1966). Reduced SH groups are also required on the ribosome for allocating transferase II (Baliga and Munro, 1971). It has been shown in Table III that sodium selenite does not react in an inhibitory fashion with the SH groups of transferases and ribosomes. This is understandable since the stoichiometry of the reaction requires four SH groups per molecule of Na_2SeO_3 and such a reaction with protein–SH groups would be highly unlikely for steric reasons.

In view of the high potency of the inhibitory compound formed between Na_2SeO_3 and glutathione on amino acid incorporation, it is suggested that the latter effect may contribute to the known toxicity of selenite.

Acknowledgments

The technical assistance of Mrs. M. Van der Steen-Kamerbeek, Mrs. J. T. M. Mekkelholt-Verdegaal, and Mr. A. J. Jonkman is gratefully acknowledged.

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Early Effect of Estradiol on the Peptide Elongation Rate by Uterine Ribosomes†

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ABSTRACT: A cell-free protein synthesis system employing uterine ribosomes was developed. The *rate* of protein synthesis ([^{14}C]leucine incorporation/10 min per 100 μg of rRNA) and the *number* of active ribosomes ([^{3}H]puromycin-peptide formed/100 μg of rRNA) were determined. The *rate* of protein synthesis by uterine ribosomes from ovariectomized mature rats increases biphasically with time after injection of estradiol *in vivo* with a maximum at 1 hr after hormone treatment which is 268% of control values. The *number* of active ribosomes increased asymptotically to a level at 12 hr which was 210% of control values. The increase in *number* of active ribosomes closely parallels the increase in *rate* of protein synthesis at all times after estradiol administration except 1 hr at which time the increase in *rate* of protein synthesis could not be accounted for by the increase in *number* of growing peptides in the ribosome preparation. The early effect of estradiol at 1 hr on protein synthesis appears to result from an increased rate of peptide elongation on each active ribosome. Uterine ribosomes from 1-hr estradiol-treated animals exhibit a greater dependency on the addition of GTP to the protein synthesis system than those from control or 4- and 12-hr estradiol-treated animals. Also, the nascent peptides released from ribosomes of 1-hr estradiol-treated animals by [^{3}H]puromycin are of a greater length than those released from control ribosomes suggesting that the *in vivo* rate of elongation has increased relative to the rate of initiation of synthesis of new peptides. The early increase in peptide elongation rate is not inhibited by actinomycin D.

Estradiol causes an increase in the rate of synthesis of protein in the uterus of the immature or ovariectomized mature rat. This effect can be seen when protein synthesis is measured in various *in vivo* as well as *in vitro* systems ranging from uterine tissue slices to isolated uterine ribosomes (Ui and Mueller, 1963; Noteboom and Gorski, 1963; Hamilton, 1964; Means and Hamilton, 1966a; Greenman and Kenney, 1964; Teng and Hamilton, 1967a,b; Suvatte and Hagerman, 1970). Most of these studies suggest that the estrogen effect on protein

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‡ Career development awardee, National Institutes of Health (HD 38,656).

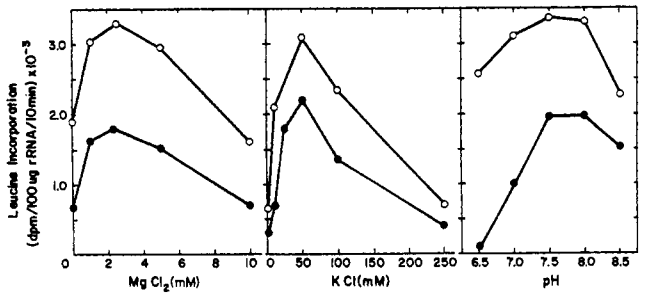

FIGURE 1: Effect of varying the concentrations of $MgCl_2$, KCl, and pH of the rate of incorporation of [^{14}C]leucine into protein by isolated uterine ribosomes. Ribosomes were isolated from uteri of control (closed circles) and 4-hr estradiol- (open circles) treated ovariectomized mature rats All conditions except the variable being tested in each experiment were held constant and were as indicated in Materials and Methods.

bisoynthesis is secondary to its effect on the synthesis of RNA, presumably mRNA, and that the response results from an increase in the numbers of active or initiated ribosomes. The present study was undertaken to determine whether or not estrogen might have an early effect on the efficiency of the ribosomal translational system above that which occurs subsequent to its effects on uterine RNA synthesis. This question is particularly important since several studies indicate that the effects of estradiol on uterine RNA synthesis are dependent on the simultaneous and/or prior synthesis of protein (Mueller *et al.*, 1961; Gorski and Morgan, 1967; Nicolette *et al.*, 1968). The strategy of the experiments to be reported was to measure both the number of growing peptide chains in a population of isolated uterine ribosomes and the rate with which the ribosomes are able to incorporate [^{14}C]leucine into protein in a standardized cell-free protein synthesis system. This permits measurement of the relative rate of peptide chain elongation in the *in vitro* system by ribosomes from uteri at various intervals of estrogen stimulation. It will be shown that at 1 hr after estradiol administration to the ovariectomized mature rat there is a transient increase in the rate of peptide elongation in the isolated ribosomes.

Materials and Methods

Preparation of Uterine Ribosomes. Mature rats (Small Animal Supply Co., Omaha) weighing 160–180 g were ovariectomized 3–4 weeks prior to use. Estradiol (5 µg) was given as a single tail vein injection in 0.5 ml of a vehicle composed of 5% ethanol in isotonic saline at the indicated intervals prior to sacrifice. Groups of ten trimmed uteri from identically treated rats were homogenized in 10 ml of *homogenizing medium* (50 mM Tris-HCl, 25 mM KCl, 5 mM $MgCl_2$, 5 mM β-mercaptoethanol, and 0.2 mg/ml of poly(vinyl sulfate), pH 7.5) containing 0.25 M sucrose. Homogenization was effected by a 30-sec treatment with a Polytron PT20 (Brinkmann Instruments, Inc.) at a setting of 4.0. The homogenate was centrifuged at 26,000*g* for 20 min and the supernatant was adjusted to 1% sodium deoxycholate followed by gentle stirring for 30 min. Aliquots (3 ml) of the deoxycholate-treated postmitochondrial supernatant were layered over 9 ml of *homogenizing medium* containing 1.5 M sucrose and were centrifuged at 165,000*g* for 3 hr. The ribosomes were suspended in 10 ml of *suspension medium* (50 mM Tris-HCl, 50 mM KCl, 2.5 mM $MgCl_2$, 5 mM β-mercaptoethanol, and 0.25 M sucrose, pH 7.5) and centrifuged at 165,000*g* for 1.5 hr. The washed ribosomes were resuspended in a small amount of *suspension medium* and the RNA content was measured as described by Wool and Cavicchi (1967). Ribosomes thus prepared have a 260/280 ratio of 1.7–1.8 and an RNA/protein ratio of 0.52. Sucrose gradient analysis of the preparations revealed the presence of polysomes, monosomes, and subunits. The yield was from 30 to 50 µg of rRNA/100 mg of uterine wet weight depending on the time after estradiol administration.

Uterine Ribosome Protein-Synthesizing System. The protein-synthesizing capacity of uterine ribosomes was determined by measuring the rate of incorporation of [^{14}C]leucine into hot Cl_3CCOOH insoluble material. The system was optimized for $MgCl_2$ and KCl concentrations and pH (Figure 1). The reaction mixture (0.5 ml) had the following composition: Tris-HCl (pH 7.5), 50 mM; KCl, 50 mM; $MgCl_2$, 2.5 mM; β-mercaptoethanol, 5.0 mM; sucrose, 250 mM; ATP, 1.0 mM; GTP, 0.4 mM; phosphoenolpyruvate, 10 mM; 19 amino acids (−leucine), 0.05 mM each; L-[^{14}C]leucine, 0.5 µCi/ml (specific radioactivity 210 Ci/mol); pyruvate kinase, 10 µg/ml; "pH 5 enzyme," 1.2 mg/ml; uterine ribosomes, 100 µg of rRNA/ml. The "pH 5 enzyme" was prepared by the method described by Moldave (1963) from livers of rats which had been fed 25 mg/day of phenobarbital in the drinking water for 5 days. Phenobarbital treatment enriched the protein synthesis supporting activity of the "pH 5 enzyme" preparation fivefold, presumably by reducing ribonuclease activity (Shortman, 1961; Louis-Ferdinand and Fuller, 1970) and thereby enhancing the tRNA levels of the livers. The reaction mixture (minus ribosomes) was equilibrated to 37° for 1 min and the reaction was initiated by the addition of ribosomes. After 10-min incubation with gentle agitation, 0.1-ml aliquots were applied to filter paper disks and immediately placed in cold 10% trichloroacetic acid. The filters were transferred to 5% trichloroacetic acid and heated at 90° for 30 min. They were then washed three times in cold 5% Cl_3CCOOH, once in 95% ethanol, once in ether, air-dried, and counted. Control assays containing all components including ribosomes, but without incubation, were performed for each ribosome preparation and the radioactivity (<35 dpm) has been subtracted from the reported values. All assays were performed in triplicate. The reaction *rate* was constant during the interval of incubation and was dependent on the amount of rRNA added to the reaction mixture. The system was dependent on the presence of a high-energy-generating system (ATP, GTP, phosphoenolpyruvate, and pyruvate kinase) and on the presence of the "pH 5 enzyme."

Determination of the Amounts of Nascent Peptides (Active Ribosomes) in Uterine Ribosomes. The relative amount or numbers of ribosomes in the various preparations of uterine ribosomes containing a growing peptide capable of forming a peptidyl puromycin complex *in vitro* was determined by a method similar to that described by Wool and Kurihara (1967) and later used by Suvatte and Hagerman (1970) to determine this parameter in isolated diabetic rat muscle and guinea pig uterine ribosomes, respectively. The reaction mixture (0.25 ml) for the formation of [^{3}H]peptidyl puromycin from [^{3}H]-puromycin had the following composition: Tris-HCl (pH 7.5), 50 mM; $MgCl_2$, 2.5 mM; KCl, 50 mM; β-mercaptoethanol, 5 mM; sucrose, 0.25 M; ATP, 1.0 mM; GTP, 0.4 mM; phosphoenolpyruvate, 10 mM; pyruvate kinase, 10 mg/ml; [^{3}H]methoxypuromycin, 10.0 µCi/ml (specific radioactivity 1.11 Ci/mmol; New England Nuclear Corp.); uterine ribosomes, 100 µg of rRNA/ml. The mixture was incubated at 37° for 40 min after which the ribosomes and peptides were precipitated by addition of 5 ml of 5% trichloroacetic acid containing 2.5% sodium tungstate. The mixture was heated at

90° for 15 min, cooled on ice for 30 min, and then collected on membrane filters (Schleicher and Schull, B-6). The filters were washed by passing 45 ml (3–15-ml aliquots) of cold 5% trichloroacetic acid through the filter followed by 30 ml (2–15-ml aliquots) of 8 M urea, 5 ml of cold 5% trichloroacetic acid, and 5 ml of 95% ethanol. The filters were then dried and counted. Control assays, prepared by addition of ribosomes after addition of cold trichloroacetic acid–tungstate, were performed for each ribosome preparation and the radioactivity (<16 cpm) was subtracted from all values before calculation of radioactivity in the peptide fraction. All assays were performed in triplicate. The formation of [^{3}H]peptidylpuromycin was ribosome dependent but was independent of the incubation times over the range of 35–45 min and the amount of [^{3}H]puromycin added over the range of 5–25 μCi/ml.

Molecular Weight Profile of Peptides Released from Uterine Ribosomes. The molecular weight profile of the nascent peptides released from uterine ribosomes in the form of [^{3}H]-peptidyl puromycin was determined by sodium dodecyl sulfate–polyacrylamide gel electrophoresis. Aliquots of ribosomes containing 200 μg of rRNA were incubated with [^{3}H]puromycin (20 μCi) as indicated above in a final volume of 2.0 ml. After 40 min, the ribosomes were sedimented by centrifugation at 165,000*g* for 1 hr. The supernatant containing [^{3}H]peptidylpuromycin and unreacted [^{3}H]puromycin was adjusted to 10% trichloroacetic acid and after 30 min at 2° the precipitated [^{3}H]peptidylpuromycin was collected by centrifugation at 165,000*g* for 30 min. The walls of the tube were wiped dry and the precipitate was solubilized by heating at 50° for 12 hr in 0.2 ml of a solution containing 1% sodium dodecyl sulfate, 6 M urea, 1% β-mercaptoethanol, and 0.1 M sodium phosphate (pH 7.0). The solubilized material was subjected to sodium dodecyl sulfate–polyacrylamide gel electrophoresis on 10% acrylamide gels as described by Palmiter *et al.* (1971).

After the tracking dye had migrated 7 cm the gels were removed from the tubes and cut into 2-mm sections from the origin to the dye front. The slices were placed into scintillation vials with 1.0 ml of NCS (Amersham/Searle Corp.) and incubated at 50° for 18 hr. Scintillation fluid (10 ml) was added and the radioactivity in each slice was determined. Recovery of total radioactivity applied to the gels was 90–95% and the results are expressed graphically as the fraction of total radioactivity contained in each gel slice. The electrophoresis system was calibrated with molecular weight marker proteins as follows: bovine serum albumin, 68,000; ovalbumin, 43,000; chymotrypsinogen, 25,700; cytochrome *c*, 11,700.

Determination of Radioactivity. Dried filter papers containing [^{14}C]protein were placed into a scintillation vial with 5 ml of a toluene-based scintillation fluid (4 g of 2,5-diphenyloxazole and 0.1 g of 1,4-bis[(2-(4-methyl-5-phenyloxazole)] in 1 l. of toluene) and counted in a Packard Tri-Carb liquid scintillation spectrometer. Counting efficiency (*ca.* 65%) was estimated by the channels ratio method. Membrane filters containing [^{3}H]peptidylpuromycin were counted as above and counting efficiency was assumed to be approximately 2.0% which was estimated by the counting efficiency of a [^{3}H]puromycin sample dried on a filter with 25 μg of bovine serum albumin. Counting efficiency of [^{3}H]peptidylpuromycin which had been solubilized and eluted from gel slices was determined by addition of [^{3}H]toluene as an internal standard and was *30–34%*.

Determination of Ribosome Composition. Protein was determined by the method of Lowry *et al.* (1951) and rRNA content was determined as suggested by Wool and Cavicchi (1967).

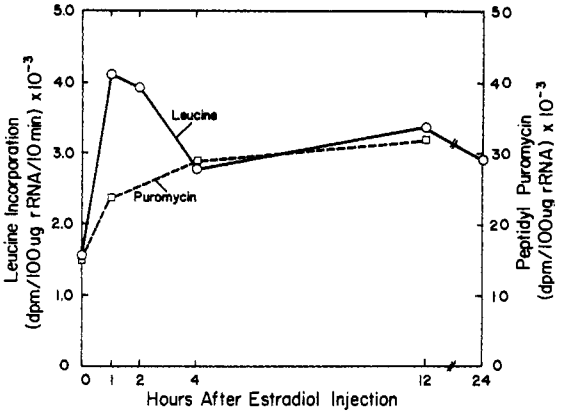

FIGURE 2: Effect of estradiol given *in vivo* on the rate of protein synthesis and the nascent peptide content of isolated uterine ribosomes. Reactions containing uterine ribosomes from control and estradiol-treated ovariectomized mature rats were incubated in either the protein synthesis system containing [^{14}C]leucine (circles) or in the reaction mixture containing [^{3}H]puromycin (squares) as indicated in Materials and Methods. All assays were performed in triplicate using uterine ribosomes prepared from groups of ten animals.

Results

Effects of Estradiol Given in Vivo on the Rate of Protein Synthesis by Isolated Uterine Ribosomes. The relative rate of protein synthesis ([^{14}C]leucine incorporation into hot trichloroacetic acid insoluble material/10 min per 100 μg of rRNA) by uterine ribosomes from ovariectomized mature rats increases biphasically as a function of time after estradiol administration (Figure 2). The rates at 1, 2, 4, 12, and 24 hr are 2.7, 2.5, 1.8, 2.2, and 1.9 times the rate observed in ribosomes from animals which did not receive estradiol. In a separate experiment (data not given) a significant increase (1.55-fold) in this parameter has been observed as early as 15 min after administration of the hormone.

Effects of Estradiol Given in Vivo on the Nascent Peptide Content of Isolated Uterine Ribosomes. The relative numbers of nascent peptides in ribosomes prepared from rat uterus at various times after administration of estradiol were determined by measuring the relative amount of [^{3}H]peptidylpuromycin which could be formed per 100 μg of rRNA using [^{3}H]puromycin. The effects of estradiol on this parameter are also given in Figure 2. It is observed that the number of growing peptides increases asymptotically to a level at 12 hr which is 2.1 times that seen in controls. The relative increase in number of growing peptides closely parallels the rate of protein synthesis at 0, 4, and 12 hr after estradiol administration suggesting that the increase in rate of protein synthesis results from an increase in the proportion of active ribosomes at these times. At 1 hr, however, the rate of protein synthesis is greater than can be accounted for by an increase in the number of active ribosomes. Presumably at this time (1 hr) the rate of elongation of each nascent peptide is increased.

From the amount of [^{3}H]peptidylpuromycin formed (15,200 dpm/100 μg of rRNA) it can be calculated that only 15.0% of the ribosomes isolated from nonstimulated rat uteri are actively engaged in protein synthesis (calculations as described by Wool and Kurihara, 1967). After 12-hr estradiol stimulation, this value increases to 31.5%.

GTP Dependency of Protein Synthesis by Isolated Uterine Ribosomes. The effects of deletion of GTP from the protein-synthesizing system on the rate of protein synthesis by uterine ribosomes prepared from ovariectomized mature rats at

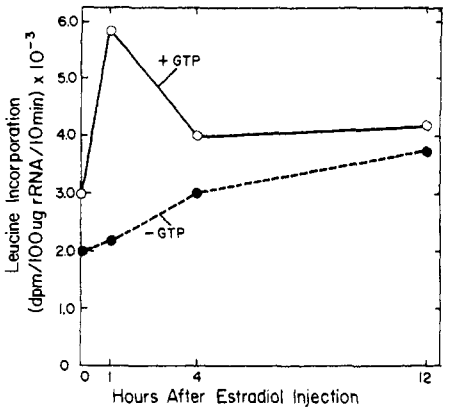

FIGURE 3: Effects of deletion of GTP from the protein-synthesizing system on the rate of protein synthesis by ribosomes isolated from the uterus of ovariectomized mature rats at various times after administration of estradiol. Protein synthesis was measured in either the complete system (open circles) or the complete system minus GTP (closed circles) as indicated in Materials and Methods. Assays were performed in triplicate using uterine ribosomes prepared from groups of ten animals. The "pH 5 enzyme" is from a second preparation which had a greater activity than that used in the experiments of Figure 1.

TABLE I: Failure of Inhibitors of the *in Vitro* Initiation Reaction to Block the Early Effect of Estradiol on Protein Synthesis by Isolated Uterine Ribosomes.[a]

Addn to Reaction Mix.	Leu Incorpn (dpm/100 μg of rRNA per 10 min) Control	1-hr Estradiol	Estrogen Effect (-Fold Increase)
None	1065	1946	1.82
NaF (5 mM)	910	1594	1.75
Aurintricarboxylic acid (0.02 mM)	900	1696	1.85

[a] Uterine ribosomes were prepared from groups of ten uteri of control and 1-hr estradiol-treated ovariectomized mature rats. The rate of [^{14}C]leucine incorporation into protein by isolated ribosomes was determined as indicated in Materials and Methods with the exception that the reaction mixtures also contained the above-indicated additions.

various times after administration of estradiol are given in Figure 3. In control ribosomes, deletion of GTP results in a 33% inhibition of protein synthesis. The GTP dependency of protein synthesis increases at 1 hr (63% inhibition by GTP removal) and thereafter the effect of GTP omission is similar to that seen in control ribosomes. In the absence of added GTP, the rate of protein synthesis increases in an asymptotical manner similar to that seen for the increase in the relative proportion of uterine ribosomes which are active after estrogen administration. In the presence of GTP, the results confirm those observed in Figure 1.

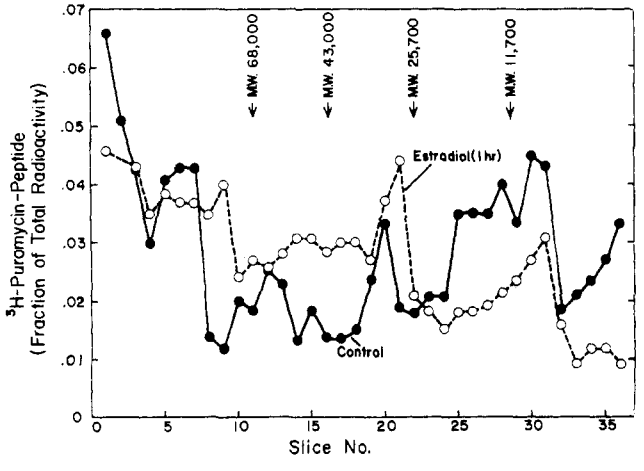

FIGURE 4: Effect of estradiol given *in vivo* on the sodium dodecyl sulfate–polyacrylamide gel electrophoretic profile of [^{3}H]puromycin-containing peptides released from isolated uterine ribosomes. The isolated [^{3}H]puromycin containing peptides were released from uterine ribosomes from control (closed circles) and 1-hr estradiol-treated (open circles) rats and subjected to sodium dodecyl sulfate–polyacrylamide gel electrophoresis as indicated in Materials and Methods. The arrows indicate the position of migration of marker proteins of various molecular weights under these electrophoretic conditions. The [^{3}H]puromycin-containing peptides applied to the gels contained 11,860 and 10,950 cpm for control and estradiol ribosomes, respectively.

Effect of Estradiol on the Molecular Weight Profile of Uterine Nascent Peptides. The sodium dodecyl sulfate–polyacrylamide gel electrophoretic profiles of the [^{3}H]peptidylpuromycin released from ribosomes of control (no estradiol) and 1 hr estradiol treated rats by [^{3}H]puromycin *in vitro* are given in Figure 4. Administration of estradiol for this period of time results in a decrease in the relative number of peptides of less than a molecular weight of 25,000 and an increase in the relative number of peptides in the molecular weight range of 30,000–80,000. Above a molecular weight of 80,000, the peptide profiles are similar.

Effect of Inhibitors of the Initiation Reaction on the 1-hr Estradiol-Induced Increase in Protein Synthesis. The effects of addition of 5 mM sodium fluoride or 0.02 mM aurintricarboxylic acid to the cell-free protein synthesis system are given in Table I. Addition of these amounts of these compounds have been shown to specifically block the initiation or reinitiation of protein biosynthesis in cell-free protein synthesis systems employing both liver and reticulocyte ribosomes (Shafritz *et al.*, 1971; Stewart *et al.*, 1971; Leader, 1972). Both inhibitors caused a 15–18% inhibition in the rate of [^{14}C]leucine incorporation into protein in uterine ribosomes from both control and 1-hr estradiol-treated rats. The estrogen effect was the same in both inhibited and control incubations indicating that the estrogen-induced increase in the rate of [^{14}C]leucine incorporation into protein was not due to the preferential initiation or reinitiation of protein synthesis in the *in vitro* protein-synthesizing system.

Effect of Actinomycin D on the 1-hr Estradiol-Induced Increase in Protein Synthesis. The effect of administration of 10 μg of actinomycin D by the intra-uterine route 15 min before administration of saline or estradiol on the rate of protein synthesis, the number of active ribosomes and the responsiveness of these two parameters to estradiol stimulation are given in Figure 5. This dose of actinomycin is two times that required to block the estradiol induction of the synthesis of uterine glucose-6-phosphate dehydrogenase and inhibits the rate of uterine RNA synthesis *in vivo* by more than 90% (Barker, 1967, 1971). The antibiotic is without effect on the estradiol-induced stimulation of protein synthesis at 1 hr.

Discussion

The early uterine response to estradiol (1 hr) is characterized by an increase in the rate of elongation of nascent peptides on the ribosomes. Evidence for this includes: (1) the rate of [^{14}C]leucine incorporation into protein in a non-initiating, cell-free protein synthesis system is increased 2.7 times while the number of growing nascent peptides is increased only 1.6 times; (2) the addition of two inhibitors of the *in vitro* peptide initiation reaction, 5 mM NaF or 0.02 mM aurintricarboxylic acid, does not eliminate the 1-hr estradiol-induced increase in the rate of protein synthesis; (3) the dependency of the cell-free protein synthesis system on the addition of GTP, an energy source and activator of the translocase reaction (McKeehan *et al.*, 1969; Lin *et al.*, 1969; McKeehan, 1972), is greater for ribosomes isolated from uteri at 1 hr after hormone treatment than at other times; and (4) there is an upward shift in the molecular weight profile of nascent peptides from isolated ribosomes at a time (1 hr) when the number of nascent peptides per milligram of isolated ribosomes has not changed greatly. The early effect on peptide elongation is seen *in vitro* in the cell-free protein-synthesizing system and based on the observed increase in the molecular weight of nascent peptides on the isolated uterine ribosomes (peptide synthesis not permitted *in vitro*) the increase in peptide elongation rate relative to the rate of initiation must also occur *in vivo*. This indicates that the *in vitro* observed effect is not the result of a preferential loss or retention of factors required for the cell-free incorporation of [^{14}C]leucine into protein by the isolated ribosomes.

The ribosome content of the uterus is increased beginning at 4 hr after administration of estradiol to the ovariectomized mature rat (Moore and Hamilton, 1964). While this effect of estradiol would result in a net increase in the uterine protein synthesis capacity, the present finding that less than 15–30% of the existing ribosomes are active suggests that factors other than the amount of ribosomes, such as mRNA, initiation factors, elongation factors, etc., are more likely candidates for the rate limiting step of uterine protein synthesis. Indeed, mRNA can be shown to be a limiting factor when ribosomes from uteri of animals given estradiol for longer periods of time (4 hr or more) are compared to ribosomes from estrogen deprived animals (Greenman and Kenney, 1964; Moore and Hamilton, 1964). Suvatte and Hagerman observed an effect of estradiol on the initiation of uterine protein synthesis (6 hr) with a later (12–18 hr) effect on the rate of elongation (translocase activity) in the guinea pig. A 40% increase in polypeptide elongation rate in the chick oviduct in response to estradiol has been observed by Palmiter (1972). The hormonal effect on the initiation process presumably required new mRNA and/or initiation factors *in vivo*. None of these studies was conducted at 1 hr where the transient effect of estradiol on the rate of peptide elongation in rat uterine ribosomes is observed.

The transient nature of this effect suggests that the causative agent is short-lived and may result from the metabolic alteration of ribosomal proteins or regulatory factors. The time course of the response coincides with the transient increase (15 sec to 1 hr) in the levels of uterine cAMP after estradiol administration (Szego and Davis, 1967). Presumably, activation of protein kinases by cAMP could have an effect on the activity of ribosomal protein synthesis by inducing a metabolically transformed structure of specific ribosomal components. This effect would presumably not be dependent on the prior synthesis of new RNA or protein. The time course of the response also coincides with the brief interval of time during which the "estrogen-activated cytosol receptor" concentration in the cytosol is high (*i.e.*, the brief interval between activation of receptors and its interaction with the nucleus) (Jensen *et al.*, 1968; Shyamala and Gorski, 1969). The estrogen-free cytosol receptor protein may act as an inhibitor of the elongation reaction or the estrogen-bound receptor could act as an activator of the elongation reaction. Presumably, the tendency for the uterine cytosol binding protein toward aggregation after interaction with the steroid might promote its interaction with organelles other than the nucleus resulting in either general or perhaps quite specific modification of organelle function. Noteboom and Gorski (1965) observed that after 2 hr about 6% of the uterine-bound [^{3}H]estradiol is associated with the microsomal fraction.

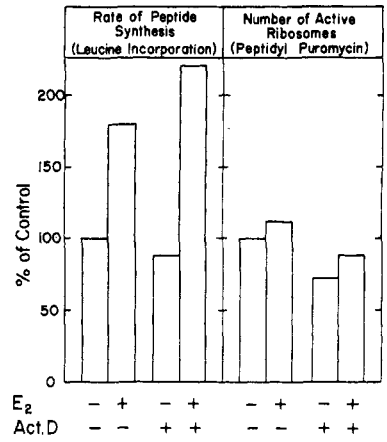

FIGURE 5: Effect of actinomycin D on the 1-hr estradiol-stimulated increase in the rate of protein synthesis and the nascent peptide content of isolated uterine ribosomes. Groups of ten rats received either 10 μg of actinomycin D (0.050 ml) or 0.3 M NaCl carrier (0.050 ml) by the intrauterine route 15 min before the intravenous treatment with either estradiol or the hormone carrier as indicated in the abscissa. Assays were as described in Materials and Methods.

It should be emphasized that the measured effect of estradiol on the rate of elongation of uterine peptides *in vitro* occurs before the effects of the steroid on the rate of incorporation of radioactive amino acids into total uterine proteins *in vivo*. The rate of incorporation of [^{14}C]glycine or [^{3}H]methionine given intraperitoneally into uterine protein does not deviate from control rates before 2 hr after administration of estradiol to either immature or mature rats (Noteboom and Gorski, 1963; Means and Hamilton, 1966b). Another report indicates that the net incorporation (not rate) of [^{14}C]glycine into uterine proteins *in vivo* is slightly elevated between 30 min to 1 hr following estrogenic stimulation (Hamilton, 1964). These measurements are, however, subject to precursor specific activity changes due to uterine permeability and amino acid pool size changes during the actual interval of protein biosynthesis and might not reflect exactly the rate of "protein synthesis." One might expect that the increase in protein synthesis seen in the present experiments might represent synthesis of a specific protein representing a minor portion (perhaps with a regulatory function) of the total uterine protein. The synthesis of a specific estrogen-induced uterine protein has been reported to be elevated nearly twofold at 1 hr and its rate of synthesis begins to decline after 2 hr (Notides and Gorski, 1966; Barnea and Gorski, 1970). Whether the effect measured herein represents synthesis of a specific uterine (perhaps regulatory) protein or a ribosome "clearing effect"

(completion of synthesis of uterine proteins synthesized in the nonstimulated state in preparation for the synthesis of a new set of estrogen-induced proteins) remains to be seen.

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