# EFFECT OF ESTRADIOL-17β ON THE DYNAMICS OF NUCLEOPROTEIN SYNTHESIS IN THE RAT UTERUS

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A single injection of a physiological dose of estradiol-17β into ovariectomized rats led to early stimulation of the synthesis of arginine-rich histones and acid proteins in the nuclei of the uterine tissues of the animals, whereas the dynamics of synthesis of lysine-rich and moderately lysine-rich histones corresponded to the dynamics of total protein synthesis in the cells of the target tissue.

Injection of estrogens is known to stimulate the template activity of the cell chromatin of target tissues, with a consequent increase in the biosynthesis of RNA and protein [1, 3]. Acid nucleoproteins and histones have also been shown to act as regulators in this process. For example, histones may bind with DNA and repress its template activity. On the other hand, acid nucleoproteins can affect the specificity of reconstruction of nucleohistone. Tissue chromatins of the same animal differ in the composition of their acid proteins [2].

The object of this investigation was to study the dynamics of synthesis of histone fractions and nuclear acid proteins following admininstration of estradiol-17β.

## EXPERIMENTAL METHOD

Noninbred female albino rats weighing 140-160 g were ovarietomized and, 2 weeks later, were given an intraperitoneal injection of estradiol-17β in a dose of 7 μg/100 g body weight in 0.1 ml isopropylene glycol. Control animals received injections of the same volume of solvent. The animals were decapitated after specified time intervals (from 30 min to 48 h), and the uterine cornua were removed and cut into small pieces which were incubated for 1 h at 37°C in Eagle's medium containing 50 μCi lysine-$C^{14}$. After incubation the tissue was washed at 0°C with 0.25 M sucrose, homogenized in a glass homogenizer, and the acid-soluble proteins were extracted by Johns' method [5]. The fraction of acid proteins was obtained by extracting the residue with 0.05 N NaOH solution. Protein was determined by Lowry's method. To study the specific activity of the preparations 0.5 mg bovine albumin was added to each fraction. Proteins were precipitated from the resulting solutions with 18% TCA. The residues were applied to Millipore filters and dried. The activity of the fractions was determined with the NAG-BM gas-flow counter.

## EXPERIMENTAL RESULTS

The experiments showed that estradiol-17β increased the incorporation of labeled lysine into the fraction of acid proteins up to 145% of the control level after 30 min. Later, between 1 and 2 h, the level of biosynthesis was close to the control, but after 4 h a further increase in incorporation of the label into the fraction of acid proteins was observed (210%). A third peak of incorporation of the label into these proteins was found 24 h after the injection of estradiol (Fig. 1). This early stimulation of acid protein synthesis revealed in these experiments evidently cannot be attributed entirely to activation of RNA synthesis by the

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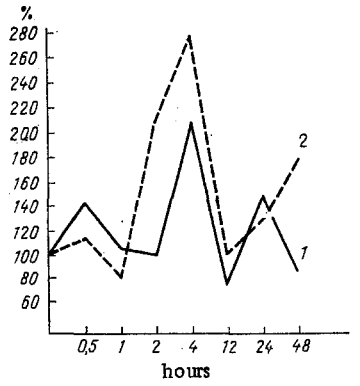


Fig. 1

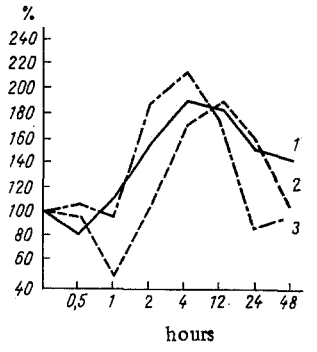


Fig. 2

Fig. 1. Dynamics of incorporation of lysine-$C^{14}$ into fractions of acid proteins and arginine-rich histones ($f_3$) after injection of estradiol-17$\beta$: 1) incorporation of label into acid protein fraction; 2) incorporation of label into fraction of arginine-rich histones. Here and in Fig. 2: abscissa, time after injection of estradiol (in h); ordinate, incorporation of label in percent of control.

Fig. 2. Dynamics of incorporation of lysine-$C^{14}$ into fractions of lysine-rich ($f_1$) and moderately lysine-rich ($f_{2a}$ and $f_{2b}$) fractions of histones after injection of estradiol-17$\beta$: 1) incorporation of label into $f_1$ histones; 2) into $f_{2a}$ histones; 3) into $f_{2b}$ histones.

hormone in the nuclei of the uterine tissues, and it must take place on account of the RNA pool already present in the cells when the hormone was injected. If the acid proteins of chromatin are regarded as potential activators of its template activity and as factors responsible for the specificity of addition of the histones to DNA [6, 7], this can evidently explain the preliminary stimulation of biosynthesis of precisely this protein fraction. The role of acid proteins as receptors of estradiol in the nuclei of the target cells likewise must not be forgotten [8]. Consequently, in either case early stimulation of acid protein synthesis must be regarded as unconnected with the mechanism of induction of template synthesis by the estrogen but as aimed at its activation.

Analysis of the changes in the specific radioactivity of the lysine-rich histones (the $f_1$ fraction) showed that the incorporation of lysine-$C^{14}$ began to increase only 1 h after injection of estradiol and reached a maximum (180-190%) after 4-12 h. This was followed by a gradual decrease in the level of labelling of the protein (Fig. 2). Characteristic changes in biosynthesis also were discovered in the histone fractions moderately rich in lysine. For instance, in the $f_{2a}$ fraction a sharp decrease in incorporation of the label (to 51% of the control) was recorded 1 h after injection of the hormone; this was followed by an increase in the specific activity of the protein to maximal values between 4 and 12 h after the injection (175 and 188% of the control). Incorporation of the label into the $f_{2b}$ fraction at this period increased to 216%, but the 48 h the incorporation of lysine had fallen gradually to the control level (Fig. 2).

The dynamics of incorporation of lysine-$C^{14}$ into histone fractions $f_1$, $f_{2a}$, and $f_{2b}$ evidently reflects the general picture of induction of protein synthesis in the target cells observed after administration of estradiol, and it can most probably be attributed to activation of the DNA-dependent RNA-polymerase reaction by the hormone through the removal of repression from individual sites on the genome.

Investigation of the incorporation of the labelled amino acid into the arginine-rich $f_3$ histone fraction showed that as early as 30 min after injection of the hormone there was a moderate increase in the incorporation of lysine-$C^{14}$ into that fraction (up to 115%). Incorporation later decreased to 60% of the control (Fig. 1). It will be clear from Fig. 1 that the second peak of incorporation of the label occurred after 4 h and reached 275% of the control. After 12 h incorporation of the label was close to the control level, but later it rose slowly to reach 180% after 48 h.

Presumably the early stimulation of biosynthesis of the $f_3$ fraction discovered in these experiments, although less marked than in the case of the acid proteins, could regulate the template activity of the chromatin somehow in that period. However, according to Barker [4], the stimulation of synthesis of this fraction is only apparent in some cases and is due to acceleration of the biosynthesis of particular fractions of acid proteins associated with the arginine-rich histone fraction.

Comparison of the results thus shows that the regulation of biosynthesis of nucleoproteins in the rat uterus under the influence of estradiol is an important link in the mechanism of action of the hormone. Activation of the acid fraction as the earliest may perhaps be responsible both for the transport of the steroid and for the specific nature of action of the hormone on chromatin.

## LITERATURE CITED


1. P. V. Sergeev, R. D. Seifulla, and A. I. Maiskii, Molecular Aspects of the Action of Steroid Hormones [in Russian], Moscow (1971).
2. R. B. Khesin, Uspekhi Sovr. Biol., 74, 171 (1972).
3. N. A. Yudaev and T. N. Protasova, Uspekhi Sovr. Biol., 72, 118 (1971).
4. K. L. Barker, Biochemistry, 10, 284 (1971).
5. E. W. Johns, Biochem. J., 92, 55 (1964).
6. S. Mizushima and M. Nomura, Nature, 226, 1214 (1970).
7. J. Paul, Symp. Soc. Exp. Biol., 25, 117 (1971).
8. C. S. Teng and T. H. Hamilton, Proc. Nat. Acad. Sci. (Washington), 63, 465 (1969).