Regulation of Polyribosome Formation and Protein Synthesis in the Uterus

EFFECT OF OVARECTOMY AND ADMINISTRATION OF OESTRADIOL-17 β ON THE AMINO ACID-INCORPORATION ACTIVITY *IN VITRO* AND THE CYTOPLASMIC CONCENTRATION *IN VIVO* OF POLYRIBOSOMAL PREPARATION

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1. The hormonal regulation of cytoplasmic protein synthesis in the uterus is described. Polyribosomal preparation from uteri of normal or ovariectomized rats was isolated by procedure 3 and assayed for [^{14}C]leucine-incorporation activity in the cell-free system, as described by Teng & Hamilton (1967). 2. Ovariectomy of normal animals caused, 3 weeks after surgery, a 50–60% increase in the amino acid-incorporation activity *in vitro* of uterine polyribosomal preparation, but a 90–95% decrease in the cytoplasmic concentration *in vivo* of the preparation. 3. Administration of 10 μg . of oestradiol-17 β to ovariectomized rats at zero time caused, 10–12 hr. later, a 100% stimulation in amino acid-incorporation activity *in vitro* of the uterine polyribosomal preparation. From 12 hr. to 36 hr. after hormone administration, the activity *in vitro* of the preparation decreased. If a second dose of hormone was administered at 36 hr., the activity *in vitro* of the preparation continued to decrease, and approached at 48 hr. and 72 hr. the lower activity observed for the preparation from normal animals. The cytoplasmic concentration of polyribosomal preparation increased by 600–700% under these experimental conditions. If a second dose of oestradiol-17 β was not administered at 36 hr., the initially elevated cytoplasmic concentration of the preparation decreased by 50% from 36 hr. to 72 hr., and the activity *in vitro* of the preparation was not fully depressed to the 'normal' value. 4. Pretreatment of ovariectomized animals with actinomycin D or cycloheximide abolished 80–90% of the stimulatory effects of hormone treatment on the amino acid-incorporation activity *in vitro* and cytoplasmic concentration *in vivo* of uterine polyribosomal preparation. 5. Two major conclusions are drawn from the results reported: that during early oestrogen action new polyribosomes having amino acid-incorporation properties different from those of the old ones appear and accumulate in the cytoplasm of the uterus; and that the regulation of cytoplasmic protein synthesis in the organ by oestrogen is of an indirect nature, with dual effects of the hormone on genetic transcription resulting in turn in a regulation of the rate and amount of genetic translation.

In the preceding paper we described the sedimentation characteristics and the amino acid-incorporation properties of the cell free system of a polyribosomal preparation isolated from the cytoplasmic fraction of the normal rat uterus (Teng & Hamilton, 1967). The polyribosomal preparation contained mixed polymeric and monomeric ribosomes isolated from the postmitochondrial supernatant fraction not treated with detergent. We concluded that uterine polyribosomes are relatively unstable, and cited results of other investigations (Greenman & Kenny, 1964; Moore & Hamilton, 1964; Means & Hamilton, 1966a) suggesting that

the number and the activity of cytoplasmic polyribosomes in the organ are oestrogen-dependent. The results of several recent investigations have pointed to the conclusion that regulation of cytoplasmic protein synthesis in the uterus is a consequence, in part at least, of genetic transcription controlled by the hormone (for reviews see Tata, 1966; Segal & Scher, 1967; Hamilton, 1968).

In the present paper we describe the regulation by oestrogenic hormone of cytoplasmic protein synthesis in the uterus of the rat. We demonstrate that, by administration of exogenous oestrogen to the ovariectomized rat, the net protein-synthesizing

capacity of the uterus can be elevated to a value near that of the organ in the normal animal. The following analyses have been performed: (a) the effect of ovariectomy in normal animals on the amino acid-incorporation activity *in vitro* of the uterine polyribosomal preparation; (b) the effect of administration to ovariectomized animals of oestradiol-17 β on the incorporation activity *in vitro*, cytoplasmic concentration *in vivo* and sedimentation profile of the polyribosomal preparation; (c) the effect of different concentrations of oestradiol-17 β in the cell-free system on the activity of the polyribosomal preparation; (d) the effect of inhibitors of RNA and protein synthesis *in vivo* on the activity *in vitro* and cytoplasmic concentration *in vivo* of the polyribosomal preparation, 4hr. after hormone treatment; (e) the effect of oestradiol-17 β *in vivo* on the activity *in vitro* of a uterine supernatant enzyme fraction, 10hr. after hormone treatment. We conclude that the regulation by oestrogen of cytoplasmic protein synthesis in the uterus is primarily indirect, occurring by an initial stimulation of genetic transcription that modulates in turn the rate and amount of genetic translation. We further conclude that the formation and accumulation in the cytoplasm of new polyribosomes having amino acid-incorporation properties different from those of the old ones occurring in the organ are important aspects of the early action of oestrogen.

METHODS AND MATERIALS

Nearly all of the methods and materials employed in this study have been described previously (Teng & Hamilton, 1967). The additional experimental details are described below.

All normal animals used were in the dioestrous phase of the oestrous cycle. Ovariectomized animals were used no sooner than 3 weeks after surgery. Oestradiol-17 β dissolved in 0.1ml. of propane-1,2-diol was administered intraperitoneally at zero time, and in certain experiments at 36hr. also. Control animals received 0.1ml. of the solvent without hormone. In experiments with inhibitors, either 250 μ g. of actinomycin D dissolved in 0.1 ml. of propane-1,2-diol or 1 mg. of cycloheximide dissolved in 0.1 ml. of buffered 0.9% NaCl was administered intraperitoneally, 1hr. before the administration of hormone or of solvent without hormone.

Uterine polyribosomal preparation was isolated according to procedure 3 described by Teng & Hamilton (1967). About 60% of the microsomal RNP* was recovered in all preparations isolated. Polyribosomal preparation was assayed for amino acid-incorporation activity in the complete cell-free system containing among other constituents a cytoplasmic enzyme fraction isolated from ovariectomized rat liver, which contained transfer factors, activating enzymes and soluble RNA. Preparation of this fraction, designated AS₇₀-enzyme fraction, and the assay of amino acid-incorporation activity *in vitro* was exactly as described by

Teng & Hamilton (1967). Specific activity of the polyribosomal preparation in the cell-free system was quantitated as counts/min. of [¹⁴C]leucine incorporated/mg. of protein of polyribosomal preparation. The protein content of the preparation was determined chemically by the procedure of Lowry, Rosebrough, Farr & Randall (1951). In certain experiments, the RNA content of the preparation was determined chemically by the procedure of Ceriotti (1955).

The cytoplasmic concentration of the polyribosomal preparation isolated was determined by the amount in μ g. of protein (Lowry *et al.* 1951) of the preparation isolated/mg. of DNA (Burton, 1956) in the whole-tissue homogenate. For several experiments, AS₇₀-enzyme fraction was prepared from the cytoplasmic fraction of pooled uteri. The procedure followed was identical with that used to obtain the enzyme fraction from liver by Teng & Hamilton (1967).

Oestradiol-17 β was obtained from Sigma Chemical Co., St Louis, Mo., U.S.A., and cycloheximide from Nutritional Biochemical Corp., Cleveland, Ohio, U.S.A. Propane-1,2-diol was obtained from Matheson, Coleman and Bell Inc., East Rutherford, N.J., U.S.A. Actinomycin D was a gift from Merck, Sharpe and Dohme Research Laboratories, Rahway, N.J., U.S.A.

RESULTS

Effect of ovariectomy. Table 1 shows the effect of ovariectomy on the amino acid-incorporation activity in the complete cell-free system of uterine polyribosomal preparation. The incorporation activity of the preparation from ovariectomized animals was consistently 50–60% greater than that of the preparation from normal animals. This result was observed whether the incorporated radioactivity in the cell-free system was quantitated per mg. of protein or per mg. of RNA of the polyribosomal preparation assayed (Table 1). In several experiments we investigated the amino acid incorporation activity *in vivo* of the uterine polyribosomal preparation by 'pulse-labelling' ovariectomized or normal animals with 20 μ C of ¹⁴C-labelled acid protein hydrolysate 10min. before they were killed. Uterine polyribosomal preparations from the two groups of animals were isolated and examined by sedimentation in sucrose density gradients as described by Teng & Hamilton (1967). The respective profiles of *E*₂₈₀ and of acid-insoluble radioactivity for the two preparations were essentially the same (see Teng & Hamilton, 1967), although the radioactivity of the fractions of the preparation from ovariectomized animals was slightly higher (5–10%) than that from normal animals. Because of the reduced concentration of cytoplasmic ribosomes in the uterus of the ovariectomized rat (Moore & Hamilton, 1964), and because of the larger size of the pool of free amino acids in the uterus of the normal rat (Kalman & Lombrozo, 1961), we abandoned investigations of amino acid incorporation *in vivo*.

* Abbreviation: RNP, ribonucleoprotein.

Table 1. [¹⁴C]Leucine-incorporation activity in the cell-free system of the uterine polyribosomal preparation isolated from the normal or the ovariectomized rat

Polyribosomal preparation was isolated, as described in the text, from the cytoplasmic fraction of pooled uteri taken from normal or ovariectomized rats each weighing 168–176 g. Recovery of microsomal RNP in the preparation isolated was 58–61% in all experiments. The preparation was assayed for [¹⁴C]leucine-incorporation activity in the complete cell-free system incubated for 30 min. at 37°, exactly as described by Teng & Hamilton (1967). Approximately 0.5 mg. of protein or 0.13 mg. of RNA of polyribosomal preparation was added to each assay tube. The amount of protein or of RNA in the preparation added was determined chemically as described in the text. The average weights of uteri are given as mean ± 0.5 of the range.

Source of uterine polyribosomes	Expt. no.	Average weight of uteri (mg.)	[¹⁴ C]Leucine-incorporation activity (counts/min./mg. of protein)
Normal animals*	1	266 ± 8	398
	2	254 ± 9	406
	3	256 ± 6	410
	4	245 ± 10	1708†
Ovariectomized animals†	1	71 ± 4	640
	2	67 ± 3	627
	3	68 ± 3	646
	4	67 ± 4	2680†

* Four rats and four uteri/expt. † 12 rats and 12 uteri/expt. ‡ Expressed as counts/min./mg. of RNA.

Effect of hormone administration. Preliminary experiments indicated that the administration of two doses of hormone, one at zero time and another at 36 hr., and a response period of 48–72 hr. were necessary to increase the weight and concentration of RNP of the uterus of the ovariectomized rat to a value close to that in the organ in the normal animal at dioestrus. Fig. 1 shows the effect of administration of one or two doses of 10 µg. of oestradiol-17β to ovariectomized animals on the amino acid-incorporation activity *in vitro* of uterine polyribosomal preparation. By 2 hr. after hormone administration *in vivo*, the incorporation activity of the uterine polyribosomal preparation had increased by 30%. By 8–12 hr. the activity had increased by about 100%. Thereafter, however, the activity declined and approached, at 72 hr., the lower activity observed for uterine polyribosomal preparation from normal animals (Table 1). It is important to note that administration of a second dose of oestradiol-17β at 36 hr. was necessary to complete the hormone-induced lowering of the activity of the polyribosomal preparation to the ‘normal’ value. The differential trend in the

Fig. 1. Effect of oestradiol-17β *in vivo* on the [¹⁴C]leucine-incorporation activity of the uterine polyribosomal preparation assayed in the cell-free system, as a function of time after the administration of the hormone to ovariectomized rats. At zero time all experimental animals were given 10 µg. of oestradiol-17β dissolved in 0.1 ml. of propane-1,2-diol. Each group consisted of 10 animals, and individuals of certain groups were given a second dose of the hormone at 36 hr. At the times indicated, animals were killed, the uteri removed and the polyribosomal preparations isolated by procedure 3, as described in the text and by Teng & Hamilton (1967). The recovery of microsomal RNP in the preparation and the assay of incorporation activity were as described in Table 1 and by Teng & Hamilton (1967). ●—●, [¹⁴C]Leucine-incorporation activity of uterine polyribosomal preparation from animals given the hormone at zero time and again at 36 hr.; ○—○, activity of the preparation from animals administered hormone only at zero time; ----, activity of the preparation from normal animals in dioestrus (see Table 1).

amino acid-incorporation activity *in vitro* of the polyribosomal preparation resulting from the action of exogenous oestrogen *in vivo* was also observed in preparations isolated under identical experimental conditions, but assayed in the cell-free system containing ¹⁴C-labelled acid protein hydrolysate rather than [¹⁴C]leucine and the other 19 unlabelled amino acids.

Fig. 2 charts the variation in amount of the polyribosomal preparation isolated from the cytoplasmic fraction of uteri, as a function of time after administration to ovariectomized animals of 10 µg. of oestradiol-17β at zero time only, or at zero time and again at 36 hr. From 2 hr. to 48 hr. there was a linear increase in the cytoplasmic concentration of the preparation. This increase was 600–700% at 48–72 hr., provided that the second dose of hormone was administered to the ovariectomized animals at 36 hr. If the hormone was administered to ovariectomized animals at zero time only, the cytoplasmic concentration of the preparation increased linearly by 400–500% during an interval of 24–36 hr., but declined thereafter to a concentration of 200–300% of that of controls at 72 hr.

Fig. 2. Effect of oestradiol-17 β *in vivo* on the cytoplasmic concentration of uterine polyribosomal preparation, as a function of time after the administration of the hormone to ovariectomized rats. Conditions of hormone administration, killing of animals and isolation of the uterine polyribosomal preparation were as described in Fig. 1 and in the text. Recovery of microsomal RNP in the preparations isolated was 58–61%. Cytoplasmic concentration of the polyribosomal preparation was determined by the amount in μ g. of protein of the preparation isolated/mg. of DNA in the whole-tissue homogenate. Protein and DNA were determined chemically as described in the text. The E_{260}/E_{280} ratio of each preparation isolated was 1.78–1.80. ●—●, Cytoplasmic concentration of the uterine polyribosomal preparation in animals given the hormone at zero time and again at 36 hr.; ○—○, concentration of the preparation in animals given the hormone only at zero time; —, concentration of the preparation determined for normal animals in dioestrus ($465 \pm 20 \mu$ g. of protein/mg. of DNA for triplicate analyses).

Fig. 3 shows the profiles on sucrose density gradients of uterine polyribosomal preparations isolated from ovariectomized animals, and from ovariectomized animals treated with hormone for 4 hr., 12 hr. or 24 hr. The profiles are similar, suggesting that an overall change in the size of polymeric ribosomes or in the relative abundances of polymeric and monomeric ribosomes did not occur during the period of hormone action examined. The sedimentation profile of the uterine polyribosomal preparation from ovariectomized animals was also similar to that of the preparation from normal animals (cf. Teng & Hamilton, 1967). This is further evidence that oestrogenic hormone in its action does not influence the size of polymeric ribosomes by altering the number of ribosomes/molecule of messenger RNA, but influences the number and activity of polymeric ribosomes in the cytoplasm (see Figs. 1 and 2).

Effect of oestradiol-17 β *in vitro*. We tested the effect on amino acid incorporation in the cell-free system of three concentrations of oestradiol-17 β . There was no evidence of stimulation of the amino acid-incorporation activity of uterine polyribosomal

preparation *in vitro* as a result of addition of oestradiol-17 β to the complete system (Table 2). At a final concentration of 10^{-6} M, the hormone had no significant effect on incorporation activity. The same was true if the more soluble oestradiol sulphate was added to the complete system to a final concentration of 10^{-6} M. With higher concentrations of oestradiol-17 β (10^{-4} M or 10^{-2} M) in the complete system, there was a 14–46% inhibition of incorporation, inhibition increasing with concentration of hormone in the system containing uterine polyribosomal preparation from normal animals. The significance and mechanism of this inhibitory effect of oestradiol-17 β on amino acid incorporation *in vitro* is unknown.

Effect of inhibitors of RNA and protein synthesis. Numerous studies have shown that many of the biochemical and physiological responses of the uterus and other tissues to steroidal hormones are blocked by pretreatment of animals with actinomycin D or cycloheximide, potent inhibitors of RNA synthesis and protein synthesis respectively (for reviews see Williams-Ashman, 1965; Tata, 1966). Table 3 shows that pretreatment of ovariectomized animals with either 250 μ g. of actinomycin D or 1 mg. of cycloheximide abolished the expected oestrogenic enhancement of the amino acid-incorporation activity *in vitro* (Fig. 1) and of the cytoplasmic concentration *in vivo* (Fig. 2) of uterine polyribosomal preparation. This demonstration that RNA synthesis and protein synthesis are prerequisites for the stimulation by oestradiol-17 β *in vivo* of the activity *in vitro* and concentration *in vivo* of the uterine polyribosomal preparation is not surprising. The inhibitor experiments do, however, point to the importance and requirement of genetic transcription for oestrogenic stimulation of genetic translation in the uterus (cf. Ui & Mueller, 1963; Gorski, 1964; Hamilton, 1964).

Effect of ovariectomy or hormone administration on the AS₇₀-enzyme fraction. In the experiments described above, an AS₇₀-enzyme fraction from the liver of the ovariectomized rat was used in the cell-free system for the assay of amino acid incorporation. We considered the possibility that oestradiol-17 β exerts an influence on the activity of the AS₇₀-enzyme fraction of the uterus and thus affects the rate of amino acid incorporation by the polyribosomes. Table 4 summarizes the data for 'criss-cross' experiments testing the activity in the cell-free system of AS₇₀-enzyme fraction, isolated from uteri of normal, ovariectomized or hormone-treated ovariectomized animals, against polyribosomal preparation from uteri of normal or ovariectomized animals. In no instance was there a significant effect of the hormone *in vivo* on the activity *in vitro* of the enzyme fraction. In the cell-free experiments shown in Table 4, 3.0 mg. of protein

Fig. 3. Sedimentation profiles of uterine polyribosomal preparations from ovariectomized rats, as a function of time after hormone administration. The preparations were isolated by procedure 3 and distributed on 10–34% (w/v) continuous sucrose density gradients, as described by Teng & Hamilton (1967). Animals were given 10 μ g. of oestradiol-17 β at zero time, and killed 4 hr., 12 hr. or 24 hr. later. (a) Profile of the uterine polyribosomal preparation from animals not treated with the hormone; (b) profile of the preparation from animals treated with the hormone for 4 hr.; (c) profile of the preparation from animals treated for 12 hr.; (d) profile of the preparation from animals treated for 24 hr.

Table 2. *Effect of the concentration of oestradiol-17 β in the cell-free system on [14 C]leucine incorporation by the uterine polyribosomal preparation*

Polyribosomal preparation was isolated from pooled uteri of normal or ovariectomized rats, as described in the text. The assay of amino acid-incorporation activity *in vitro* was exactly as described by Teng & Hamilton (1967). Approximately 0.5 mg. of protein of the polyribosomal preparation was added to each assay tube. Oestradiol-17 β was dissolved in ethanol before addition to the tubes. Control tubes received the same amount of ethanol without the hormone. The other experimental details were as already described.

Incubation system	[14 C]Leucine-incorporation activity (counts/min./mg. of protein)
Uterine polyribosomal preparation from normal animals	
Complete system without oestradiol-17 β	416
Complete system containing 10 $^{-6}$ M-oestradiol-17 β	408
Complete system containing 10 $^{-4}$ M-oestradiol-17 β	349
Complete system containing 10 $^{-2}$ M-oestradiol-17 β	722
Uterine polyribosomal preparation from ovariectomized animals	
Complete system without oestradiol-17 β	680
Complete system containing 10 $^{-6}$ M-oestradiol-17 β	394
Complete system containing 10 $^{-4}$ M-oestradiol-17 β	368
Complete system containing 10 $^{-2}$ M-oestradiol-17 β	355

Table 3. *Effect of actinomycin D or cycloheximide treatment in vivo on the stimulation of the amino acid incorporation activity in vitro and cytoplasmic concentration in vivo of the uterine polyribosomal preparation by oestradiol-17 β*

Polyribosomal preparation was isolated as already described from the cytoplasmic fraction of uteri taken from ovariectomized rats treated as designated. Recovery of microsomal RNP in the polyribosomal preparations isolated was 60–62%. Each control or experimental group consisted of six animals each weighing 174–179 g. To each animal was administered 10 μ g. of oestradiol-17 β dissolved in 0.1 ml. of propane-1,2-diol, or 0.1 ml. of the solvent without hormone. In experiments with inhibitors, each animal was given either 250 μ g. of actinomycin D or 1 mg. of cycloheximide 1 hr. before the administration of hormone or of the solvent without hormone. All animals were killed 4 hr. after the administration of hormone or of the solvent. The assay of the incorporation activity *in vitro* was as already described, approximately 0.5 mg. of protein of polyribosomal preparation being added to each assay tube. The cytoplasmic concentration *in vivo* of polyribosomal preparation was determined by the amount in μ g. of protein isolated/mg. of DNA in the whole-tissue homogenate (see Fig. 2). The other experimental details were as described in the text and by Teng & Hamilton (1967). The results are representative of replicate analyses.

Treatment of animals	Average weight of uteri* (mg.)	[¹⁴ C]Leucine-incorporation activity (counts/min./mg. of protein)	Cytoplasmic concentration of polyribosomal preparation (μ g. of protein/mg. of DNA)
Control	66 $\pm$ 3	641	43
Oestradiol-17 β	104 $\pm$ 4	956	74
Actinomycin D	71 $\pm$ 3	352	45
Actinomycin D and oestradiol-17 β	74 $\pm$ 5	678	51
Cycloheximide	76 $\pm$ 4	548	40
Cycloheximide and oestradiol-17 β	78 $\pm$ 5	697	46

* Mean $\pm$ 0.5 of the range of six uteri/expt.

Table 4. *Effect of ovariectomy and administration of oestradiol-17 β on the amino acid incorporation activity in vitro of the uterine AS₇₀-enzyme fraction*

AS₇₀-enzyme fraction was isolated from pooled uteri of six normal rats, of 30 ovariectomized rats or of 20 ovariectomized rats that had been given 10 μ g. of oestradiol-17 β 10 hr. before they were killed. The procedure for isolating the enzyme fraction was as described in the text and by Teng & Hamilton (1967). The assay of incorporation activity *in vitro* was as already described, with the exception that 3.0 mg. of protein of uterine enzyme fraction, rather than 1.5 mg. of liver enzyme fraction, was added to the incubation medium. Uterine polyribosomal preparation was isolated as already described, and approximately 0.5 mg. of protein of this preparation was added to each assay tube.

Source of AS ₇₀ -enzyme fraction ...	[¹⁴ C]Leucine-incorporation activity (counts/min./mg. of protein)		
	Uteri of normal rats	Uteri of hormone-treated ovariectomized rats	Uteri of ovariectomized rats
Source of uterine polyribosomal preparation			
Normal rats	302	278	279
Ovariectomized rats	535	537	497

of the uterine AS₇₀-enzyme fraction was added to the incubation medium. Preliminary experiments had shown that the enzyme fraction of the uterus was about 50% less active than the enzyme fraction isolated from the liver of the ovariectomized animal. Finally, the data of Table 4 demonstrate again that polyribosomal preparation from the uterus of the ovariectomized animal is 50–60% more active in

the cell-free system than the preparation from the uterus of the normal animal (cf. Table 1).

DISCUSSION

Some insight into the problem of oestrogenic regulation of cytoplasmic protein synthesis is gained by estimating the net protein-synthesizing

Fig. 4. Effect of oestradiol-17 β *in vivo* on the net protein-synthesizing capacity of uteri of ovariectomized rats, as a function of time after hormone treatment. This Figure combines the data of Fig. 1 with data of Fig. 2. Net protein-synthesizing capacity was estimated by converting each value for specific activity (in counts/min./mg. of protein) in Fig. 1 to μ moles of [14 C]leucine incorporated/mg. of protein of polyribosomal preparation, and multiplying that value by the corresponding amount in μ g. of protein of polyribosomal preparation/mg. of DNA in the whole-tissue homogenate (Fig. 2). ●—●, The uterine net protein-synthesizing capacity of animals given the hormone at zero time and again at 36 hr.; ○—○, the uterine protein-synthesizing capacity of animals administered hormone only at zero time; ----, the uterine protein-synthesizing capacity of normal animals in dioestrus.

capacity of the uterus as a function of time after ovariectomized rats are treated with hormone. This can be estimated by combining the information on the incorporation activity in the cell-free system of the polyribosomal preparation (Fig. 1) with that on the cytoplasmic concentration of the preparation (Fig. 2). Fig. 4 shows the effect of oestrogen on the net protein-synthesizing capacity of the uterus, with the capacity estimated as μ moles of [14 C]leucine incorporated/mg. of DNA in the whole-tissue homogenate. From 2 hr. to 24 hr. after hormone treatment, the uterine capacity for protein synthesis increased by 300%, and levelled off thereafter to 72 hr. Uterine protein-synthesizing capacity, elevated as a result of hormone treatment at zero time, decreased by more than 50% from 36 hr. to 72 hr., if the second dose of hormone was not administered at 36 hr. The essentially constant protein-synthesizing capacity exhibited by the organ between 24 hr. and 72 hr. was a result of the decrease in amino acid-incorporation activity of the polyribosomal preparation (Fig. 1) being balanced by an increase in concentration of the preparation during this time-interval (Fig. 2). This suggests that an equilibrium between formation and function of polyribosomes in the uterus occurs during the later period of oestrogen action, and that the equilibrium is achieved by the hormone

controlling the number and the activity of the polyribosomes in an inverse relation.

Although the precise mechanism of control is unknown, a considerable body of evidence indicates that cytoplasmic protein synthesis in the uterus is regulated in part at least by hormonal control of genetic transcription (Mueller, 1965; Segal, Davidson & Wada, 1965; Tata, 1966; Hamilton, 1967; Segal & Scher, 1967). Several investigations have shown that synthesis of RNA is a major aspect of early oestrogen action in the uterus of the immature or ovariectomized rat, and, further, that pretreatment of animals with actinomycin D prevents this effect of the hormone (cf. Ui & Mueller, 1963; Gorski, 1964; Hamilton, 1964; Moore & Hamilton, 1964). Actinomycin D has proved to be a potent inhibitor of ribosomal RNA as well as of messenger RNA (Reich & Goldberg, 1964; Trakattellis, Axelrod & Montjar, 1964). It has been suggested that the inhibitory effect of actinomycin D on oestrogen-stimulated RNA synthesis is a result of the action of corticosteroidal hormones released by the antibiotic, and not a result of suppression of RNA synthesis by the antibiotic (Lippe & Szego, 1965). Recent experiments, however, with animals both adrenalectomized and ovariectomized indicate that the inhibitor abolishes most of the oestrogen-stimulated RNA synthesis in the uterus (Nicolette & Mueller, 1966). In a comparative study of uterine synthesis of RNA and protein rapidly labelled *in vivo* in the ovariectomized rat, Means & Hamilton (1966a,b) demonstrated that stimulation of nuclear RNA synthesis from 2 min. to 20 min. is one of the earliest biosynthetic responses of the organ after the administration of a single dose of oestradiol-17 β . Stimulation of cytoplasmic protein synthesis was not observed until after 2 hr. of hormone action.

The present results are in agreement with the sequence of events described above. The earliest effect of oestrogen *in vivo* on the activity *in vitro* or concentration *in vivo* of the uterine polyribosomal preparation that we have detected occurs at 2 hr. (Figs. 1 and 2). Our finding that pretreatment of animals with actinomycin D or cycloheximide inhibits both of the stimulatory effects of oestrogen (Table 3) indicates further the requirement of RNA and protein synthesis for the effects of oestrogen on genetic translation described above. The absence of a stimulatory effect of oestrogen *in vitro* on the incorporation activity of the uterine polyribosomal preparation in the cell-free system (Table 2) may be considered evidence that the hormone does not exert direct effects on genetic translation in the organ. The absence of an effect of the hormone on the activity *in vitro* of the transfer factors, activating enzymes and soluble RNA of the AS₇₀-enzyme fraction (Table 4) also points to this conclusion.

A consensus now exists that the synthesis of ribosomal RNA and of ribosomes is a characteristic feature of the early action of many hormones in their characteristic target organs (Karlson & Sekeris, 1966; Tata, 1966). The nucleolus appears to be the primary site of synthesis of ribosomal RNA and of ribosomal components, if not of ribosomes (Busch, Byvoet & Smetana, 1963; Perry, 1963; McConkey & Hopkins, 1964). The nuclear RNA synthesized early in the action of oestrogen in the uterus of the ovariectomized rat is predominately of a ribosomal type (Hamilton, Widnell & Tata, 1965). Laquens (1964) has shown by electron-microscopic investigations that from 6 hr. to 24 hr. after oestrogen administration there is an increase in the size and number of nucleoli in uterine smooth-muscle cells. From 24 hr. to 72 hr. the nucleoli continue to increase in size and move to a position against the nuclear membrane, in association with an increased concentration of cytoplasmic RNP particles. It is quite probable that the dual effects of oestrogen on the activity and formation of uterine polyribosomes that we have described are a result of effects of the hormone on genetic transcription in association with nucleolar function, whereby synthesis and transport to the cytoplasm of RNP particles are regulated. This conclusion is compatible with the recent evidence that a continuous generation of ribosomes or ribosomal components is necessary for transport of messenger RNA into the cytoplasm (Girard, Latham, Penman & Darnell, 1965; Henshaw, Revel & Hiatt, 1965; McConkey & Hopkins, 1965).

We conclude that oestrogen regulates the rate and amount of genetic translation in the uterine cytoplasm by differentially controlling genetic transcription for resulting variations in the activity and number of functional polyribosomes. It is further concluded that this regulation is by a modulation of the otherwise normal mechanism of synthesis and transport to the cytoplasm of ribosomes or ribosomal components (see Hamilton, Widnell & Tata, 1967). The effect of oestrogen on messenger RNA synthesis in this process remains uncertain (Tata, 1966; Hamilton, 1967). There are now indications that ribosomes or ribosomal components may actively participate in the process of messenger RNA synthesis (cf. Shin & Moldave, 1966; Summers, Noteboom & Mueller, 1966), but this possibility has not yet been investigated for transcriptional activities known to be influenced by hormones. The observed differences in activities of the uterine polyribosomal preparations attributable to oestrogen action *in vivo* could be due to variations in rate of messenger RNA synthesis (Liao & Williams-Ashman, 1962), to coded ribosomes (Byrne, Levin, Bladen & Nirenberg, 1964) or

to both. However, the sedimentation profile of the uterine polyribosomal preparation appears not to be altered by hormone action *in vivo* (Fig. 3). This implies no significant increase in the proportion of polymeric to monomeric ribosomes. How ribosomes might be coded is uncertain. Earl & Korner (1966) observed a 62% inhibition of the incorporation of [14 C]leucine into protein in the cell-free system with ribosomes isolated from heart muscle of rats treated with actinomycin D 12 hr. before they were killed. They concluded that actinomycin D caused a defect in the ribosome itself. We find that actinomycin D and cycloheximide, in addition to blocking the stimulatory effects of oestrogen *in vivo*, cause 45% and 15% inhibitions respectively of the [14 C]leucine-incorporation activity *in vitro* of the uterine polyribosomal preparation isolated from ovariectomized animals 5 hr. after administration of the inhibitors (Table 3). It remains to be investigated whether the observed variations in amino acid-incorporation activity of polyribosomes *in vitro* resulting from treatment with oestrogen or inhibitor or both *in vivo* reflect selective alterations in translational mechanism(s) controlling the initiation of new polypeptide chains and the completion of old ones (Lin, Mosteller & Hardesty, 1966; Teng & Hamilton, 1967).

Finally, our conclusion that during early oestrogen action in the uterus new polyribosomes appear in the cytoplasm having template or structural properties or both for amino acid incorporation different from those of old polyribosomes occurring in the organ has a precedent in the findings of Tata & Widnell (1966) for thyroid hormone acting in rat liver. A precedent can also be drawn from the investigations of Korner (1960) and of De Venuto & Lange (1966) concerned with the effect of adrenalectomy on rat liver.

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