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Control by Estrogen of Genetic Transcription and Translation

Binding to chromatin and stimulation of nucleolar RNA synthesis are primary events in the early estrogen action.

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The physiological effects of estrogen in the mammalian uterus are mediated by increases in the synthesis of RNA and protein (*1–3*). By 1964 it was clear that an acceleration of synthesis of ribosomal RNA and of ribosomes was an essential feature in the initial or early action of estrogen in its characteristic target organ (*4*, *5*). We also knew, as a result of the experiments of Segal and his co-workers (*3*), that growth of the estrogen-deficient uterus was induced by treatment *in utero* with RNA extracted from the organ stimulated by the hormone. However, the role of messenger RNA synthesis in this action of estrogen was and still remains uncertain (*6*, *7*). Furthermore, it was also uncertain whether, for early estrogen action, genetic transcription was truly stimulated prior to enhancement of cytoplasmic genetic translation, as expected by the sequence hypothesized: hormone→genome→RNA→protein. Before 1964 there was no technique for separating the nuclei from the cytoplasmic fraction of the uterus. Since we knew that nearly all of the RNA in the mammalian cell was synthesized in the nucleus and transported to the cytoplasm where most of the cellular RNA resided, it was obvious that isolation of nuclei from the uterus was a prerequisite to studies of estrogen-stimulated RNA synthesis. In 1964 Widnell and Tata (*8*) developed an excellent technique for isolating intact and enzymically active nuclei from homogenized rat liver. In 1965 Widnell, Tata, and I (*9*, *10*) adapted the technique for application to rat uterus, obtaining a meaningful partition of the nuclear and cytoplasmic RNA and protein in the organ. A series of investigations of the metabolism of RNA and protein in vivo and in vitro during the course of the action of estrogen in the uterus were carried out first in Tata's laboratory at the National Institute for Medical Research in London, and later in my laboratory at the University of Texas (*9–16*).

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In this article I review the metabolism of RNA and protein in the nuclear and cytoplasmic fractions of the uterus, as a function of time after administration of exogenous 17β-estradiol (1,3,5-estratriene-3,17β-diol) to the ovariectomized adult rat. Variations in the metabolism of RNA and protein in the uterus of the normal rat, from the diestrous to estrous phase of the estrous cycle, mimic in certain particulars the variations observed during early estrogen action. The topics deal with the synthesis of RNA and protein in the nucleus, the binding in vivo of tritiated 17β-estradiol to chromatin and the latter's template activity assayed in vitro, the transport of RNA from nucleus to cytoplasm, the formation of polyribosomes, and the variation in the incorporation of amino acid by the polyribosomes as assayed in the cell-free system. Although the precise and primary molecular mechanisms involved in early estrogen action remain undiscovered, the broad outlines of the hormone's control of the formation and the activity of the protein-synthesizing apparatus of the organ are clear. The original theory of a genomic site for one of the primary actions of estrogen remains valid, but the inducer theory (*17*) for the mechanism of action of this hormone strictly according to the Jacob-Monod model for microbial enzyme induction (*18*) appears in need of revision.

Metabolism of RNA and Protein

With regard to the variation in concentration of DNA, RNA, and protein, the uterus of the normal rat in estrus contains about twice the amount of RNA and six times the amount of

Table 1. Effect of ovariectomy, hormone treatment, and phase of estrous cycle on the ratio of RNA to DNA and the ratio of protein to DNA in the uterus or the nuclei of its individual cells (*16*). Ovariectomized adult rats were given (intraperitoneally) 10 μg of 17β-estradiol at zero time, and at later times in the experiments designated. Animals were killed, the uteri were weighed, and the nuclei were isolated (*10*). Each nuclear preparation was washed twice prior to determinations of ratios of RNA to DNA and of protein to DNA (*16*). Portions of the tissue homogenate were taken for DNA determination. Each control or experimental group consisted of six to eight animals, and their uteri were pooled for the subsequent determinations. With the exception of the data on uterine DNA, each value is the average of duplicate or triplicate experiments with the range of variation being less than 5 percent. The other experimental details have been described (*11, 12, 16*).

Interval after hormone treatment (hr)	DNA in uterus (mg)	Nuclear ratios		Whole-tissue homogenate ratios		Uterus wet weight (mg)
		RNA/DNA	Protein/DNA	RNA/DNA	Protein/DNA	
		Ovariectomized animals				
0	0.98	0.23	2.20	0.43	2.10	68 ± 5
0.5	.91	.24	2.05	.41	2.66	86 ± 7
1	.85					79 ± 7
2	.96	.28	2.32	.52	2.80	87 ± 6
4	.92	.27	2.30			102 ± 10
12	.97	.29	2.30	.71	3.98	117 ± 9
24*	1.10	.32	3.36	.82	7.82	130 ± 8
36	1.22					135 ± 9
48*	1.20	.32	3.64			140 ± 11
72*	1.24	.33	3.78			210 ± 19
		Normal animals				
Diestrus	1.26	0.32	3.54	0.58	4.86	213 ± 16
Estrus	1.49	.34	3.28	.92	24.80	630 ± 44

*Hormone administered again at intervals of 24 hours each.

protein that occurs in the organ of the ovariectomized animal, as shown in Table 1. There is a three- to sixfold increase in the protein content of the organ taken from the normal animal in estrus, compared to that in the diestrous phase. The ratios of nuclear RNA to DNA and of protein to DNA exhibit only minor decreases after ovariectomy, and only slight increases during the estrous phase. These observations indicate that both ovariectomy and decreased hormone titer result in a marked decline in the cytoplasmic concentration of ribonucleoprotein in uterine cells. The DNA content of the organ decreases only slightly as a result of ovariectomy, and there is only a minor increase on passing from the diestrous to estrous phase. In estrus, the endogeneous titers of ovarian hormones are possibly at their highest (*19*). The consistent but minor variation in the DNA content during the cycle indicates a small turnover either in the number of cells in the organ or in the DNA content of the cells responsive to estrogen. The physiological significance of this turnover is unknown (*10*). Table 1 shows also that the biochemical deviations are restored to normal by administration of 17β-estradiol to the ovariectomized animal. The decrease in the concentration of ribonucleoprotein in the cytoplasm and the absence of any significant decrease in the DNA content of the organ after ovariectomy are in accordance with the well-known observation that hormone-dependent cells shrink, undergo atrophy, and become dormant when deprived of hormone (*20*). It is now established that DNA templates in the nucleus are the sites of synthesis of nearly all the RNA occurring in animal cells (*21*). Thus the observation that in the uterus concentration of cytoplasmic RNA, but not of nuclear RNA, is altered markedly by ovariectomy, estrous cycle, or hormone treatment is one line of evidence that estrogen in some way regulates nuclear synthesis of RNA and its transport to, and its accumulation in, the cytoplasm.

Nuclear Synthesis of RNA

Previous studies had shown the involvement of RNA synthesis in early estrogen action in the uterus of the ovariectomized or immature rat (*22*), and Gorski had described (*23*) the stimulation in vivo by estrogen of the RNA polymerase reaction (*24*) for an enzyme preparation obtained from nuclei isolated from the organ. Utilizing the technique for isolating intact nuclei from the uterus (*10*) and the procedure of Marushige and Bonner (*25*) for isolating and assaying the activity of chromatin preparation, we investigated estrogen-stimulated RNA synthesis according to (i) rapidly labeled nuclear RNA synthesized in vivo, (ii) RNA polymerase in intact nuclei assayed in vitro, and (iii) the template activity of chromatin preparation assayed in vitro with added *Escherichia coli* RNA polymerase.

The early effects of estrogen on synthesis of uterine nuclear RNA rapidly labeled in vivo are shown in Fig. 1. The incorporation of tritiated uridine into RNA was increased by more than 500 percent 20 minutes after the hormone was administered. Thereafter, the specific activity of rapidly labeled nuclear RNA decreased, but remained higher than that of the control from 40 minutes to 24 hours of hormone action. In another series of investigations (*14*) we were able to show that, as early as 2 minutes after administration of the hormone, the specific activity of the nuclear RNA was increased over that of the control by 40 percent. The effect of hormone treatment on the uptake by the uterus of the tritiated uridine, and on the proportion of this total radioactivity that is not incorporated into material precipitable by perchloric acid (that is, the acid-soluble fraction), is also shown in Fig. 1. A consistent observation in our studies has been that estrogen, in differentially increasing the specific activity of nuclear RNA in vivo from 2 minutes to 24 hours, also increases differentially the uptake by the organ of the radioactive precursor (*9–11, 13*). Correlated with this increase in uptake is a drop in the proportion of radioactivity in the acid-soluble fraction, and an increase in the acid-insoluble fraction. It is difficult to say whether the variation in specific activity of the nuclear RNA is a cause or consequence of the variation in uptake of the precursor. However, kinetic studies suggest that synthesis anticipates uptake in estrogen action, with the radioactive precursor entering the uterine cells in response to depletion of the uridine pool, as a result of acceleration of the rate of RNA synthesis (*26*).

The RNA polymerase in isolated uterine nuclei was studied in the presence of divalent magnesium (Mg^{2+}) ions at low ionic strength, or in the presence of divalent manganous (Mn^{2+}) ions and 0.4*N* ammonium sulfate. The principal characteristics and kinetics for the incorporation of ^{14}C-labeled adenosine triphosphate into RNA under both conditions were similar to those reported for liver nuclei (*27, 28*). Both RNA polymerase reactions assayed in uterine nuclei in vitro were dependent upon the presence of all four nucleoside triphosphates, and they were in-

hibited by deoxyribonuclease, ribonuclease, or actinomycin D. The data obtained (Table 2) for the base composition and nearest-neighbor base frequencies of the reactions indicate that the RNA formed by the Mg^{2+}-activated reaction is a ribosomal type of RNA. The ratio of the base pairs (adenine and uracil to guanine and cytidine) was less than 0.8. The product of the reaction activated by Mn^{2+} and $(NH_4)_2SO_4$ is more like that of DNA (that is, the base-pair ratio is equal to or greater than 1.0). These observations are in good agreement with the findings of Widnell and Tata (*20*) and of McGregor and Mahler (*28*) concerning RNA synthesis in isolated nuclei from rat liver.

The stimulation of the two DNA-dependent RNA polymerase reactions by estrogen acting in the uterus of the ovariectomized rat is shown in Fig. 2. The Mg^{2+}-activated reaction was stimulated at 1 hour, as reported by Gorski (*23*). The greatest increase, 100 to 125 percent, was reached at 12 hours and the peak was maintained until 24 hours after hormone treatment. No change was observed in the polymerase reaction activated by Mn^{2+} and $(NH_4)_2SO_4$ before 12 hours of hormone action. From 12 to 24 hours after hormone treatment a slight but consistent (50 to 60 percent) stimulation of this polymerase reaction was observed. The same sequential stimulation of the two RNA polymerase reactions, but on a more protracted time scale, was also observed for liver nuclei stimulated in vivo by thyroid hormone (*29*). Dissociations of the stimulations of RNA polymerase activities in nuclei incubated in the presence or absence of ammonium sulfate have also been reported for the actions in vivo of testosterone (*30*) and growth hormone (*29–31*) and for diethylstilbestrol in vitro (*32*).

More recently, we have undertaken a study of the effect of ovariectomy or estrogen administration on the template activity of uterine chromatin assayed in vitro (*33*). Barker and Warren (*34*) had shown that this activity was stimulated 2 hours after hormone treatment, and that the activity rises and falls (*35*) in a way that could be correlated with the estrous and diestrous phases of the estrous cycle. This observation of cyclical variation in template activity of chromatin assayed in vitro paralleled our previous observation (*11*) that the Mg^{2+}-activated RNA polymerase re-

Table 2. The base composition and frequency of the nearest-neighbor base of the product of RNA polymerase in nuclei isolated from uterine cells (*12*). For each experiment (in duplicate), nuclei were isolated from pooled uteri taken from ten normal adult rats. For analysis of base composition, all four nucleoside triphosphates were labeled with carbon-14, and 0-3 μ-mole of each was added to the reaction mixture and incubated for 15 minutes (*12*). For the reaction in the presence of 0.4*M* ammonium sulfate, the radioactive nucleoside triphosphates were added after 15 minutes of preliminary incubation. For analysis of nearest-neighbor base frequency, 0.06 μmole of α-^{32}P-adenosine triphosphate was added to the reaction mixture containing 0.3 μmole each of the nonradioactive nucleoside triphosphates. For the Mn^{2+}-$(NH_4)_2SO_4$-activated reaction, the labeled adenosine triphosphate was added after the reaction mixture was incubated for 15 minutes with 0.3 μmole each of the nonradioactive nucleoside triphosphates. Conditions of incubation, the other experimental details, and the calculation of the frequencies have been described (*12*). UMP, GMP, CMP, and AMP are uridylic, guanylic, cytidylic, and adenylic acids, respectively.

RNA polymerase activation	Recovery of radioactivity (%)				AMP + UMP
	UMP	GMP	CMP	AMP	GMP + CMP
	Base composition				
Mg^{2+}	21	31	27	21	0.72
	22	30	28	20	0.72
Mn^{2+} and $(NH_4)_2SO_4$	23	26	23	28	1.04
	26	26	22	26	1.08
	Nearest-neighbor base frequency				
Mg^{2+}	18	32	29	21	0.64
	20	33	27	20	0.67
Mn^{2+} and $(NH_4)_2SO_4$	25	26	21	28	1.13
	23	24	20	33	1.27

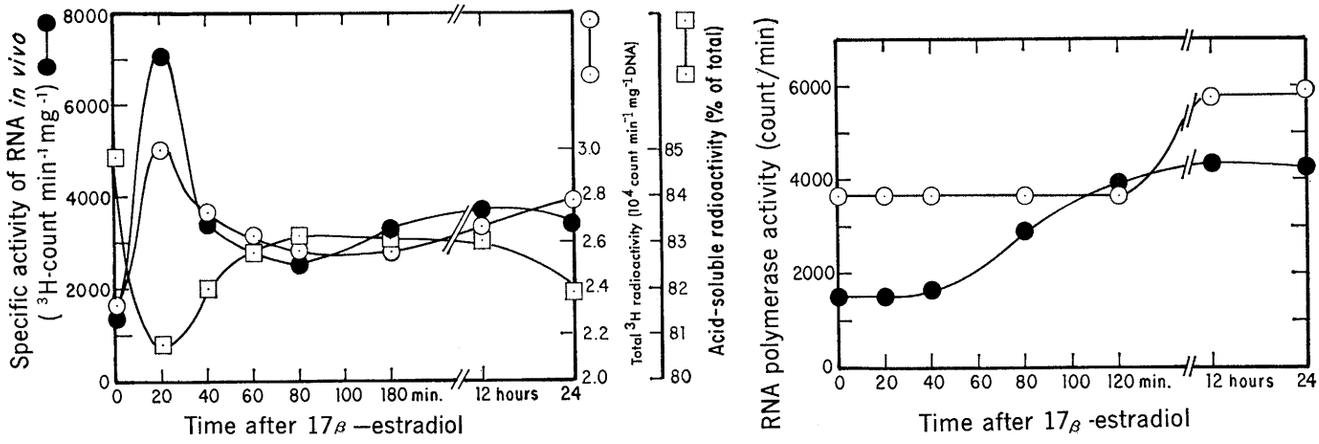

Fig. 1 (left). Time course for the effect of a single dose of 17β-estradiol on the incorporation of ^{3}H-uridine into nuclear RNA rapidly labeled in vivo in the uterus of the ovariectomized rat (*12*). All animals were given 100 μc of the radioisotope intraperitoneally 10 minutes before they were killed. All animals were given 10 μg of 17β-estradiol intraperitoneally at zero time and killed at the indicated time thereafter. Three to five uteri were pooled for homogenization and isolation of nuclei for each experiment. Uptake of the radioisotope by the uterus was measured as the total radioactivity per milligram of DNA in the whole-tissue homogenate. Determination of the total radioactivity and that in the acid-soluble fraction and of the specific activity of RNA in the nuclei isolated has been described (*12*). Fig. 2 (right). The effect of administration of a single dose of 17β-estradiol on polymerase reactions in RNA nuclei isolated from the uterus of the ovariectomized rat (*12*). All animals were given 10 μg of the hormone intraperitoneally at zero time. The animals were killed as indicated, and the uteri were excised, pooled, and homogenized. The nuclei were isolated and assayed for RNA polymerse activity in the presence of 5 m*M* Mg^{2+} and in the absence of $(NH_4)_2SO_4$, or in the presence of 4 m*M* Mn^{2+} and 0.4*M* $(NH_4)_2SO_4$. Solid circles, Mg^{2+}-activated RNA polymerase; open circles, Mn^{2+} and $(NH_4)_2SO_4$-activated RNA polymerase.

Table 3. Chemical composition and template activity of uterine chromatin (*33*) from ovariectomized and intact animals. Ovariectomized adult rats were given (intraperitoneally) 10 μg of 17β-estradiol and killed at the time indicated. The uteri of each group of ovariectomized or intact animals were pooled, and the chromatin isolated and purified. The chemical composition and template activity of the chromatin were determined as described (*33*). The template activity was expressed in picomoles (pm) of ^{14}C-adenylic acid incorporated per 0.25 ml of the reaction mixture containing 10 μg of DNA in the form of chromatin.

Ovariectomized animals*			Intact animals†	
Control	Hormone-treated		Diestrus	Estrus
	8 hours	48 hours		
		DNA (mass ratio)		
1.0	1.0	1.0	1.0	1.0
		RNA (mass ratio)		
0.12	0.14	0.17	0.17	0.21
		Total protein (mass ratio)		
1.12	1.14	1.32	2.45	2.65
		Histone (mass ratio)		
0.70	0.55	0.65	0.92	0.75
		Template activity of chromatin (pm)		
14.5	30.5	22.5	12.5	16.0

*Six animals per group. †Three animals per group.

action shows cyclical variation in preparations of nuclei from uterine cells at the two prominent phases of the cycle.

The variation in template activity in vitro of uterine chromatin, isolated and purified at various intervals after administration of estrogen to the ovariectomized rat, is shown in Fig. 3. The principal characteristics and the kinetics of the "template reaction" in the presence or absence of *Escherichia coli* RNA polymerase were similar to those reported for liver chromatin (*25*). Thirty minutes after hormone treatment, the template activity of the isolated uterine chromatin had increased 26 percent over that of the control. Eight hours later, this activity increased to about 300 percent more than that of the control. Thereafter, to 72 hours, the activity decreased, although at all intervals of time examined it remained greater than that of the control. There was a small but repeatable stimulatory effect of the hormone on the template activity of chromatin preparations isolated from the livers of the animals used (Fig. 3). This stimulation was also maximum at 8 hours, but represented an enhancement of activity of only 5 to 10 percent compared to the control. The mammalian liver is generally considered not to be a target organ of estrogen, and the physiological significance of the stimulation observed remains to be discovered.

An unexpected observation (Fig. 4) was that the ratio of RNA to DNA of the chromatin isolated from the uterus and purified showed a significant increase after only 15 minutes of estrogen action in vivo, seemingly anticipating the stimulation of the preparation's template activity first detected at 30 minutes. From 12 to 24 hours the ratio of protein to DNA in the chromatin also increased (Fig. 4), in parallel with the rise in the ratio of protein to DNA noted for intact nuclei (Table 1).

That the chemical composition as well as the template activity of chromatin isolated from the uterus is regulated by both exogenous and endogenous estrogen acting in vivo is shown in Table 3. The chromatin isolated from the ovariectomized rat showed increased template activity, increases in the ratios of RNA to DNA and of total protein to DNA, and a decrease in the ratio of histone to DNA. The same alterations of template activity and of chemical composition were observed for the chromatin isolated from intact rat in estrus, compared to that from the animal in diestrus. Whether the hormone-induced increase in template activity is

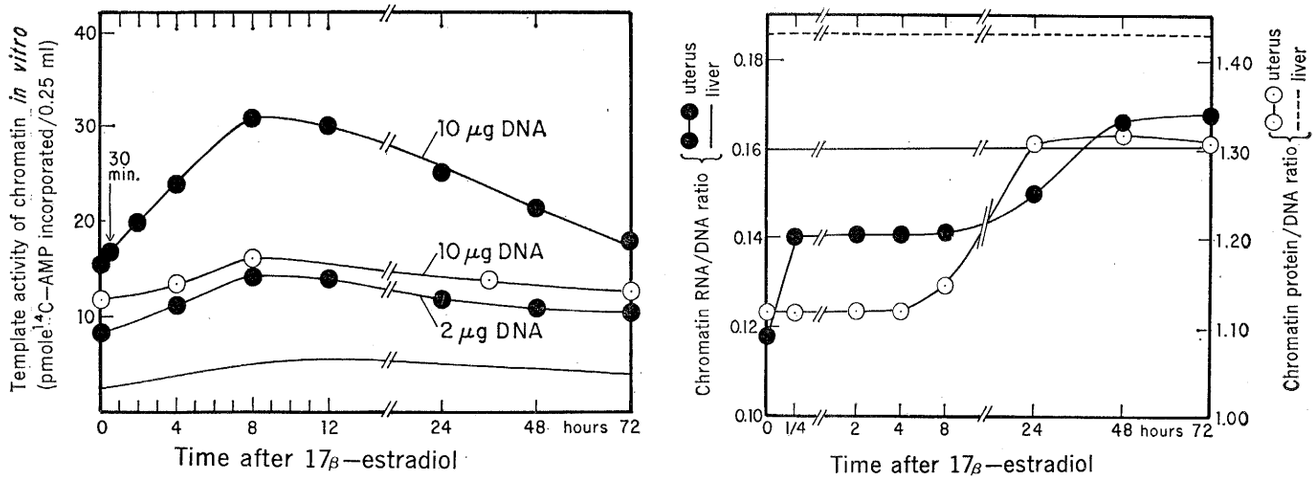

Fig. 3 (left). Rate of RNA synthesis directed by chromatin isolated from the uterus or liver of the ovariectomized rat, as a function of time after treatment with estrogen in vivo (*33*). All animals were given 10 μg of 17β-estradiol intraperitoneally at zero time. At the times indicated, the animals were killed; the uteri and fragments of the liver were excised and pooled respectively for homogenization. The chromatin was isolated and purified, by a modification of the technique of Marushige and Bonner (*25*), and assayed for template activity in vitro, with the same amount of RNA polymerase of *Escherichia coli* being added to each reaction tube. Template activity is expressed as picomoles (pmole) of ^{14}C-adenylic acid incorporated per 0.25 ml of reaction mixture containing the amount of DNA indicated in the form of chromatin. The base line indicates the activity observed for uterine chromatin (10 μg of DNA) assayed in the absence of RNA polymerase. The constituents of the reaction mixture and the remaining experimental details have been described (*33*); solid circles, uterine chromatin; open circles, liver chromatin. Fig. 4 (right). Ratios of RNA to DNA and of protein to DNA in chromatin isolated from the uterus or liver of the ovariectomized rat, as a function of time after treatment with estrogen in vivo (*33*). The experimental procedure is described in Fig. 3, with the exception that the chromatin isolated and purified was determined chemically for DNA, RNA, and protein (*33*). Results are representative of replicate experiments.

a cause or consequence of the changes in chemical composition is unknown.

These observations suggest that the estrogen-induced increase in the ratio of RNA to DNA for uterine chromatin is a result of an early synthesis of chromatin-associated RNA, and not a result of contamination or of redeployment of other RNA occurring within the nucleus. First, the chromatin preparations isolated from the livers of the animals used in these experiments did not show an increase in this ratio (Fig. 4). Second, 15 minutes after hormone treatment the specific activity of RNA labeled in vivo with tritiated uridine and contained in the isolated chromatin is also increased over that of the control (*33*). Third, the ^{32}P-labeled RNA contained in the chromatin preparation distributed by centrifugation in 2.09*M* cesium chloride indicates by sedimentation profiles a heavy, DNA-associated RNA peak and a lighter RNA peak associated with the histone of the preparation (*33*). It remains to be determined if an early increase in the synthesis and accumulation of chromatin-associated RNA in the uterus is a prerequisite for hormonal stimulation of the template activity of the organ's chromatin.

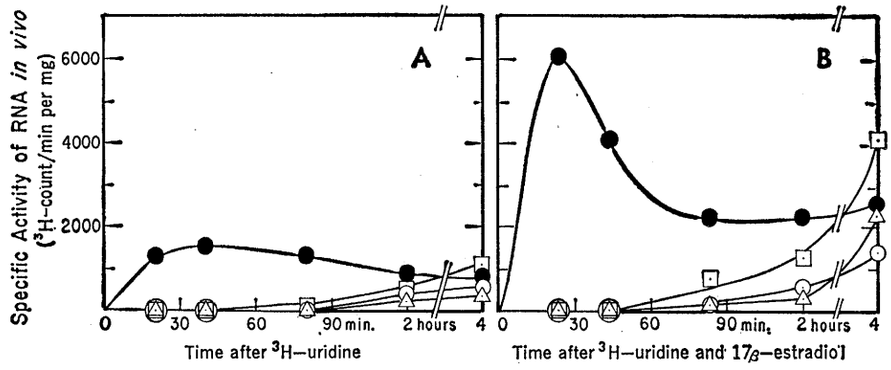

Fig. 5. Effect of a single dose of 17β-estradiol on the incorporation of ^{3}H-uridine into RNA in the uterus of the ovariectomized adult rat (*12*). All animals were given either 100 μc of the radioisotope (A) or 100 μc of the radioisotope and 10 μg of the hormone (B) intraperitoneally at zero time. At the time indicated, the animals were killed, and the uteri were excised and pooled for homogenization. The subcellular fractions were then isolated for extraction of RNA and determination of its specific activity. Specific activity is expressed as the number of counts per minute per milligram of RNA. The other details have been described (*12*). Solid circles, nuclear RNA; open circles, mitochondrial RNA; open squares, microsomal RNA; open triangles, cell-sap RNA.

Transport of RNA from Nucleus to Cytoplasm

The turnover of newly synthesized RNA from the nucleus to the cytoplasm in vivo in the uterus has been examined for effects of ovariectomy and estrogen administration (*12*). The characteristics of incorporation of tritiated uridine into RNA of the uterus of the ovariectomized rat, as a function of time after administration of the precursor are shown in Fig. 5A. Tritiated uridine was rapidly incorporated into nuclear RNA, with the specific activity being maximum at 20 to 40 minutes. The appearance of newly formed RNA in the cytoplasm was not observed until 1 hour and 20 minutes, at which time the RNA of the microsomal fraction demonstrated a significant incorporation of the radioactive precursor. Incorporation of radioactivity into mitochondrial and cell-sap RNA was not observed before 2 hours. At 4 hours, the specific activity of RNA of all cytoplasmic fractions was significantly elevated, with the specific activity of nuclear RNA now being less than that of the microsomal RNA. The effect of coadministration of 17β-estradiol with tritiated uridine (Fig. 5B) was to increase further the specific activity of nuclear RNA and to accelerate the entry of newly formed RNA into the cytoplasm. Twenty minutes later the specific activity of nuclear RNA was increased threefold over that observed in the absence of the hormone. At 1 hour and 20 minutes, all cytoplasmic fractions of RNA were significantly labeled, and by 4 hours the activity of microsomal RNA was about 300 percent more than that of the RNA labeled in the absence of the hormone. In general, hormone treatment caused the rates of synthesis and transport of RNA in the organ to return to values similar to those observed when labeled precursor was administered to the normal rat (*12*).

These observations on the intracellular distribution of RNA labeled in vivo are in good agreement with the evidence for transport of RNA from the nucleus to the cytoplasm in mammalian cells (*36*), as well as with the evidence for variation in uterine content of whole-tissue and nuclear RNA (Table 1).

Synthesis of Protein in vivo

Earlier investigations had shown (*37*) a general stimulation of synthesis of total protein during early estrogen action in the uterus, usually 4 hours after hormone administration. Since total protein was extracted from homogenates of the organ previously exposed to radioactive amino acid for a relatively long period of time, these studies provided no information on variations in subcellular rates of protein synthesis. Also, the synthesis of rapidly labeled proteins could not be dissociated from the labeling of structural protein of a membranous or organelle nature.

The effect of estrogen administration on synthesis of nuclear, mitochondrial, microsomal, and cell-sap protein rapidly labeled in vivo (*31, 26*) with radioactive methionine is shown in Fig. 6. First there was a 35 to 60 percent depression of the rate of incorporation of tritiated methionine into protein of the several subcellular fractions. This depression was detectable from 20 to 30 minutes after hormone treatment, and persisted for about 1 to 1½ hours. The early depression of the rate of amino acid incorporation due to estrogen action at 30 minutes was also observed if glycine, tryptophan, or lysine was used as a radioactive precursor (*15*). At 2 hours, the stimulation of synthesis of protein in both nuclear and microsomal fractions was apparent. From 2 hours to 4 hours, there was an enhancement of nuclear protein synthesis which anticipated the stimulation of synthesis of cytoplasmic protein, and particularly of that of the microsomal fraction. At 8 hours, the rate of protein synthesis in the cytoplasmic fractions was accelerated by 200 to 300 percent. After 8 to 24 hours of hormone action, cytoplasmic protein synthesis remained elevated, whereas the amount of synthesis of nuclear protein decreased to less than that of the control. The uptake of tritiated methionine by the uterus and the proportion of radioactivity that was acid soluble were consistent with the

variation in specific activity of the protein fractions, with exception of the activity of the nuclear protein fraction in the later period of estrogen action.

Why the rate of nuclear protein synthesis in the uterus of the ovariectomized rat should first be depressed, then accelerated, and depressed again during the course of estrogen action is uncertain. Perhaps the early effect of the hormone on nuclear RNA synthesis at 2 to 20 minutes provides a major drain on the concentration of adenosine triphosphate in the cells, thereby diminishing the intracellular energy available for protein synthesis. Aaronson, Natori, and Tarver have reported (*38*) that during early estrogen action in the ovariectomized or immature rat a linear decrease in the adenosine triphosphate content of the uterus is detectable from 30 minutes to 4 hours after hormone treatment. The stimulation from 2 to 8 hours in the rate of nuclear protein synthesis, preceding stimulation of protein synthesis in the cytoplasm, may well be a reflection of enhanced synthesis of the protein programed for transport to the cytoplasm in conjunction with the accelerated formation of ribosomes or of their components (*16*). The decrease in rate of nuclear protein synthesis later in the action of the hormone is correlated both with the accumulation of polyribosomes in the cytoplasm and with the decrease in their activity in incorporating amino acids in vitro, as discussed below.

Formation and Activity of Ribonucleoprotein Particles

Cell-free systems for the study of protein synthesis by preparations of ribonucleoprotein extracted from the rat uterus or chick oviduct has been described (*3, 39*). Segal and Scher (*2*) have pointed out the disappointments in these early efforts, although the studies cited demonstrated enhancement of amino acid incorporation activity of ribonucleoprotein particles isolated from the target organs, as a result of estrogen action in vivo. One weakness in these studies was the absence of information on the sedimentation characteristics of the ribonucleoprotein particles isolated for assay in the cell-free system. For this and other reasons, we undertook a complete study of the sedimentation characteristics of ribonucleoprotein particles in the cytoplasmic fraction of the uterus and of the activity in vitro of the particles with respect to incorporation of amino acid, as influenced by ovariectomy or hormone treatment (*40, 41*). A mixture of polymeric and monomeric ribosomes, designated polyribosomal preparation, was isolated from the microsomal fraction of the uterus (that is, a homogenate of the organ minus remaining cells, cell membranes, nuclei, and mitochondria (*42, 43*). Most of the polymeric and monomeric ribosomes in the microsomal fraction appear to be unattached to membranes and to be relatively unstable (*41, 44*). The recovery in the particles isolated of the total ribonucleoprotein in the microsomal fraction was 58 to 61 percent. The same values were obtained even if the microsomal fraction was 1.3 percent with respect to potassium deoxycholate (*40*). Table 4 describes the principal characteristics of the cell-free system containing, in addition to the usual constituents (*45*), ^{14}C-leucine and the polyribosomal preparation. The complete system is dependent upon the presence of adenosine triphosphate, guanosine triphosphate, polyribosomal preparation, a supernatant fraction (which contains activating enzymes, transfer factors, and soluble RNA), and the 20 amino acids. Cycloheximide, puromycin, ribonuclease, and sodium fluoride inhibit amino acid incorporation in the system. Table 4 also shows the activity in the cell-free system of washed monomeric ribosomes previously treated with ribonuclease, sodium deoxycholate, or sodium fluoride. Ribosomes treated with sodium fluoride have a higher amino acid incorporation activity than those treated with ribonuclease. Sucrose density-gradient sedimentation indicated, however, that sodium fluoride was more effective than ribonuclease in dissociating polymeric ribosomes (*40*). Lin, Mosteller, and Hardesty (*46*) also observed that ribosomes treated with sodium fluoride incorporate amino acids very easily in the rabbit reticulocyte cell-free system. Sodium fluoride is thought to dissociate polyribosomes into single ribosomes, with messenger RNA remaining attached to some of the monomers (*41, 47*). If true, this conceivably could be useful in studying the effects of estrogen on the synthesis of messenger RNA.

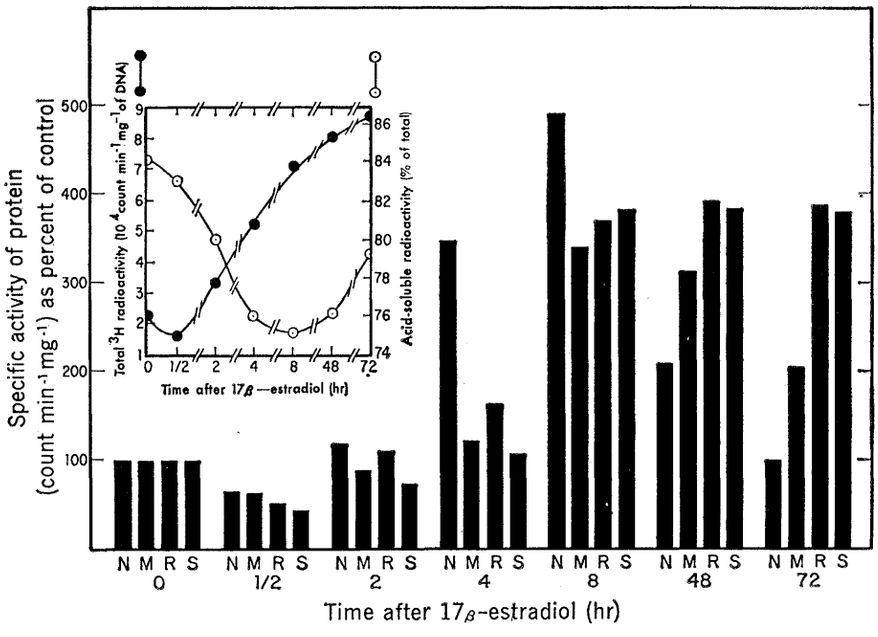

Fig. 6. Rates of synthesis of rapidly labeled protein of subcellular fractions of the uterus of the ovariectomized rat, as a function of time after treatment with estrogen in vivo (*13, 26*). All animals were given 83 μc of tritiated L-methionine intraperitoneally 10 minutes before they were killed. Experimental animals were similarly given 10 μg of the hormone at zero time, and killed at the time indicated. Certain animals were given doses of the hormone at intervals of 24 hours thereafter. Uteri were pooled and homogenized, and the subcellular fractions were isolated for determination of protein and its radioactivity. Specific activity was expressed in counts per minute per milligram of protein. The data are expressed as percentages of the control values, which are nuclear protein (N), count/min 340; mitochondrial protein (M), 548 count/min; microsomal protein (R), 514 count/min; supernatant protein (S), 409 count/min. The insert shows the variation in tissue radioactivity and in the proportion of radioactivity that was in the acid-soluble fraction. The other experimental details have been described (*13, 26*).

Effect of Ovariectomy and Hormone Treatment

The ^{14}C-leucine incorporation activity of the preparation from ovariectomized animals was consistently observed to be 50 to 60 percent greater that that of the preparation from the normal animal (Table 5). This was true whether the incorporated radioactivity in the cell-free system was evaluated for each milligram of protein or each milligram of RNA contained in the polyribosomal preparation assayed.

The effect of administration of estrogen to the ovariectomized rat on the incorporation of ^{14}C-leucine in vitro by the polyribosomal preparation is shown in Fig. 7. Two hours after hormone treatment, this activity had increased 30 percent. At 8 to 12 hours, the activity had increased by about 100 percent. Thereafter, however, the activity declined, and at 72 hours it approached the lower activity observed for the preparation obtained from normal animals (Table 5). A second dose of the hormone administered at 36 hours further lowered the activity of the preparation, to a value closely approaching "normal."

Variation in concentration of the polyribosomal preparation isolated from the uterus of the ovariectomized rat, as a function of time after hormone treatment, is shown in Fig. 8. From 2 to 48 hours there was a linear increase in the concentration of the polyribosomes. This increase was 600 to 700 percent at 48 to 72 hours if a second dose of hormone was administered at 36 hours. If the hormone was administered only at the start, the concentration of the preparation from cytoplasm increased linearly by 400 to 500 percent during the first 36 hours, but decreased thereafter to 200 to 300 percent of the control. This is further evidence for estrogenic control of cytoplasmic concentration of ribonucleoprotein particles in the organ (*3*, *4*), and is one indication of their relative instability (*40*). Profiles on sucrose-density gradients of the polyribosomal preparations isolated from uteri of ovariectomized animals treated with the hormone for 4, 12, or 24 hours were similar to each other, as well as to that of the preparation isolated from normal animals (*48*).

The uterus could be considered a factory for the production of protein. For this interpretation I can make an analogy to an industrial factory. There the output or net manufacturing capacity is controlled by the number of working units and the rate with which these units work or are run. I believe a new view of estrogen regulation of protein synthesis in the cytoplasm of uterine cells can be obtained by estimation of the net protein-synthesizing capacity of the organ, as a function of time after the ovariectomized rat is treated with the hormone. This capacity can be estimated by combining the information on the incorporation activity in the cell-free system of the polyribosomes (Fig. 7) with that of their concentration in the cytoplasm (Fig. 8). The effect of hormone treatment on the net protein-synthesizing capacity of the uterus is shown in Fig. 9. The capacity is estimated as the number of picomoles of ^{14}C-leucine that would have been incorporated in 30 minutes in the cell-free system, if all of the polyribosomes isolated from the organ homogenate containing 1 milligram of DNA had been incubated. From 2 to 24 hours after hormone treatment, this capacity for protein synthesis increases 300 percent. Thereafter to 72 hours, the capacity holds constant. The elevated protein-synthesizing capacity decreases more than 50 percent from 36 hours to 72 hours if a second dose of the hormone is not administered at 36 hours. The constant protein-synthesizing capacity exhibited by the organ between 24 hours and 72 hours, which results from serial administrations of estrogen, is achieved by the decrease in incorporation activity of the polyribosomal preparation (Fig. 7) being balanced by an increase in the amount of the preparation (Fig. 8). This observation indicates an equilibrium between the formation and the activity of uterine polyribosomes during the later period of estrogen action. This equilibrium for cytoplasmic genetic translation in the uterus is maintained by estrogen controlling the number and the activity of the polyribosomes in an inverse relation.

These observations lead to the conclusion that an essential feature of the early action of estrogen in the uterus is the appearance in the cytoplasm, of new polyribosomes, having amino acid incorporation properties different from the old ones occurring there. This conclusion is consistent with the previous studies (*3*, *4*) suggesting that both the number and activity of ribosomes in the organ are estrogen-dependent.

Table 4. Principal characteristics of the uterine cell-free system for protein synthesis (*40*). Polyribosomal preparation was isolated from homogenized pooled uteri of normal rats and assayed for amino acid incorporation activity (*40*). The ^{14}C-leucine incorporation activity was expressed as counts per minute per milligram of protein of polyribosomal preparation added to the reaction mixture.

Alterations to reaction mixture	Radioactivity incorporated (count/min)
None	406
Minus polyribosomal preparation	17
Minus 19 unlabeled amino acids	196
Minus ATP	62
Minus GTP	250
Minus supernatant enzyme fraction	28
Plus puromycin ($4 \times 10^{-4} M$)	48
Plus cycloheximide (50 μg)	244
Plus cycloheximide (100 μg)	164
Plus ribonuclease (5 μg)	58
Plus sodium fluoride (5 mM)	360
Plus sodium fluoride (10 mM)	250
Plus sodium fluoride (20 mM)	255
Plus ribonuclease-treated ribosomes, minus polyribosomal preparation*	197
Plus sodium fluoride-treated ribosomes; minus polyribosomal preparation*	302
Plus potassium deoxycholate-treated ribosomes, minus polyribosomal preparation*	209

* Monomeric ribosomes were prepared by incubation of the polyribosomal preparation in the presence of the enzyme, salt, or detergent (potassium deoxycholate). After this incubation the ribosomes were washed twice, and collected for assay in the cell-free system (*40*).

Table 5. Effect of ovariectomy on the ^{14}C-leucine incorporation activity of polyribosomal preparation in the cell-free system (*41*). Polyribosomal preparation from pooled uteri of normal or ovariectomized rats was isolated and assayed in the complete cell-free system as described in Table 4. The ovariectomized animals were used not less than 3 weeks after surgery, and 12 uteri were pooled for each experiment. The normal animals were in diestrus, and four uteri were pooled for each experiment. Activity in the cell-free system was expressed as counts per minute of ^{14}C-leucine incorporated per milligram of protein of the polyribosomal preparation added to the reaction tubes.

Average weight of uteri* (mg)	Radioactivity incorporated (count/min)
Normal animals	
266 ± 8	398
254 ± 9	406
245 ± 10	1708†
Ovariectomized animals	
71 ± 4	640
67 ± 3	627
67 ± 4	2680†

* Mean values, plus and minus one-half the range of measurements. †Expressed by counts per minute per milligram of RNA of polyribosomal preparation added to the reaction mixture.

Binding of Radioactive Estrogen to Chromatin

Another approach to the study of the early if not primary sites and mechanisms of action of estrogen is based on the concept that estrogen is bound only in its characteristic target organs, and that this binding is related to the subsequent physiological actions of the hormone. In particular, Jensen and his co-workers have emphasized this concept (*49, 50*). Both cytoplasmic (*51*) and nuclear binding sites (*52*) within the uterine cell have been proposed for estrogen. Autoradiographic studies have shown the occurrence of labeled estrogen in the uterine nucleus (*53*), and Maurer and Chalkley (*54*) have described some properties of a nuclear binding site for 17β-estradiol in calf endometrial cells. They estimated 2000 to 2500 binding sites per nucleus for the hormone and an equilibrium constant of $2 \times 10^{-8}M$, and they concluded that the binding of the hormone is stereospecific and noncovalent in the nature of its linkage. They suggest that the estrogen binds to a protein component of chromatin, and is not contamination as a result of cytoplasmic protein being isolated with the chromatin.

As a result of our finding of very early effects of estrogen on nuclear RNA synthesis in vivo and on the ratio of RNA to DNA in uterine chromatin (see Figs. 1 and 4), we measured the time course of the binding in vivo of radioactive estrogen to uterine and liver chromatin in the ovariectomized rat (*33*). In the 2-minute interval after administration of tritiated 17β-estradiol in vivo, the hormone's molecules entered the uterine cell, penetrated the nucleus, and were bound to the chromatin (Fig. 10). This was also the earliest time that we could detect a stimulation of nuclear RNA synthesis by the hormone (*14*). Radioactive 17β-estradiol was considered bound to chromatin if it was not released by dialysis for 18 hours at 0°C against 0.03*M* tris [tris(hydroxymethyl)aminomethane], *p*H 7.3 (*54*). Maximum binding occurred at 8 hours, and it is useful here to recall that, in the studies of estrogen-stimulated template activity of uterine chromatin, the peak stimulation occurred at 8 hours (Fig. 3). At this time in the action of estrogen in the uterus the template activity of the isolated chromatin is maximum (Fig. 3) and its content of RNA and histone are respectively elevated and reduced (Table 3). The causal mechanisms responsible for these genomic variations in template activity, chemical composition, and hormone binding remain to be elucidated. Substantial binding of the hormone to hepatic chromatin also occurred, although the amount of the binding was less than that bound to uterine chromatin (Fig. 10).

The relation of the binding of 17β-estradiol to uterine chromatin (*33, 54*) to the binding of the hormone to 9.5*S* and 5*S* cytoplasmic and nuclear receptor molecule(s) of the organ (*50, 52*) remains to be investigated. The role of the nuclear membrane in early estrogen action also remains to be clarified, and there is evidence that steroid hormones bind to the nuclear membrane (*49*). A comparable conclusion can be inferred from the findings of Kanno and Lowenstein (*55*) concerned with changes in nuclear membrane potentials of insect cells treated with ecdysone. In terms of molecular structure and binding phenomena, the major characteristics of the nuclear binding site of 17β-estradiol remain undescribed. The receptor

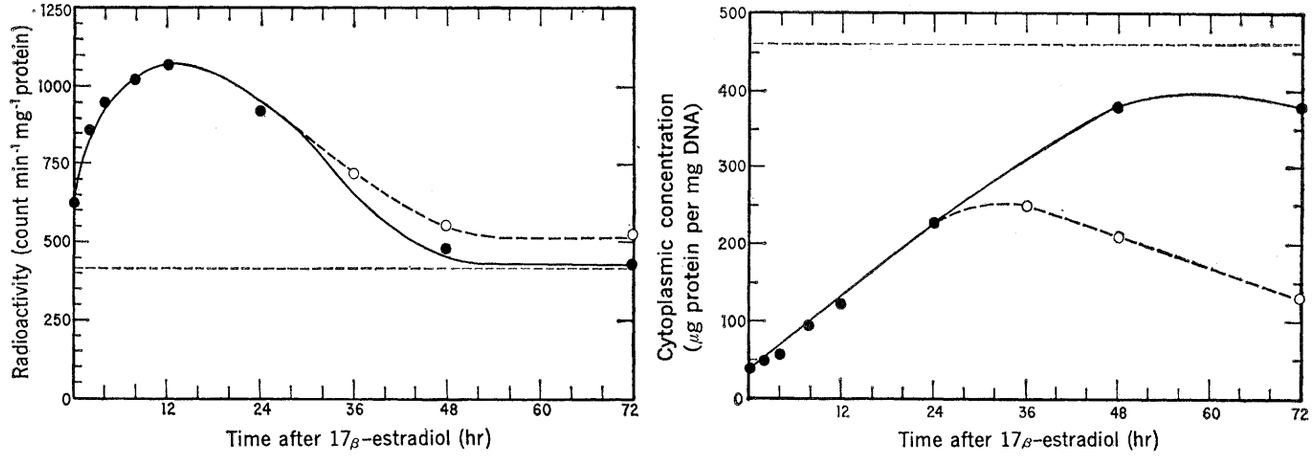

Fig. 7 (left). Effect of administration of 17β-estradiol on the ^{14}C-leucine incorporation activity in the cell-free system of the polyribosomal preparation isolated from the uterus of the ovariectomized rat (*41*). At zero time all experimental animals were given 10 μg of the hormone intraperitoneally. Each group consisted of ten animals, and individuals of certain groups were given a second dose of the hormone at 36 hours. At the times indicated the animals were killed, the uteri were removed, and the polyribosomal preparation was isolated. Recovery of the microsomal ribonucleoprotein in the polyribosomal preparation was 58 to 61 percent. The constituents of the cell-free system and the procedure of assay of incorporation activity were described in Table 4 and by Teng and Hamilton (*41*). Solid circles, incorporation of ^{14}C-leucine by the polyribosomal preparation isolated from uterine cells of animals given the hormone at zero time and again at 36 hours; open circles, from animals given the hormone at zero time only; broken line, from normal animals in diestrus. Fig. 8 (right). Effect of administration of 17β-estradiol on the cytoplasmic concentration of uterine polyribosomal preparation, as a function of time after administration of 17β-estradiol to the ovariectomized rat (*41*). The procedure and recovery of microsomal ribonucleoprotein in the polyribosomal preparation isolated were as described in Fig. 7 and elsewhere (*41*). Concentration of the polyribosomes in the cytoplasm was determined by the number of micrograms of protein of the preparation isolated per milligram of DNA in the tissue homogenate. Protein and DNA were determined chemically. Tht ratio of absorbance at 260 mμ to that at 280 mμ for each preparation isolated was 1.78 to 1.80. Solid circles, concentration of polyribosomes in cytoplasm of uterine cells of animals given hormone at zero time and again at 36 hours; open circles, concentration when hormone was given at zero time only; broken line, concentration for normal animals in diestrus (465 $\pm$ 20 μg of protein per milligram of DNA for triplicate analyses).

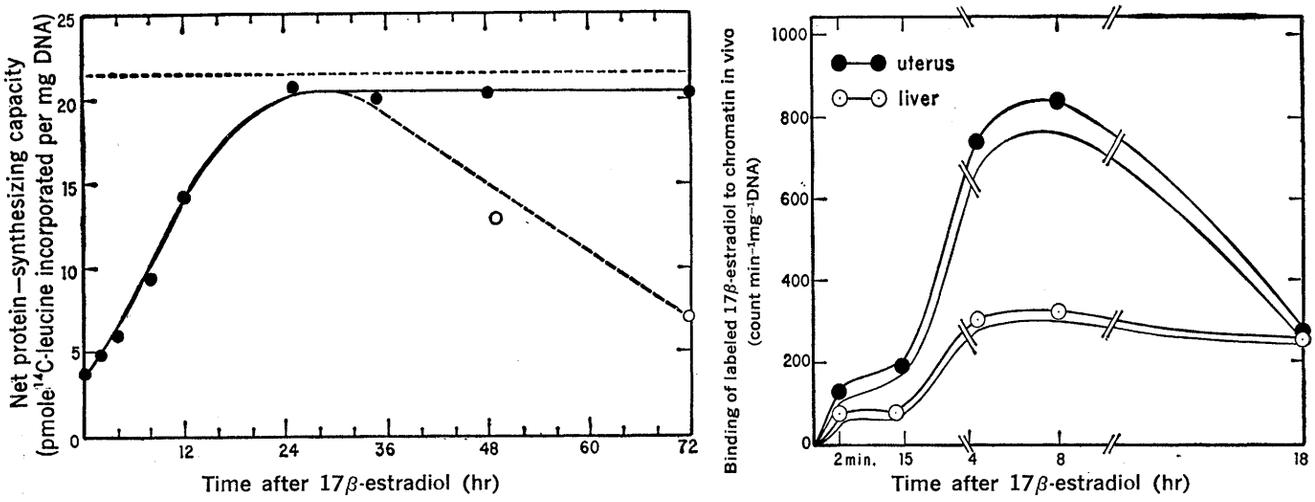

Fig. 9 (left). Effect of 17β-estradiol on the net protein-synthesizing capacity of the uterus of the ovariectomized rat (*41*). This figure combines the data of Fig. 7 with that of Fig. 8. Net protein-synthesizing capacity was estimated by converting each value for specific activity in Fig. 7 to the number of picomoles of ^{14}C-leucine incorporated per milligram of protein of polyribosomal preparation, and multiplying that value by the corresponding number of micrograms of protein of polyribosomal preparation per milligram of DNA in the tissue homogenate (Fig. 8). Solid circles, net protein-synthesizing capacity of uteri of animals administered 17β-estradiol at zero time and again at 36 hours; open circles, the net protein-synthesizing capacity of uteri of animals treated with hormone at zero time only; broken line, the net protein-synthesizing capacity of uteri of normal animals in diestrus. Fig. 10 (right). Binding of tritiated 17β-estradiol to chromatin isolated from the uterus or the liver of the ovariectomized rat (*33*). At zero time all animals were given 1 μg (150 μc) of the radioactive estrogen. At the times indicated thereafter the animals were killed, and the uteri and the liver fragments were respectively excised, pooled, and homogenized for isolation and washing of the chromatin (*33*). The binding was expressed as the number of counts of tritiated 17β-estradiol per minute per milligram of DNA of chromatin preparation. The thin lines indicate the binding values determined again after samples of the preparations previously isolated and washed had been dialyzed for 18 hours at 0°C against 0.003*M* tris, *p*H 7.3.

molecule in chromatin for the hormone appears to be a protein (*54*), but whether it is a histone is still uncertain. Evidence for the binding of testosterone (*56*) and hydrocortisone (*57*) to histone has been reported. However, now that techniques are available (*58*) for the electrophoretic separation of histones in polyacrylamide gel and for their chemical determination, some answers to these problems may soon be found.

Discussion and Conclusions

Considered together, the various studies surveyed and summarized in Fig. 11 indicate that genetic transcription is activated prior to sequential stimulations of nuclear and cytoplasmic genetic translation during the early action of estrogen in the uterus. The genetic program activated by the hormone early in its action apparently has as a primary function the initiation of biosynthetic and nuclear-to-cytoplasmic transport activities, which result in a restoration of the organ to a higher and "normal" level of protein-synthesizing capacity. Figure 11 also shows that the sequential administration of several doses of the hormone to the ovariectomized adult rat restores to the uterus the biochemical balance observed for the organ in the diestrous phase of the estrous cycle of the intact animal.

Although it is clear that in order to enter the nucleus estrogen must first pass or be transported (*49–52*) through the membranes, and the cytoplasm of the uterus, there appears to be one primary site of action of the hormone within the genome of the organ's cells (*14, 33, 54, 59*). Five observations support this conclusion. (i) Two minutes after injection of radioactive 17β-estradiol to the ovariectomized rat the hormone is tightly bound to uterine chromatin (Fig. 10). The kinetics of this binding and some general properties of the binding site have been described for chromatin isolated from endometrial cells of the calf uterus (*54*). (ii) During the same and very early period of hormone action, the rate of synthesis of nuclear RNA in the rat uterus is accelerated by 40 percent (*14*). (iii) The ratio of RNA to DNA of the chromatin preparation isolated 15 minutes after hormone treatment is also increased (Fig. 4). (iv) The incorporation of tritiated uridine into the RNA of the chromatin preparation is also enhanced by the hormone during the first 15 minutes of its action (*33*). (v) Church and McCarthy have discovered that, within minutes after estrogen administration, there is a qualitative and quantitative increase in the nuclear RNA of the uterus of the ovariectomized rabbit, as evidenced by analysis of RNA hybridized with DNA (*59*).

These observations leave little doubt that a stimulation of synthesis of nuclear RNA is a primary event in the early action of estrogen. The RNA thus synthesized is now best considered only in general terms as representing one or more types of chromosomal and ribosomal RNA (*6, 7, 12, 32, 60*). The function of the nuclear or nucleolar RNA synthesized early in the action of estrogen or testosterone, or during liver regeneration, may be more diverse than attributed commonly to "ribosomal RNA," as implied by the work of Trachewsky and Segal (*61*), Barton and Liao (*62*), Church and McCarthy (*63*), and others. Precise characterization of the RNA formed early in estrogen action, and its relation to the unstable nuclear RNA described by Harris and his co-workers (*64*) and to the chromosomal RNA described by Bonner and Widholm (*65*), remain to be determined. Of interest but yet uncertain significance here is the observation that cyclic 3',5'-adenylic acid stimulates uri-

dine incorporation in the uterus, taken from the ovariectomized rat and incubated in vitro for 1 hour in the presence of the nucleotide (*66*). It has also been reported that generation of the cyclic nucleotide is stimulated at 15 seconds after the rat uterus is stimulated by estrogen (*67*).

Current Models and Theories of Estrogen Action

Tata has reviewed (*7*) recent advances in the understanding of hormonal mechanisms. He points out that evidence accumulated since 1963 necessitates a revision of Karlson's idea (*17*) that the primary action of many growth-promoting and developmental hormones involves induction of messenger RNA synthesis. Karlson followed explicitly the Jacob-Monod model (*18*), whereby inducers cause derepression of specific operons for synthesis of messenger RNA and a resulting control of enzyme synthesis. The new evidence may be summarized as follows.

First, work from several laboratories (*36*) has indicated that messenger RNA in nucleated cells is transported from the nucleus to the cytoplasm only when it is attached to the smaller ribosomal component. In the cytoplasm, this complex appears to be united to the larger ribosomal component for the formation of a polyribosome. The majority of monomeric ribosomes isolated from the cytoplasm are thought to be derived from polyribosomes. Thus the mechanism of polyribosome formation must be considered in interpreting effects of hormone on messenger RNA synthesis and its transport to the cytoplasm, which determines the specificity of hormone action (*7*, *12*). Second, there is now evidence that insulin as well as adrenocorticophic and gonadotrophic hormones may control the translational activity of cytoplasmic polyribosomes (*68*). These translational effects of hormones seem to occur independently of effects on nuclear synthesis of RNA, and Tata emphasizes the probability that many hormones may have multiple rather than single primary actions and effects on the protein-synthesizing apparatus of the cells of their respective target organs. Taking the foregoing ideas into account, let us now discuss the mechanisms of estrogen action.

The nucleolus is the major site of synthesis of ribosomal RNA and of ribosomal components or precursors (*69*). Much of the nuclear RNA synthesized early in the action of estrogen in the uterus of the ovariectomized rat is of a ribosomal type (*9*, *12*). Laquens (*70*) has shown by electron microscopy that from 6 to 24 hours after estrogen action begins there is an increase in the size and number of nucleoli in the smooth muscle cells of the uterus; from 24 to 72 hours the nucleoli continue to increase in size and move closer to the nuclear membrane. At the same time there is an increase in the number of cytoplasmic ribonucleoprotein particles. The differential effects of estrogen on the activity and the formation of uterine polyribosomes (Figs. 7 and 8) probably represent dual effects of the hormone on genetic transcription in association with nucleolar as well as chromosomal function. The early stimulation of the Mg^{2+}-activated RNA polymerase reaction (Fig. 2), the product of which is a ribosomal type of RNA (Table 1), also points to this conclusion.

There is some positive evidence that messenger RNA synthesis is stimulated after an initial increase in the synthesis of chromosomal and ribosomal RNA during early estrogen action. For example, the relatively small but delayed enhancement of the reaction of RNA polymerase activated by Mn^{2+} and $(NH_4)_2SO_4$ in vitro only occurs after the cytoplasmic concentration of ribosomes is already rapidly increasing (Figs. 3 and 8). Since the product of this polymerase reaction is a more DNA-like type of RNA (Table 1), it can be assumed that the enhancement of this nuclear reaction is associated with the increased synthesis of messenger RNA geared to increased formation of ribosomal components programmed for transport to the cytoplasm for polyribosome formation. Supporting this assumption is the observation that between 12 hours and 24 hours, the ratio of RNA to DNA and that of protein to DNA increase in the uterine nucleus about 20 to 60 percent (Table 1), with values similar to those observed for the enhancement of the Mn^{2+}-$(NH_4)_2SO_4$-activated polymerase reaction during the same period (Fig. 11).

These parallel observations of en-

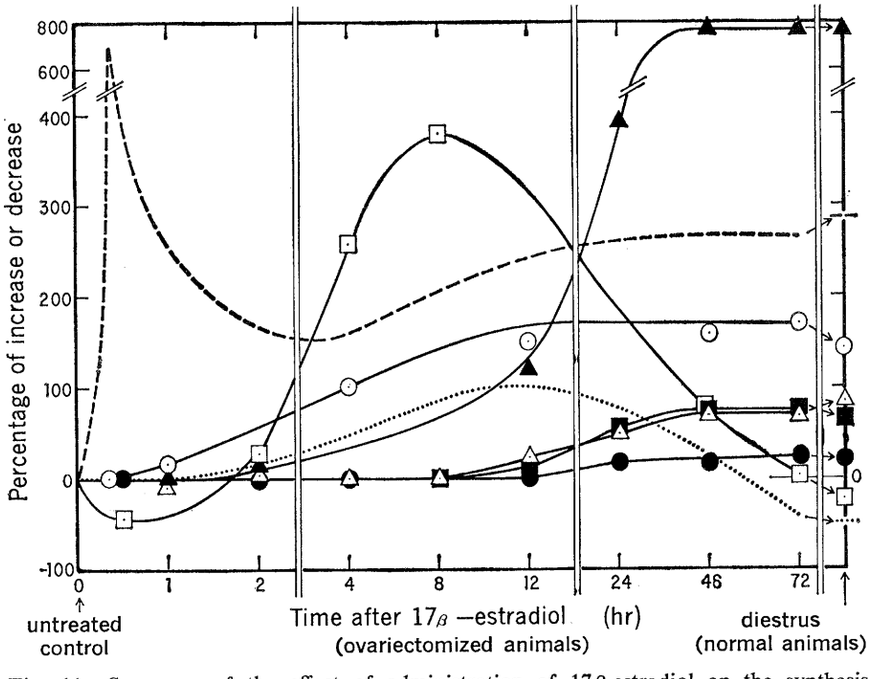

Fig. 11. Summary of the effect of administration of 17β-estradiol on the synthesis and accumulation of RNA and protein in the uterus of the ovariectomized rat. All animals received 10 μg of the hormone at zero time, and certain animals were given additional hormone at intervals of 24 hours thereafter. The symbols on the right ordinate indicate the biochemical data on uteri of normal animals in diestrus, similarly expressed as percentages of the control values. Open circles, activity of the Mg^{2+}-activated RNA polymerase reaction; open triangles, activity of the Mn^{2+}-$(NH_4)_2SO_4$-activated RNA polymerase reaction; open squares, specific activity of rapidly labeled nuclear protein in vivo; solid circles, ratio of nuclear RNA to nuclear DNA; closed squares, ratio of nuclear protein to DNA (values prior to 8 hours are not shown); closed triangles, concentration of ribonucleoprotein in the cytoplasm; broken line, specific activity of nuclear RNA rapidly labeled in vivo; dotted line, incorporation of ^{14}C-leucine in the cell-free system of cytoplasmic polyribosomes. The last two curves are taken from refs. *12*, *13*, and *16*.

hanced polymerase activity and nuclear content of RNA and protein relatively late in estrogen action suggest that another effect of the hormone leads to an increase in the protein-synthesizing capacities of subnuclear fractions, by increasing their content of ribonucleoprotein particles. Indeed, an amplification of the capacity of the nucleus to synthesize RNA (messenger as well as chromosomal and ribosomal RNA) and protein (RNA polymerase as well as structural ribosomal protein) may be necessary for the "underwriting" of the synthesis of the ribosomal components which turn over to and accumulate in the cytoplasm in polyribosomes. McCarty and his co-workers (*71*) have shown that ribosomes isolated from subfractions of the rat-liver nucleus synthesize protein by essentially the same mechanism as those found in the cytoplasm. Further, recent studies in my laboratory (*72*) and that of Allfrey and Mirsky (*73*) have shown by electron microscopy and autoradiography that the Mg^{2+}-activated RNA polymerase resides in the nucleolus, whereas the Mn^{2+}-$(NH_4)_2SO_4$-activated RNA polymerase is restricted almost entirely to the extranucleolar chromatin of the nucleus.

Whether a preferential synthesis of new messenger RNA molecules is necessary for the early effects of estrogen on protein synthesis in the uterine nucleus and cytoplasm is still unknown. However, the studies on early estrogen action can be interpreted without resorting to the idea of induction of synthesis of messenger RNA only by the hormone (*17*). On the contrary, one of the earliest effects of estrogen in its action may well be the stimulation or activation of some rate-limiting process which in turn controls the activity of the cistrons controlling the synthesis of several species of RNA. Thus the hormone would do no more than cause an acceleration of the synthesis of the same or several species of RNA molecules. For the present, the cistrons that control the synthesis of chromosomal and ribosomal RNA seem likely candidates for some of the primary sites of action of the hormone (*74, 75*).

In my laboratory we now follow the hypothesis that estrogen in its early action accelerates first the synthesis of chromosomal and ribosomal RNA and then the formation of ribosomal components or precursor particles. These particles probably then "pick up" messenger RNA molecules from their respective DNA sites (*76*), and transport them to the cytoplasm for completion of ribosomal synthesis and thus of polyribosome formation. This hypothesis is compatible with the demonstration that *Escherichia coli* ribosomes stimulate the capability of the DNA-RNA polymerase complex of the system to synthesize RNA (*77*). The hypothesis is in agreement with the work indicating that either the small ribosomal subunits (*36*) or "informosome" particles (*78*) transport messenger RNA from the nucleus to the cytoplasm. It is also consistent with the demonstration that hydrocortisone causes an accelerated rate of transfer of 45*S* ribonucleoprotein particles from the nucleus to the cytoplasm in liver (*79*). The hypothesis also falls into line with Tata's suggestion (*7*) that a coordinated proliferation of a unit of membrane, ribosomes, and messenger RNA geared to the rate of protein synthesis is a common property of multicellular control systems (*80*).

Stimulation of genetic transcription precedes that of genetic translation during the early action of estrogen—insofar as a genetic program for increased nuclear-to-cytoplasmic transfer of ribonucleoprotein particles and an increase in the protein-synthesizing capacity of the uterine cytoplasm is concerned. This is not to say that this is the only genetic program activated or controlled by the hormone in the organ. Evidence for the synthesis in vitro by estrogen of specific proteins now exists (*75, 81*) for several other systems influenced by the hormone, and these studies and the techniques may be of value in further studies of the mechanisms through which estrogen controls the synthesis of new proteins. Separate, hormone-dependent genetic programs may exist in uterine cells responsive to hormones: one for quantitative control in overall RNA and protein metabolism such as described, and one for the qualitative control of messenger RNA synthesis or translation for the specific synthesis of individual proteins. As Tata (*7*) has suggested, growth-promoting hormones such as estrogen may have multiple rather than single primary mechanisms or sites of action. The estrogen-deficient uterus of the ovariectomized adult rat should not be expected to exhibit a sequential array of new and specific proteins synthesized de novo during its initial response to hormonal stimulation. A return to a higher and more efficient state of protein synthesis with a corresponding increase in the number of cytoplasmic organelles necessitates only an expansion of pre-existing biosynthetic and transport mechanisms. However, the development of the immature rat uterus or of the chick oviduct (*32, 82*) may demand the synthesis of new proteins. Correspondingly, the uterus of the adult rat encounters a new hormonal environment during its transition from estrus to the pregnancy state. A new constellation of proteins may well appear at that time, correlated with the new functions of the organ (*83*). In this context it is important to note that recently two groups reported (*83a*) that estrogen induces the synthesis of new species of nuclear RNA in the chick oviduct, as indicated by studies of RNA hybridized with DNA.

Prospects

The uterus undoubtedly has several if not many genetic programs permitting a wide array of metabolic responses to variations in hormone titer and diversity, and in terms of molecular mechanisms we know almost nothing of this control. Of particular interest concerning the direct or indirect effect of estrogen on the activity of the uterine genome is the recent isolation from *Escherichia coli* systems of repressor proteins (acid type) by Gilbert and Müller-Hill (*84*) and by Ptashne (*85*). Investigations of the roles of chromosomal RNA (*86*) as well as of acidic (*87*) and basic (*58, 88*) proteins in the nucleus during early estrogen action in the uterus should provide basic information. Solution of related problems dealing with the interaction between 17β-estradiol molecules and the nuclear receptors or protein components of uterine chromatin, should reveal the nature of the repressor-inducer interaction in this system. Such findings would also aid us in understanding the molecular mechanisms regulating genic activity in multicellular organisms. There are already hints (*13, 26*) of the synthesis of protein or proteins having repressor or suppressor functions in control of RNA synthesis in the estrogen-deficient uterus.

Summary

Although the precise molecular mechanisms are almost totally unknown, recent studies of RNA and protein synthesis in the uterus of the ovariectomized adult rat suggest the following intracellular sequence for some of the events occurring during

the early action of 17β-estradiol: (i) binding of the hormone to the chromatin in the nucleus; (ii) stimulation of synthesis of chromosomal and ribosomal RNA in conjunction with chromosomal and nucleolar activity; (iii) an acceleration of the rate of formation of ribosomal precursor particles; (iv) an acceleration of transport of the ribosomal precursor particles with attached messenger RNA to the cytoplasm; and (v) an accumulation of new polyribosomes in the cytoplasm having different amino acid incorporation properties compared to those of the old ones. The effect of estrogen on cytoplasmic protein seems indirect, with effects of the hormone on genetic transcription resulting in a regulation of the rate and amount of genetic translation.

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on the fractional distribution of ribosomes and their membrane attachments and respective amino acid incorporation activities in vitro. He concludes that the regulation of protein synthesis by growth and developmental hormones may involve a simultaneous control of the rates at which cytoplasmic RNA and membranes are proliferated as functional units within the cell. This may have important implications for our finding (Figs. 7 and 8) that new polyribosomes appear in the cytoplasmic fraction of the homogenized uterus during early estrogen action, with different amino acid incorporation properties compared to the old ones. There is evidence that phospholipid synthesis is stimulated during early estrogen action (*81*), and this occurs approximately at the time that we find new polyribosomes appearing and accumulating in the cytoplasm (*41*).

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Undergraduate Achievement and Institutional "Excellence"

Traditional indices of institutional quality do not appear to contribute to student achievement.

Alexander W. Astin

Although the American system of higher education is noted for its diversity, most of its institutions pursue a common quest for quality or "excellence." Among the attributes which are generally regarded as indices of excellence are a select student body, a highly trained faculty, an institutional emphasis on scholarship, a large library, a high faculty-student ratio, and a vigorous program of research. Perhaps the most important benefit presumed to derive from these attributes concerns the intellectual development of the student. In the folklore of higher education, it is assumed that the student's learning and intellectual development will be enhanced if he attends a "high-quality" institution. The principal purpose of the research reported here was to test this assumption empirically, by means of a longitudinal study of undergraduate students attending colleges of varying degrees of "quality."

Design of the Study

Studies of the comparative effects of collegiate institutions on the student's development are difficult to design, primarily because students are not distributed randomly among institutions. On the contrary, particular types of students are attracted to particular types of institutions. Under these circumstances, observed variations among colleges in the average achievement of their students may result from differences in ability that existed prior to matriculation, as much as from the differential impact of the institutions themselves. The nonrandom character of college attendance necessitates the use of "natural experiments," in which the comparative influence of different institutions is examined only after some attempt is made to compensate for differences in the average academic ability of the entering students.

The need for controlling differential student inputs is well illustrated by the history of studies of institutional "Ph.D. productivity" (*1*). In general, this research has shown that an adequately controlled study requires the following three types of data. (i) Student output information—performance or achievement of the student; these data represent the dependent variable or variables of concern in the study; (ii) student input (control) information—characteristics of the student, at the time of college entrance, that might affect his subsequent performance on the output measures; (iii) environmental data—characteristics of the student's college that might affect his subsequent output performance.

Sample of Students

The sample of 669 students used in the analysis was drawn from a larger sample comprising the freshman classes entering a stratified national sample of 248 accredited, 4-year colleges and universities in the fall of 1961 (*2*). A subject was included in the study if he satisfied the following four conditions: (i) he was among the random samples of approximately 250 students at each college who were selected for a follow-up study conducted in 1962 (*3*); (ii) his institution was one of the 38 in our sample that administered the area tests from the Graduate Record Examinations to its seniors in 1965; (iii) he could be positively identified by name among those students from whom Graduate Record Examination scores were available; (iv) he had taken the National Merit Scholarship Qualifying Test while in high school, and his scores could be obtained from the files of the National Merit Scholarship Corporation. This last requirement was considered essential, since the student's academic ability before entrance into college was expected to be a major determinant of his subsequent performance on the Graduate Record Examination (*4*).

The author is director of research, American Council on Education, Washington, D.C., and is currently on leave as a fellow at the Center for Advanced Study in the Behavioral Sciences, Stanford, California. This article is adapted from chapter 3 of *The Educational and Vocational Development of American College Students*, to be published in the fall by the American Council on Education.