in the kinetics

![Graph](http://www.jbc.org/)

**Fig. 5.** Time course for the effect of a single dose of estradiol-17\(\beta\) on the incorporation of \(^{3}H\)-uridine into rapidly labeled nuclear RNA in the uterus of the ovariectomized adult rat. All animals were given 10 \(\mu\)g of the hormone at time zero and 100 \(\mu\)C of the radioisotope 10 min before they were killed. At the times indicated, animals were killed (three to five per group), and the uteri were excised and pooled for homogenization. Determination of total tissue and acid-soluble radioactivity in the homogenate and of specific activity of RNA in the nuclei isolated was as already described. 

- , rapidly labeled nuclear RNA; , total tissue radioactivity; , acid-soluble radioactivity.

![Graph](http://www.jbc.org/)

**Fig. 6.** Time course for the effect of a single dose of estradiol-17\(\beta\) on RNA polymerase in isolated nuclei from the uterus of the ovariectomized adult rat. All animals were given 10 \(\mu\)g of estradiol-17\(\beta\) at time zero. At the times indicated, the animals were killed (six to eight per group), and the uteri were excised and pooled for homogenization. The nuclei were isolated and assayed for RNA polymerase activity in the presence of Mg\(^{2+}\) ions and the absence of ammonium sulfate, or in the presence of Mn\(^{2+}\) ions and the salt at a concentration of 0.4 M. The other experimental details and the expression of enzyme activity were as already described. 

- , Mg\(^{2+}\)-activated RNA polymerase; , Mn\(^{2+}\)-\((NH_4)_2SO_4\)-activated RNA polymerase.

**Fig. 7.** Dose-response curve for the effect of estradiol-17\(\beta\) on RNA polymerase in isolated nuclei. Ovariectomized adult rats were given the dose of estradiol 17\(\beta\) indicated. Two hours later the animals were killed (six to eight per group), and the uteri were excised and pooled for homogenization. The nuclei were isolated and assayed for Mg\(^{2+}\)-activated RNA polymerase as already described.

**Fig. 8.** Effect of actinomycin D or cycloheximide on estrogen-stimulated nuclear RNA synthesis in the uterus of the ovariectomized adult rat. In experiments concerned with specific activity of rapidly labeled nuclear RNA formed in \(vivo\), animals were treated for 1 hour with 200 \(\mu\)g of actinomycin D or 1 mg of cycloheximide, and then treated for 20 min with 10 \(\mu\)g of estradiol 17\(\beta\) or with the hormone carrier only. All animals were given 100 \(\mu\)C of \(^{3}H\)-uridine 10 min before they were killed. In experiments concerned with activity of RNA polymerase assayed in isolated nuclei, animals were treated with the inhibitors for 1 hour, and then treated with hormone or with the hormone carrier only for 2 hours before death. In both groups of experiments, control animals were correspondingly pretreated with inhibitor and hormone carrier only. Preparation of the uterine homogenates, determination of specific activity of nuclear RNA rapidly labeled in \(vivo\), and assay of Mg\(^{2+}\)-activated RNA polymerase in \(vivo\) were as already described. The data are representative of replicate experiments and are presented as percentage of control values which were: specific activity of nuclear RNA, 2156 cpm per mg of RNA; activity of RNA polymerase, 2160 cpm of \(^{14}C\)-ATP incorporated into RNA per mg of DNA; uterine weight for animals used in the polymerase experiments, 71 ± 3 mg, wet weight. The uterine weights for animals used in the experiments concerned with RNA labeled \(in vivo\) are given in Table VI.
or principal characteristics of either RNA polymerase reaction. In addition, no change could be detected, either as a result of ovariectomy or following estrogen administration, in the base composition or nearest neighbor base frequency of the RNA product. Furthermore, no effect of estradiol-17β was observed in vivo, even if the nuclear preparations were first preincubated with the hormone.

The dose-response curve for the effect on Mg²⁺-activated RNA polymerase, measured 2 hours after a single injection of estradiol-17β to ovariectomized rats, is shown in Fig. 7. The maximum response was observed with doses of either 1 or 10 μg. A larger dose of 100 μg, which is presumably nonphysiological, resulted in a lower stimulation. Estradiol and estrone, at a dose level of 100 μg, were observed to stimulate RNA polymerase. Neither, however, was as effective as estradiol-17β at the dose level of 1 to 10 μg.

**Effect of Inhibitors of RNA and Protein Synthesis**—Previous investigations have shown that actinomycin D and cycloheximide or puromycin are, respectively, effective inhibitors of estrogen-stimulated synthesis of RNA and protein in the uterus of immature or ovariectomized rats (1-5, 26), or of animals both adrenalectomized and ovariectomized (27). These studies primarily concerned the determination of specific activities of total RNA or protein extracted 4 hours following hormone treatment, while the effects of the inhibitors on uterine uptake of the radioactive precursors used were not examined. Fig. 8 shows the effects of actinomycin D and cycloheximide in vivo on estrogen-stimulated RNA synthesis in the uterus of the ovariectomized rat. The effects were determined both for rapidly labeled nuclear RNA synthesized in vivo and for Mg²⁺-activated RNA polymerase assayed in vitro. Actinomycin D almost completely blocked the effect of estrogen on RNA synthesis in vivo, 20 min after hormone treatment. Cycloheximide blocked about 80% of the estrogen-stimulated RNA synthesis in vivo, provided the stimulatory effect of cycloheximide alone was discounted. Both inhibitors blocked the effect of estrogen on RNA polymerase by 80 to 90% in nuclei isolated 2 hours after hormone treatment. In general, the effect of the inhibitors on uterine uptake of ³H-uridine and its incorporation into acid-insoluble material paralleled the effect on specific activity of nuclear RNA (Table VI).

<table>
<thead>
<tr>
<th>Treatment of animals</th>
<th>Radioactivity</th>
<th>Activity of Mg²⁺-activated RNA polymerase reaction in vitro</th>
<th>Uterine weight</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>cpm</td>
<td>mg, wet wt</td>
<td>mol, wet wt</td>
</tr>
<tr>
<td>Control</td>
<td>18,190</td>
<td>14</td>
<td>86</td>
</tr>
<tr>
<td>Estradiol-17β</td>
<td>22,260</td>
<td>21</td>
<td>79</td>
</tr>
<tr>
<td>Actinomycin D</td>
<td>8,870</td>
<td>8</td>
<td>92</td>
</tr>
<tr>
<td>Actinomycin D; estradiol-17β</td>
<td>10,390</td>
<td>9</td>
<td>91</td>
</tr>
<tr>
<td>Cycloheximide</td>
<td>12,040</td>
<td>18</td>
<td>82</td>
</tr>
<tr>
<td>Cycloheximide; estradiol-17β</td>
<td>14,220</td>
<td>21</td>
<td>70</td>
</tr>
</tbody>
</table>

**Effect of histamine, antihistamine, or histamine releaser on estrogen-stimulated nuclear RNA synthesis**

Immature rats were treated as designated with the hormone or the amine, or both. Uteri from the groups of animals (8 to 10 per group) were pooled and homogenized. The nuclei were isolated for extraction of nuclear RNA or for assay of RNA polymerase. In experiments concerned with effects on synthesis of rapidly labeled nuclear RNA in vivo (A), animals were given amine, 2 hours, and hormone, 20 min before they were killed. All animals were given 25 μC of ³H-uridine 10 min before death. In experiments concerned with effects on RNA polymerase (B), animals were given amine or hormone, or both, 2 hours before they were killed. The control values cited are representative of seven replicate experiments. The remaining experimental details were as described under “Materials and Methods.”
of shock when histamine was coadministered. Prior treatment of animals with any of the compounds failed to alter in any detectable manner the specific activity of rapidly labeled nuclear RNA synthesized in vivo, or the response of the system to estradiol-17β 20 min after hormone treatment. None of the compounds significantly increased or decreased the activity of Mn²⁺-activated RNA polymerase in uteri nuclei isolated 2 hours after administration. Furthermore, none of the compounds prevented estradiol-17β from increasing the activity of RNA polymerase in uteri nuclei assayed 2 hours after coadministration with estradiol-17β. That both histamine and "48-80" were affecting the uterus in these experiments was indicated by the increase in weight of the organ, probably due to water imbition.

**DISCUSSION**

In Fig. 9 are summarized the sequential stimulations or accumulations of nuclear and cytoplasmic RNA and protein that we have observed as a function of time following administration of a single dose of estrogen to the ovariectomized adult rat. At 20 min after estrogen administration, the rate of nuclear RNA synthesis in vivo was maximal, or 500 to 600% of control (Fig. 5). This is possibly the earliest biologically significant response of the uterus to a single dose of estrogen. Means and Hamilton (10) have further shown that the increase in specific activity of nuclear RNA was 40% over control as early as 2 min following hormone administration. At 1.3 hours, the Mn²⁺- activated RNA polymerase reaction was stimulated 100%, and at 12 hours the RNA polymerase reaction, in nuclei incubated in the presence of Mn²⁺-ions and ammonium sulfate, was stimulated 50 to 60% (Fig. 6). Similar dissociations of the stimulation of RNA polymerase activities in nuclei incubated in the presence or absence of ammonium sulfate have been reported for estrogen (5), testosterone (33), growth hormone (33-35), and thyroid hormone (14). The first appearance of newly formed cytoplasmic RNA was detected in the microsomal fraction between 1.3 and 4 hours after 3H-uridine administration. Specific activities of RNA of mitochondrial and cell sap fractions also increased between 2 and 4 hours after the hormone was given (Fig. 4). That the specific activity of nuclear RNA rapidly labeled in vivo decreased between 20 min and 1.3 hours, whereas that of mitochondrial RNA was increasing, suggests the transfer of a part of the newly synthesized RNA from the nucleus to the cytoplasm. This is in accordance with the increased accumulation of whole tissue RNA and protein between 2 and 24 hours without any detectable increase in RNA:DNA and protein to DNA ratios of the nuclei (Table IV). Finally it is important to note that the relatively smaller but delayed enhancement of the Mn²⁺-(NH₄)₂SO₄-activated RNA polymerase reaction (Fig. 6) was only detected after the cytoplasmic concentration of ribosomes was already rapidly increasing (32).

The effects of estrogen on RNA polymerase in intact nuclei isolated from the uterus, described here and elsewhere (11), correlate approximately with those described (36, 37) for effects of the hormone in vivo on the activity of isolated chromatin preparations from the organism.

Actinomycin D has been found to be a most effective inhibitor of ribosomal as well as of messenger RNA synthesis (38, 39). That prior treatment of ovariectomized animals with this inhibitor blocks estrogen-stimulated RNA synthesis in the uterus (Fig. 8) is therefore not surprising, but points to the importance of RNA synthesis during the early action of the hormone (40, 41). The stimulation by estrogen of RNA synthesis in uteri of immature animals treated with antihistamine and the failure of either histamine or histamine releaser to mimic such a stimulation of RNA synthesis (Table VII) indicate, at the minimum, that the differential effects on the rate of RNA synthesis described are specific hormone-induced responses.

A particular feature of our observations on the early effects of estrogen on nuclear RNA metabolism in the uterus is that the uptake of the organ of 3H-uridine increased concomitantly with the specific activity of nuclear RNA (Fig. 5, Table V). Such an increase in the uptake of the isotope was accompanied by a drop in the proportion of radioactivity which is acid-soluble (Fig. 5). Our data provide no information on whether the rise in total tissue radioactivity was a cause or consequence of the increase in specific activity of nuclear RNA. It is interesting that cycloheximide, a potent inhibitor of protein synthesis (26, 42), decreased in control animals the uptake of 3H-uridine while simultaneously causing a stimulation of the incorporation of the isotope into nuclear RNA (Table VI, Fig. 8). A similar observation has been made previously and its significance is also unknown (1-4, 9).

A remarkable parallel exists between the sequence of nuclear RNA synthesis observed during the early action of estrogen in the uterus and the sequence reported (14) for thyroid hormone acting in the liver. The major difference between the two sets of findings is the more protracted or delayed response of the liver nuclei to hormonal stimulation. In both cases there is a striking similarity in the sequential stimulations of rapidly labeled nuclear RNA synthesis in vivo and RNA polymerase assay in vitro. Much of the nuclear RNA, the synthesis of which was initially stimulated by either hormone, resembles
ribosomal RNA, whether determined by sucrose density gradient sedimentation or by base composition analysis. Furthermore, in the case of growth hormone (41, 43) and thyroid hormone acting in liver (14), testosterone in seminal vesicles (44), and estrogen in the uterus (10, 45, 46), it was observed that enhancement of cytoplasmic protein synthesis occurred after a preceding stimulation of nuclear RNA synthesis and was accompanied by the accelerated entry into the cytoplasm of new ribosomes (47).

Two lines of evidence suggest that the ribosomal RNA synthesis, initially stimulated in the nucleus by estrogen, is nucleolar in origin. Firstly, in a variety of multicellular systems the major site of ribosomal RNA synthesis has been found to be the nucleoli and its associated chromatin (48–51). In addition, regulation of nucleolar synthesis of ribosomal RNA has been implicated in the actions of androgen and estrogen in tissues sensitive to the hormones (52, 53). Secondly, recent investigations by high resolution autoradiography indicate that the Mg²⁺-dependent RNA polymerase reaction largely reflects nucleolar RNA synthesis, whereas the activation of the reaction by ammonium sulfate was found to be the nucleoli and its associated chromatin (48–51). Electron microscopic studies (56) also indicate that at 4 hours after estrogen administration the uterine nucleoli are enlarged. At 12 hours, in correlation with an increase in the concentration of ribonucleoprotein particles in the cytoplasm, the nucleoli were still larger and were now more adjacent to the nuclear membrane. It is important to note that the Mg²⁺-(NH₄)₂SO₄-activated RNA polymerase reaction, the product of which is a more DNA-like RNA, was found to be stimulated only at this latter time interval (Fig. 6). Whether this is a reflection of an amplification of chromosomal potential for messenger RNA synthesis in a relatively delayed response to the hormone remains to be determined.

We conclude that regulation of synthesis of ribosomal RNA is a major aspect of the early action of the hormone in the uterus, and that the transport of at least part of this RNA from the nucleus to the cytoplasm is also accelerated. The turnover of RNA to the cytoplasm probably occurs in conjunction with the formation of ribosomes or of ribosomal components, a process which may be the rate-limiting step in the growth response of the uterus to estrogen (8, 11). Our conclusion is in general agreement with recent studies which emphasize the importance of nuclear synthesis of ribosomal RNA in the early action of a variety of growth-promoting and developmental hormones in their characteristic target organs (for reviews see References 40 and 41). Furthermore, our conclusion is compatible with recent evidence that the formation of ribosomes or their components must be continually occurring for the transport of messenger RNA to the cytoplasm (21–23), which would determine the specificity of the hormone action (8, 57).

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REFERENCES
