Journal of Steroid Biochemistry, 1977, Vol. 8, pp. 1213–1220. Pergamon Press. Printed in Great Britain

PROGESTERONE RECEPTORS IN RABBIT UTERUS—II CHARACTERIZATION AND ESTROGEN AUGMENTATION

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(*Received* 28 *December* 1976)

SUMMARY

Progesterone-binding protein in uterine, endometrial and myometrial cytosol of normal and estrogen (diethylstilbestrol)-primed, immature rabbits has been characterized using sucrose gradient centrifugation and charcoal adsorption techniques. Estrogen priming daily for five days increased the concentration of uterine progesterone receptors 32-fold as compared to progesterone binding in serum. The number of receptor binding sites in the uterus reached a maximum on day ten of 51,700 per cell. This result compares favorably with the best progestational response, which occurs on days 10 and 11 in McPhail's[1] bioassay. The mean dissociation constant for progesterone and progesterone receptor complexes was estimated at 3.3×10^{-10} M ($n = 18$). We observed a higher concentration of progesterone receptors in myometrial than in endometrial cytosol; both levels were less than that in uterine cytosol.

In addition to quantitative changes, we observed a qualitative change in the sedimentation constant of progesterone-binding protein from a 4-5*S* to a 5–6*S* complex after ten days of estrogen administration; the complex changed irreversibly to the 4–5*S* form in the presence of 0.4 M KCl. Among the natural steroids tested, the highest relative binding affinity was observed with 5α-pregnan-3,20-dione (15%) when progesterone was 100%. Synthetic progestational compounds showed a wide range of binding affinities for progesterone receptors.

INTRODUCTION

The evaluation of changes in the uterine histology of immature rabbits which have been primed with estrogen and then administered progestins is used as a bioassay for progestin activity [2]. However, the mechanism by which estrogen-induction enables the progestins to elicit a measurable response is unknown. Generally, intracellular steroid-binding proteins or receptors† have been implicated in the elicitation of the hormonal response; more specifically, the presence of specific progesterone receptors has been demonstrated in the rabbit uterus [3–6].

In this study, we characterized progesterone receptors from the uteri of normal and estrogen-treated immature rabbits. The effect of estrogen pretreatment on the concentration of these receptors was determined and a comparison made of their distribution in the cytosols of endo- and myometrium. The quantitative progesterone receptor determination was supplemented with qualitative studies. In addition, we compared the binding affinities of natural steroids and several synthetic compounds.

MATERIALS AND METHODS

[1,2,6,7-^{3}H]-Progesterone (New England Nuclear, S.A. 80 Ci/mmol) having a radiopurity of at least 98% as measured by silica gel chromatography in acetone–chloroform [7] was used. Non-radioactive steroids, bovine serum albumin, gamma globulin and monothioglycerol were obtained from Sigma Chemical Co. Synthetic progestational compounds were donated by Searle, Syntex and Upjohn Companies. Phenol reagent, diphenylamine and "Spectranalyzed" grade solvents and toluene (T313) for the preparation of scintillation fluid were obtained from Fisher. 2,5-Diphenyloxazole (PPO) and *p-bis*-[2-(5 phenyl-oxazoyl)] benzene (POPOP) were purchased from Research Products International. Six-week old, female rabbits weighing 700–900 g were purchased from Eldridge Rabbitry, St. Louis. Dextran D (M.W. 20,000) and RNase-free sucrose were purchased from Schwarz/Mann and Norit A from Matheson, Coleman and Bell.

Preparation of tissue fractions. After various numbers of daily subcutaneous injections of estrogen (diethylstilbestrol-100 μg in 0.1 ml sesame oil), 20 ml

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† The experimental demonstration of specific cytoplasmic steroid-binding proteins in comparable processes to those outlined here has given rise to the use of receptor as a convenient short term. Use of the term in this paper is not meant to imply a definition of receptor as currently used in pharmacology.

of blood was collected from each rabbit by heart puncture and was allowed to clot. We centrifuged the clotted blood at 1000 *g* for 10 min to separate the serum from the clot. After the blood was drawn, the rabbits were killed by a blow on the head. The uterus, a portion of spleen, and the ovaries were excised and weighed. The tissues were rinsed repeatedly in cold buffer A (10 mM Tris–HCl, pH 8 containing 1 mM disodium EDTA, 12 mM monothioglycerol and 10% glycerol) and minced with scissors. We prepared a tissue homogenate with 25% (w/v) tissue in buffer A using a Polytron homogenizer (Brinkman). The homogenate was centrifuged at 3500 *g* for 10 min at 4°C and the pellet and supernatant were treated separately. The latter was centrifuged at 130,000 *g* for an additional 90 min; the vol. of the resulting supernatant (referred to as cytosol) was then measured and its protein content was determined according to Lowry *et al.*[8]. The cytosol was either used on the same day or frozen over dry ice and stored in small aliquots at −20°C for a maximum of 7 days prior to use. In preliminary experiments we had observed no detectable change in progesterone binding activity after 7-day storage in the frozen state. The original 3500 *g* pellet of only uterine homogenate was washed twice in buffer A by centrifugation at 5000 *g* for 15 min each time and the final pellet was resuspended in 10 volumes of distilled water and homogenized thoroughly; the total vol. of the homogenate was noted. DNA content was measured by the method of Burton[9]. In some experiments the uterus was cut open longitudinally and the endometrium was separated from myometrium by scraping with a scalpel the endometrium off the myometrium. Observation of endometrial scrapings under the microscope showed that they were free of myometrium. For the preparation of myometrium the uterine horn was cut into sections starting from outside and cutting towards the uterine lumen. The myometrial sections which were clearly devoid of endometrial cells were used as myometrial fractions. Cytosols from each tissue were separately prepared.

Radioactive counting procedure. Radioactivity was measured with a Packard Tri-Carb Model 3373 in scintillation fluid (18.9 g of a mixture of 98% PPO and 2% POPOP to 3.7 l of toluene). In practice, 10 ml of scintillation fluid was added to a 1 ml radioactive sample. It was shaken vigorously at room temperature and allowed to cool for at least 90 min at 4°C before counting. Maximum counting efficiency was 52% and quenching was estimated by channel ratio. Sample to sample variation was less than 3% and the samples were counted for a sufficiently long period to achieve a counting error of less than 2%.

Separation of unbound from protein-bound steroid. A dextran coated charcoal suspension (0.5% norit plus 0.025% dextran in 0.01 M Tris–HCl, pH 8.0) was used to separate unbound from protein-bound steroid. We followed the assay procedure described previously [10].

Comparison of progesterone binding activity. [^{3}H]-Progesterone binding activity to serum and cytosols from spleen, ovary and uterus was determined using two protein concentrations in triplicate. Labeled progesterone and the protein samples were incubated in 0.5 ml of buffer A for 16 h at 4°C. Unbound steroid was removed by charcoal adsorption. We measured the protein-bound radioactivity and calculated the binding activity per unit protein. To compare the binding activity in the samples, we used the average of the values obtained for the two protein concentrations.

Estimation of progesterone binding sites. To determine the number of progesterone binding sites in the uterine, endometrial, and myometrial cytosol, we used the following procedure. A quantity of cytosol containing 300–900 μg of protein and capable of binding 6000 to 10,000 c.p.m. of labeled progesterone was incubated for 18 h at 4°C with [^{3}H]-progesterone and increasing amounts of non-labeled progesterone. Unbound steroid was separated from protein-bound steroid by charcoal adsorption technique. The calculated molarity of bound progesterone was plotted against the bound/free ratio of labeled progesterone, according to the method of Scatchard[11]. From the slope of the resulting curve, the dissociation constant (K_D) and the number of binding sites in terms of moles of steroid was computed. We then calculated the number of moles of bound steroid in the total tissue cytosol. Assuming that there is a single binding site per protein molecule and a cellular DNA content of 5.3 pg in rabbit [12], the number of binding sites per cell was calculated using Avogadro's number (6.025×10^{23} molecules/mol).

Some experiments were performed in the presence of cortisol; in these cases the ratio of cortisol to labeled progesterone was 100:1.

In some spleen and ovarian cytosols and in serum, the dissociation constants were estimated from Scatchard plots; however, we used a higher protein concentration in these samples than in uterine tissue cytosols.

Sucrose gradient centrifugation. We analyzed [^{3}H]-progesterone binding to uterine cytosol proteins by sucrose gradient centrifugation, according to a procedure described previously [10]. Cytosol was incubated with an excess of [^{3}H]-progesterone and then centrifuged in an IEC, model B-60 centrifuge (rotor SB-405) at 50,000 rev./min for 16 h at 3°C. The amount of radioactive incorporation in the fractions was measured. Simultaneously gamma globulin and bovine serum albumin were centrifuged in separate tubes and the protein content in each fraction was measured by Lowry method [8]. The sedimentation coefficients for progesterone receptors were determined by comparison with gamma globulin (7.5*S*) and bovine serum albumin (4.6*S*) according to Martin and Ames[13].

In some experiments, uterine cytosol incubated with [^{3}H]-progesterone was centrifuged on a 5%–20%

Table 1. Progesterone binding activity

Days of estrogen treatment	Uterine cytosol (A) c.p.m./mg protein ($\times 10^{-3}$)	Serum (B) c.p.m./mg protein ($\times 10^{-3}$)	Ratio A/B
0	5.3 ± 2.1*	0.7 ± 0.2	8
1	7.2 ± 1.8	1.4 ± 0.7	5
3	10.8 ± 1.3	0.6 ± 0.3	18
5	12.8 ± 0.9	0.4 ± 0.2	32
10	47.9 ± 3.8	2.0 ± 0.9	24
15	64.3 ± 2.1	2.2 ± 0.8	29
20	42.1 ± 7.0	4.3 ± 1.4	10

* Mean ± S.D. of four experiments performed at two protein concentrations (see Method section).

sucrose gradient prepared in buffer containing 0.4 M KCl. They were fractionated and the radioactivity in one of the gradients was measured. We pooled the fractions containing the radioactive peak from the second gradient and the sample was vacuum dialysed against a large vol. of buffer A in a collodion bag; they were again centrifuged on sucrose gradient prepared in buffer A without KCl.

Evaluation of binding specificity and relative binding affinity. A sample of [^{3}H]-progesterone containing 30,000 c.p.m. was equilibrated at 4°C with uterine cytosol containing 200–475 μg of protein and varying quantities (0–100 ng) of various unlabeled competing compounds in buffer A; the total vol. was 0.5 ml. Cytosol preparations employed throughout this study gave an unbound to bound ratio varying between 2 and 4. Relative binding affinity was calculated for each competing compound by the method of Korenman[14]. The assay and the calculation were performed according to our previously published method [10].

RESULT

None of the excised ovaries contained corpora lutea and their weight, which varied between 0.2 and 0.3 gm, showed no appreciable change after estrogen treatment.

Progesterone binding activity

The progesterone binding activity of cytosols prepared from uterus, spleen and ovary was compared to the activity in serum as a function of the number of daily estrogen treatments. Progesterone binding activity in the spleen and ovary cytosols was consistently lower than in serum. Additional experiments indicated that progesterone binding activity in the cytosols of spleen and ovary is similar to that observed in serum; these results are presented later in this section. Only the data on [^{3}H]-progesterone binding to uterine cytosol and serum are given in Table 1. Progesterone binding activity increased in both tissues, although the serum response showed no consistent pattern as a function of the number of estrogen treatments. The uterine progesterone binding activity, on the other hand, showed a maximum 32-fold increase over that of serum on day 5.

Estimation of progesterone binding sites

After 3 days of DES treatment, we observed a large increase in high affinity progesterone receptors (Table 2, Column 2). The number of binding sites reached a maximum of 51,700 per cell after ten days of estrogen treatment (Table 2, Column 4). We also determined progesterone receptor levels in uterine cytosol of immature rabbits which received estrogen

Table 2. Concentration of progesterone binding sites* in uterus

Days of estrogen treatment	Uterine cytosol Pmol / total protein	Uterine DNA Pmol / milligram	Sites† / cells
0	0.7	0.10	300
1	0.9	0.12	400
3	1.4	0.20	600
5	11.5	1.60	5,100
10	165.5	16.20	51,700
11‡	136.1	—	—
15	127.0	8.90	28,400
20	65.8	8.30	26,500

* Determined after charcoal adsorption (ignoring endogenous progesterone) and calculated from Scatchard plots. † Sites per cell calculated assuming the binding ratio of progesterone to protein to be 1, no cellular polyploidy and the DNA content of single rabbit uterine cells to be 5.3 pg (Vendrely and Vendrely, 1949). ‡ Daily DES treatment for 6 days and rabbits killed on day 11. DNA determinations and calculations of sites/cell were not done.

Table 3. Progesterone binding sites* in cytosol

Days of estrogen treatment	Endometrium		Myometrium	
	Pmol / mg DNA	Sites / cell	Pmol / mg DNA	Sites / cell
5	0.32	1,000	0.54	1,700
10	3.83	12,200	7.66	24,500
15	2.63	8,400	5.18	16,500

* As per Table 2.

daily for six days followed by five days of no treatment. Although DNA content in these uterine tissues was not measured, the total cytosol receptor concentration is given in Table 2 (column 2).

Table 3 shows our data on the number of progesterone binding sites in endo- and myometrial cytosols prepared from animals treated daily for 5, 10 or 15 days with estrogen. The response of endo- and myometrial cytosols was similar to that observed in uterine cytosols; samples from animals treated with estrogen for 10 days had the highest number of binding sites. There was nearly a two-fold difference in the number of progesterone binding sites in myometrial cytosol compared to endometrial cytosol.

The dissociation constant for the progesterone–receptor complex, calculated using a number of uterine cytosols, varied from 2.9×10^{-10} M to 5.1×10^{-10} M, with a mean of 3.3×10^{-10} M ($n = 18$). The dissociation constant was not significantly affected by the presence or absence of cortisol (Fig. 1). The progesterone–receptor complex in endo- and myometrial cytosols from animals treated daily for 5, 10 or 15 days with estrogen had a dissociation constant similar to that observed in uterine cytosols.

Dissociation constants of progesterone and progesterone-binding protein in serum and cytosols prepared from spleen and ovary were higher than 3.2×10^{-8} M.

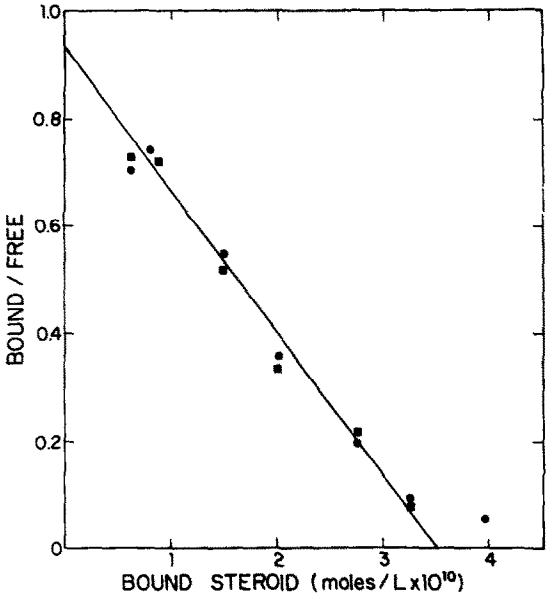

Fig. 1. Uterine cytosol prepared from immature rabbit treated with estrogen (diethylstilbestrol) for 10 days was incubated with [^{3}H]-progesterone plus an increasing mass of non-labeled progesterone (●) and [^{3}H]-progesterone plus a 100-fold excess of cortisol and an increasing mass of non-labeled progesterone (■) for 18 h at 4°C. Unbound steroid was separated from protein-bound steroid by charcoal adsorption. The results were analysed according to Scatchard (1949). In the figure the steroid receptor binding to only the high affinity protein is shown. The dissociation constant for progesterone and progesterone receptor complexes either with or without cortisol was calculated to be 3.76×10^{-10} M. The mass of cortisol in the incubation mixture was ignored in the calculations.

Qualitative estimation of progesterone receptors

The sedimentation profiles of the progesterone-binding macromolecules in cytosols incubated with [^{3}H]-progesterone alone, [^{3}H]-progesterone plus cortisol and [^{3}H]-progesterone plus cortisol and progesterone are presented in Fig. 2. [^{3}H]-Progesterone binding was displaced only by non-labeled progesterone.

Progesterone binding to uterine cytosols increased following estrogen treatment of immature rabbits and a maximum binding occurred after 10 days of estrogen treatment. [^{3}H]-Progesterone bound to the uterine cytosol from either untreated immature rabbits or rabbits treated with estrogen for up to 5 days, sedimented as a single 4–5*S* complex. After 10 days of estrogen treatment, however, the sedimentation constant changed from 4–5*S* to 5–6*S*. We also observed differences in the sedimentation constant of the progesterone–receptor complex when we analysed cytosols containing either an identical protein concentration or a low concentration of protein (1/5).

When endometrial and myometrial cytosols were examined, both sedimented as 4–5*S* complexes during the first 5 days of estrogen treatment; after 5 days only the latter had a tendency to change to a "heavier" complex.

The 5–6*S* progesterone receptor complex sedimented as a 4–5*S* complex, either when incubated with [^{3}H]-progesterone in buffer A containing 0.4 M KCl and centrifuged in a normal sucrose gradient, or incubated in buffer A and centrifuged in a sucrose gradient prepared in buffer A containing 0.4 M KCl. Removal of KCl by dialysis did not result in the reformation of the "heavier" complex; however, there was some loss of progesterone binding (Fig. 3).

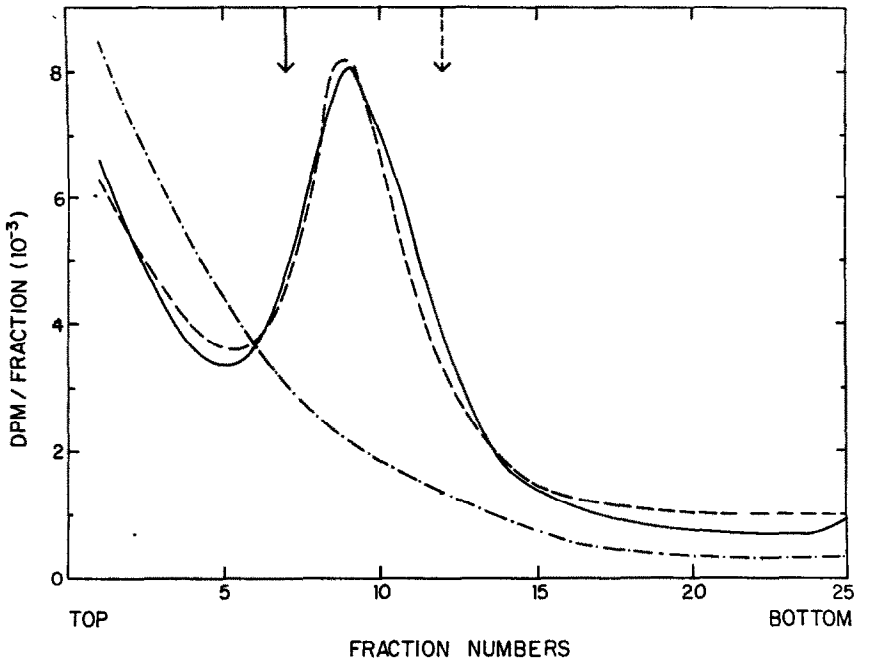

Fig. 2. Uterine cytosol prepared from immature rabbit treated with estrogen for 15 days was incubated with [^{3}H]-progesterone (——), [^{3}H]-progesterone plus non-labeled cortisol (– – –) and [^{3}H]-progesterone plus non-labeled progesterone (·—·—·) for 8 h at 4°C. The samples (200 μl) were layered on a 5% to 20% sucrose gradient and centrifuged at 50,000 rev./min for 16 h at 3°C. Peak fractions containing bovine serum albumin (↓) and gamma-globulin (↓) centrifuged simultaneously in separate tubes are shown.

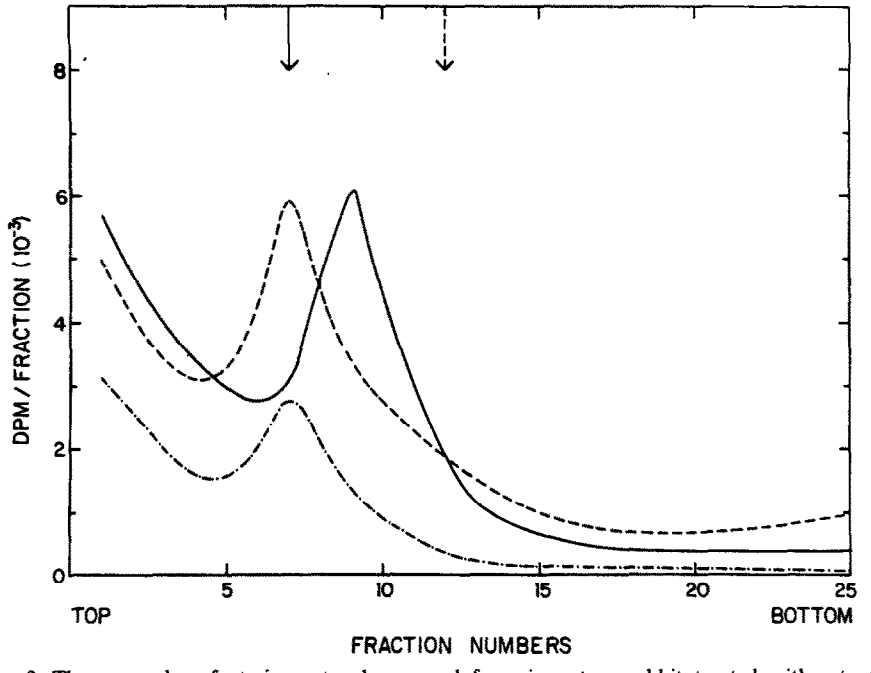

Fig. 3. Three samples of uterine cytosol prepared from immature rabbit treated with estrogen for 10 days were incubated with [^{3}H]-progesterone for 5 h at 4°C. Two samples were centrifuged in sucrose gradients prepared in buffer A containing 0.4 M KCl and the third was centrifuged in sucrose gradient prepared in buffer A alone. Centrifugation was at 50,000 rev./min for 16 h at 3°C. All the gradients were fractionated. Radioactivity was measured in the fractions from one gradient prepared with KCl buffer (– – –) and the gradient with normal buffer (——). Fractions 5–11 from the third gradient prepared in KCl were pooled and dialysed (see material and methods) to remove the KCl. The sample was again centrifuged as the first time, but in a sucrose gradient prepared in buffer A without KCl (·—·—·). Peak fractions containing bovine serum albumin (↓) and gamma-globulin (↓) centrifuged in separate sucrose gradient tubes are shown.

Table 4. Relative binding affinities* of selected compounds

Steroid or compound	Percent
Progesterone	100
17α-Hydroxy-6α-methyl pregn-4-en-3,20-dione 17 acetate (Medroxy progesterone acetate)	121
6α-Methyl progesterone	114
17α-Acetoxy-6-methyl-16-methylene pregn-4,6-diene-3,20-dione (U-21, 240)	105
17α-Ethyl-17-hydroxy-19-nor-androst-4-en-3-one (northandrolone)	77
17α-Acetoxy-6-methyl pregn-4,6-diene 3,20-dione (Megestrol acetate)	70
17α-proply-17-hydroxy-18-nor-androst-4-en-3-one	67
17α-Hydroxy-6α-methyl-pregn-4-en-3β-ol, 20-one 17 acetate	65
9α-Fluoro-11β-hydroxy-pregn-4-en-3,20-dione 17 acetate	61
17α-Acetoxy-6α-fluoro progesterone	57
17α-Ethynyl-17-hydroxy-19-nor-androst-4-en-3-one (northindrone)	41
17α-Ethynyl-7α-methyl-19-nor-androst-4-en-3-one (7α-methyl northindrone)	28
17α-Ethynyl-17-hydroxy-5(10)-estren-3-one (norethynodrel)	28
17α-Hydroxy progesterone acetate	17
5α-Pregnan-3,20-dione	15
6α-Methyl-17α-hydroxy progesterone	7

* Binding quantified by charcoal technique. Relative binding affinity was calculated according to the method given previously (Rao *et al.*, 1973).

A number of compounds had relative binding affinities of less than 5%: estrone, estradiol-17β, estriol, testosterone, 20α-hydroxy-pregn-4-en-3-one, 20β-hydroxy-pregn-4-en-3-one, 17α-OH-progesterone, 11α-OH-progesterone, 5β-pregnanedione, corticosterone, cortisol, cholesterol.

Ligand specificity of cytosol progesterone-binding protein

We examined the binding of [^{3}H]-progesterone to the uterine cytosols from immature rabbits treated daily for either 5 or 10 days with estrogen, in the presence of a number of both naturally-occurring steroids and synthetic compounds. Compounds which competed for progesterone binding sites showed a linear dose-response relationship ($r > 0.96$) and we determined the mass of each substance required to displace 50% of bound [^{3}H]-progesterone; from these data we calculated their relative binding affinities (Table 4). No significant difference in the specificity of progesterone-binding proteins of cytosols from endo-, myometrium and whole uterus for the above compounds was observed.

DISCUSSION

Although estrogen priming is a prerequisite in eliciting a uterine progestational response, the preparatory changes induced by estrogen in the uterus which account for this response are unknown. If some mediation by progesterone receptors is necessary, then one would expect a correlation between the concentration of such receptors and the progestational response.

In order to see whether the uterine cytosol progesterone binding activity could be due to the presence of serum progesterone binding proteins in the uterine cytosol preparations, we compared the [^{3}H]-progesterone binding activity of serum and uterine cytosol prepared from the same animal, at identical protein and [^{3}H]-progesterone concentrations. Two protein concentrations in triplicate were used to minimize the error in the estimation of progesterone binding activity. The bound over free ratio of steroid varied between 0.15–0.23, indicating that sufficient excess of steroid was present in all the samples. In all the animals there was a higher progesterone binding activity in the uterine cytosol than in the serum (5–32-fold; see Table 1). There was no reduction in the binding of [^{3}H]-progesterone in all the uterine cytosol samples analysed in the presence of 100-fold excess of cortisol. Some uterine cytosols were assayed for the number of progesterone binding sites in the presence and absence of 100-fold excess of cortisol and analysed by Scatchard plot. Neither the number of progesterone binding sites nor the dissociation constant of progesterone–receptor complex varied in the presence of excess of cortisol. These results together with the result on specificity of uterine cytosol progesterone receptor (see Table 4) demonstrate that we were assaying the specific progesterone receptor in the uterine cytosol.

In this study, estrogen treatment of immature rabbits increased the concentration of uterine, myometrial and endometrial progesterone receptors; of the latter two, the myometrium demonstrated the higher concentration. The maximum concentration of uterine progesterone receptors occurred after daily administration of estrogen for 10 days. Progesterone receptor concentration in uterine cytosol prepared from rabbits treated for six days with estrogen followed by no treatment for 5 days, was lower than that of 10 days estrogen treatment but higher than levels following either 5 or 15 days of treatment. It is also of interest that McPhail[1] in his systematic analysis of progestational response in immature rabbits observed that the maximal changes in endometrial histology occurred on day 10 and 11 when estrogen is administered daily for 5 or 6 days followed by a single dose of progesterone.

The patterns of reduction in the number of progesterone binding sites in immature rabbits after 10 days of estrogen treatment are similar in the cytosols of uterus, endometrium and myometrium. Davies *et al.*[15] observed a correlation between a reduction in the number of progesterone receptors in myometrial cytosol and an increase in serum progesterone levels in pregnant rabbits. A similar correlation between the number of progesterone binding sites and the serum progesterone level has been observed in the rat [16] and the guinea-pig [17]. We have not attempted such a correlation in this investigation. However, the absence of corpora lutea in the ovaries indicates that the serum progesterone level may be expected to be minimal in the immature rabbits used in this study. Therefore the serum progesterone level may not have caused the reduction in the progesterone binding sites in immature rabbit uterine cytosol. Another factor which may have influenced the result, is the synthesis of progesterone binding inhibitors which interact with the receptors. Though the presence of inhibitors has not been reported, we have observed that, during the purification of progesterone receptors, recombination of a partially purified fraction containing progesterone receptors with either extensively dialysed or nondialysed original cytosol results in the reduction of progesterone binding sites. We suspect that the receptor protein interacts with another macromolecule, leading to the inactivation of progesterone binding activity [18].

The present investigation does not provide evidence to indicate whether an increase or decrease in progesterone receptors occurs after estrogen treatment in all cell types or only in certain cells, particularly of endometrium. The over-all response as estimated in this study, may depend on the proliferative rates of various cell types. To examine these possibilities, cell types must be separated prior to the estimation of progesterone receptors, and the proliferation rates of the various cell types after estrogen priming must be determined. The progesterone receptor level in the myometrial cytosol was higher than in the endometrial cytosol in immature rabbits after estrogen treatment. This difference in the distribution of progesterone receptors between the two major uterine tissues may be due to a difference in their response to estrogen action. Other possible explanations include differences in the solubility of progesterone receptors during the preparation of cytosol from these tissues and/or differences in the cellular density per unit mass of the tissues.

Toft and O'Malley[19] reported a maximum cellular concentration of progesterone receptors in the immature chick oviduct after ten days of estrogen treatment. The number of progesterone binding sites increased similarly to that reported here following estrogen priming of immature rabbits. Interestingly, there was also a decrease in the number of progesterone binding sites per unit DNA on day 15 compared to day 10 of estrogen treatment in chick oviduct.

In addition to quantitative changes in the concentration of progesterone receptors, there were also qualitative changes. After 10 days of daily estrogen treatment, the 4–5*S* receptor complex changed to a slightly "heavier" form (5–6*S*). This change in the sedimentation constant is not due to analytical conditions in which a high protein concentration was used [20]. Although the "heavier" macromolecule was changed to a 4–5*S* form in the presence of high concentrations of KCl, it was not reversible to a 5–6*S* form after the removal of KCl. The "heavy" (5–6*S*) form may be due to the aggregation of receptors which, once disaggregated, lose their capacity to reaggregate.

We have previously reported the presence of an 8*S* progesterone binding component in uterine cytosol of mature spayed rabbits which were estrogen-primed and injected with labeled progesterone *in vivo* [3,4,10]; however, we never observed this 8*S* component in *in vitro* incubations of labeled progesterone from uterine cytosols of either mature rabbits, regardless of spaying or estrogen-priming, or immature, estrogen-primed rabbits. Similar results have been reported by some authors [6, 15, 21], although others have reported the presence of an 8*S* binder after *in vitro* incubation [22, 23]. We have no satisfactory explanation for such a discrepancy in sedimentation coefficient values.

Of all the naturally-occurring steroids, progesterone had the highest affinity for the receptor. Synthetic steroids, on the other hand, not only showed a wide range of affinities but also, with the exception of three compounds, had a lower affinity than did progesterone. We previously reported that there was no correlation between the affinity to cytosol receptors prepared from human and mature rabbit uteri and the progestational activity of various progestins [3, 4, 24, 25]. A similar conclusion may be drawn from the present investigation with regard to the uterine cytosol from estrogen-primed, immature rabbits.

Smith *et al.*[26] and Terenius[27] have estimated the binding affinity of a number of progestins by adding either non-labeled cortisol or non-labeled cortisol plus estradiol-17β respectively, to their incubation mixtures. In these studies, the rationale for including cortisol was to minimize binding of progestins to "non-specific" binders. Estradiol-17β was used to inhibit compounds possessing both estrogenic and progestational activity from binding to estrogen receptors. The conclusion drawn from both of these studies was similar to ours, i.e., that compounds which possess progestational activity compete with labeled progesterone for binding sites on the receptor; however, there is no correlation between the binding affinity of progestins to receptors and their biological potency.

Acknowledgements—The technical assistance of Ms. Susan Aycock, Susan Pepples, Susan Reichman and Mary Ann Rafle is gratefully acknowledged. Sincere thanks are extended to Drs. R. Burton and S. Eliasson for use of

equipment and Drs. A. Nakeff and F. Valeriote for critical review of the manuscript. We wish to thank Searle, Syntex and Upjohn companies for their generous supply of synthetic compounds.

This investigation was supported by grants HD-05680, 1POCA13053-05 awarded by the National Cancer Institute—DHEW and a Washington University General Research Support Grant.

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