*TEMPLATE CAPACITY OF UTERINE CHROMATIN: CONTROL BY ESTRADIOL**

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The uterine response of the ovariectomized rat to a single injection of estradiol-17β is characterized by a rapid increase in RNA and protein synthesis.[1-4] The *in vitro* synthesis of RNA by isolated, whole uterine nuclei is clearly elevated 1 hr after estrogen treatment of the intact immature rat.[5] Whether the site of estrogen activation in this system involves RNA polymerase, inactivation of repressors,[6] nuclear membrane permeability, or chromatin template capacity, is not known. It has been demonstrated that one steroid hormone, hydrocortisone, alters the template activity of chromatin purified from a target organ.[7] It will be shown below that estradiol-17β administered *in vivo* is capable of stimulating the capacity of purified uterine chromatin to act as a template for DNA-dependent RNA polymerase.

Materials and Methods.—Treatment of rats: Holtzman-strain female rats, weighing 180–200 gm, were ovariectomized by the dorsal approach under ether anesthesia 4 weeks prior to use. The animals were killed by cervical fracture 2–12 hr after a tail-vein injection of 5.0 μg of estradiol-17β (Sigma, grade A) in 0.5 ml of 5% ethanol in isotonic saline. Control animals received only the vehicle prior to their sacrifice. Uteri from three or four similarly treated rats were trimmed and pooled for each chromatin preparation.

Preparation of purified chromatin: Chromatin was prepared from each set of uteri immediately after removal by the procedures of Marushige and Bonner[8] with modifications to allow preparation from 300 to 600 mg of tissue. The tissue was homogenized in 30 ml of saline-EDTA (0.05 *M* NaCl and 0.016 *M* Na_2EDTA, pH 8.0) and 0.15 ml of 2-octanol in a VirTis model 23 homogenizer for 1 min at 110 v and 4 min at 70 v. The homogenate was strained through two layers of Miracloth (Chicopee Mfg. Co., Milltown, N.J.) and centrifuged at 1500 *g* for 15 min. The sediment was washed with 12 ml of saline-EDTA and 12 ml of tris buffer (0.05 *M*, pH 8.0), followed each time by centrifugation at 1500 *g* for 15 min. The sediment was suspended two times in 5 ml of Tris buffer (0.05 *M*, pH 8.0) by aspiration through a small-bore pipette, and centrifuged at 10,000 *g* for 15 min. The pellet was taken up in 5 ml of tris buffer (0.05 *M*, pH 8.0), stirred for 30 min, and layered over 25 ml of 1.7 *M* sucrose in tris buffer (0.01 *M*, pH 8.0). The upper two thirds was gently mixed and the preparation centrifuged at 30,000 *g* for 2 hr. The gel-like pellet was washed two times in 10 ml of tris buffer (0.01 *M*, pH 8.0) and centrifuged after each wash at 30,000 *g* for 30 min. The final pellet was suspended in Tris buffer (0.01 *M*, pH 8.0). DNA content was determined and the chromatin was assayed immediately for template capacity. All preparative steps were carried out at 0–4°C.

Assay of template capacity: The reaction mixture for RNA synthesis was in accord with the conditions specified by Bonner and Huang[9] and contained in a final volume of 0.25 ml: 10 μmoles tris buffer (pH 8.0), 1.0 μmole $MgCl_2$, 0.25 μmole $MnCl_2$, 3.0 μmoles β-mercaptoethanol, 0.1 μmole each of CTP, UTP, and GTP, 0.1 μmole of 8-C^{14}-ATP (1000 cpm/mμmole), chromatin or DNA (amount as indicated in legends), and purified DNA-dependent RNA polymerase. The enzyme was purified from early log phase cells of *E. coli* strain B (General Biochemicals) by the method of Chamberlin and Berg[10] to the stage of their fraction 3. Following incubation at 37°C for 10 min, the reaction was stopped by the addition of cold 6% trichloroacetic acid (TCA). Acid-insoluble material was collected on membrane filters (Schleicher and Schuell, B-6) and washed seven times with 3-ml portions of cold 3% TCA. The filters were dried, glued to aluminum planchets, and counted in a Nuclear-Chicago windowless gas flow counter. Sufficient counts were collected to give a counting error not exceeding 2%.

DNA determinations: An aliquot of purified chromatin was hydrolyzed in 0.5 *M* perchloric acid at 70°C for 15 min. The hydrolysate was cooled and centrifuged at 2000 *g* for 15 min. The optical density of the supernatant was measured at 260 mμ, corrected for light scattering as measured at 320 mμ, and DNA concentrations were obtained by comparison with a standard DNA solution. The concentration of DNA in the preparation was verified the following day using the diphenylamine reaction, and was always within 5% of the amount determined by the ultraviolet absorption method.

Chemical composition: DNA was determined by the diphenylamine reaction of Burton[11] using salmon-sperm DNA (Calbiochem Co.) as a standard, and RNA was determined by the orcinol reaction by the method of Dische and Schwarz[12] using purified yeast RNA (Calbiochem) as a standard. Histone and nonhistone proteins were separated by the method of Marushige and Bonner,[8] and their amounts were determined by the method of Lowry *et al.*[13] using purified rat liver histone and bovine serum albumin (Sigma) as a standard.

Ribonuclease assay: Ribonuclease was assayed as described by Dahmus and Bonner[7] using C^{14}-labeled RNA (33,000 cpm/mg RNA). Chromatin containing 12 μg of DNA was incubated with 30 μg of the C^{14}-labeled RNA for 10 min at 37°C. The reaction was stopped by adding a known volume of cold 6% TCA, and the reaction mixture was passed through a membrane filter. An aliquot of the filtrate was plated on an aluminum planchet, dried, and counted in a windowless gas flow counter.

Results.—Purified chromatin was prepared from whole uteri of ovariectomized rats 12 hr after a single intravenous injection of 5.0 μg of estradiol-17β. Similar treatment has been shown to induce a twofold increase in the total uterine activities of glucose-6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase by the twelfth hour of stimulation.[14] A comparison of the template capacity of purified chromatin from control and estrogen-primed uteri is presented in Figure 1. These data indicate that in the presence of added DNA-dependent RNA polymerase, the capacity of purified chromatin (12 μg DNA) from the uteri of estrogen-treated animals to act as a template for RNA synthesis is about 70–80 per cent greater than that from control uteri. The addition of estradiol-17β (3 μM) to the RNA synthesis assay did not affect the template capacity of uterine chromatin from the nontreated animals. A twofold increase in the concentration of the ribonucleoside triphosphates had no effect on the observed template capacity of uterine chromatin, and doubling the amount of RNA polymerase doubled the rate of incorporation of ATP into the TCA-insoluble precipitate.

Ribonuclease activity of purified chromatin prepared from both estrogen-primed and control uteri was found to be negligible (<0.03 % of the added RNA per 10 min when assayed as described in *Methods*) in both preparations. The purified chromatin from both control and estrogen-primed uteri was essentially free of endogenous RNA polymerase activity (see Fig. 2).

The chemical compositions of uterine chromatin from control and 12-hr estradiol-17β-treated animals are presented in Table 1. There is no appreciable difference in the amount of the various components between the two preparations.

The template capacity of uterine chromatin has been found to be significantly elevated at the second hour following estradiol-17β administration (Fig. 2). The template capacities observed at 2, 4, and 12 hr after estradiol injection were significantly greater than control values, as measured by the *t* test, at $P < 0.05$, $P < 0.01$, and $P < 0.001$, respectively.

Table 2 indicates that the estrogen-induced increase in template capacity of chromatin is specific for uterus. Estrogen treatment did not simulate the template capacity of either liver or lung chromatin. It is also apparent that the unstimulated

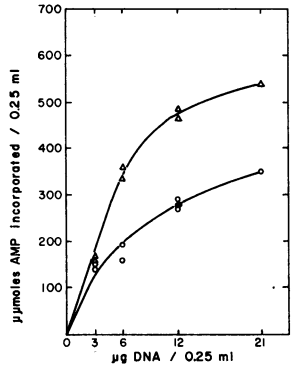

FIG. 1.—Template capacity of rat uterine chromatin purified from 12 control rats (O-O) and 12 rats injected 12 hr prior to sacrifice with 5.0 μg of estradiol-17β (△-△). Incubations were carried out as described in *Methods* with various concentrations of DNA in the form of purified chromatin. The added polymerase was capable of incorporating 11,640 μμmoles AMP/0.25 ml when 50 μg of purified salmon-sperm DNA was used as a primer and the incorporation without primer (393 μμmoles AMP/0.25 ml) has been subtracted from the values reported. Each point represents one incubation. Effects of adding estradiol-17β (3 μM) to incubation mixture containing 12 μg of DNA in the form of purified chromatin from control rats are shown (■).

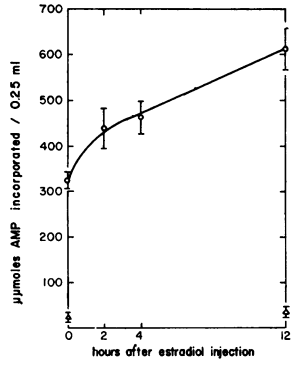

FIG. 2—Template capacity of purified rat uterine chromatin prepared from rats at various times after a single injection of 5.0 μg of estradiol-17β (O-O). Incubations were carried out as described in *Methods* with 12 μg of DNA in the form of purified chromatin. Added polymerase was capable of incorporating 9040 μμmoles AMP/0.25 ml when 50 μg of purified salmon-sperm DNA was used as a primer and the incorporation without primer (405 μμmoles AMP/0.25 ml) has been subtracted from the values reported. Each chromatin preparation was made from three or four uteri, and the number of preparations represented is 7, 4, 4, and 6 for the 0-, 2-, 4-, and 12-hr points, respectively. Template capacity of each chromatin preparation was determined in duplicate. Incorporation without added polymerase by 12 μg of DNA in the form of purified chromatin is shown (△). The data are presented as mean ± standard error of the mean.

uterus provides chromatin of a relatively low template capacity as compared to liver and lung.

Discussion.—These data clearly indicate that a single intravenous administration of 5.0 μg of estradiol-17β to ovariectomized rats causes an increase in template capacity of uterine chromatin for RNA synthesis. The fact that addition of estradiol-17β to the RNA assay system immediately prior to assay does not alter the rate of RNA synthesis indicates that the action is not merely an immediate effect on the

TABLE 1

CHEMICAL COMPOSITION OF RAT UTERUS CHROMATIN

	Mass Ratios*	
Component	Control	Estradiol-17β
DNA	1	1
RNA	0.38	0.34
Histone	1.45	1.43
Nonhistone protein	0.71	0.83
OD_{280}/OD_{260}	0.58	0.58

* Average of duplicate determinations. Chromatin was purified from the tissues of 12 control and 12 estradiol-17β-treated (12 hr before sacrifice) rats. Ratios of optical density at 280 mμ to that at 260 mμ were determined on chromatin samples in 0.7 mM potassium phosphate buffer (pH 7.4) and are corrected for light scattering as measured at 320 mμ.

TABLE 2

TEMPLATE CAPACITY OF PURIFIED CHROMATIN PREPARED FROM UTERUS, LUNG, AND LIVER OF OVARIECTOMIZED RATS

Chromatin source	μμMoles AMP Incorporated/0.25 ml		
	Control	Estradiol-17β	Control (%)
Uterus	144	261	181*
Lung	761	727	96
Liver	1800	1628	91

* Significant at $P < 0.05$. Chromatin was purified from the tissues of 12 control and 12 estradiol-17β-treated (12 hr before sacrifice) rats. Incubations were carried out as described in *Methods* with 12 μg of DNA in the form of purified chromatin. The added polymerase was capable of incorporating 8450 μμmoles AMP/0.25 ml when 50 μg of purified salmon-sperm DNA was used as a primer and incorporation without primer (387 μμmoles AMP/0.25 ml) has been subtracted from the values reported. Each chromatin preparation was assayed in duplicate.

assay itself. The *in vivo* hormone effects have been shown to be tissue-specific, since the template capacity of liver chromatin and lung chromatin from the same animals is not elevated by the estrogen treatment. These observations parallel those of Dahmus and Bonner[7], who found increased template capacity of chromatin prepared from the liver of the adrenalectomized, hydrocortisone-treated rat and noted no immediate effect of the steroid on the assay system.

Whole nuclei isolated from the uteri of immature rats 2 hr after intraperitoneal administration of estradiol-17β have twice the RNA-synthesizing capacity of nuclei from untreated controls.[5] Although this observation has been attributed to increased RNA polymerase activity rather than elevated template capacity, use of an intact nuclear system precludes a definite decision. In the present study, chromatin isolated from the uteri of mature ovariectomized rats 2 hr after estrogen injection displayed a 35 per cent increase in template capacity when compared with controls. The difference in magnitude of response of these two RNA-synthesizing systems may be due to: (1) the use of ovariectomized mature rats as compared to immature rats, (2) the administration of the hormone by the intravenous route as compared to the intraperitoneal route, or (3) the use of 5.0 μg of estradiol-17β in a 200-gm rat as compared to a 50–60-gm rat. It is also possible that the greater magnitude of response in the nuclear system is, in part, the result of increased activity of endogenous RNA polymerase. Nevertheless, the present observations clearly indicate that activation of RNA synthesis after administration of estradiol-17β involves an increase in the template capacity of uterine chromatin and is not *merely* an increase in RNA polymerase activity.

It was also noted, in the study by Gorski,[5] that the addition of 0.4 *M* $(NH_4)_2SO_4$ to the nuclear incubation medium stimulated the synthesis of RNA by uterine nuclei from untreated animals but not by those from estrogen-primed animals. Since elevated ionic strength is classically used to separate histones from nucleohistone complexes, it seems quite possible that the elevated salt concentration could well have activated the chromatin as opposed to increasing the RNA polymerase activity. Further, it has been shown by Widnell[15] that rat liver nuclei synthesize a more "DNA-like" RNA (similar base ratios) when the synthesis is done in 0.4 *M* $(NH_4)_2SO_4$. This again suggests that the action of the salt may be to expose more DNA to the transcription activities of endogenous polymerase.

Summary.—Intravenous administration of estradiol-17β to ovariectomized mature rats results in a tissue-specific increase in the template activity of uterine chromatin.

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