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PROPERTIES OF TWO PROGESTERONE-BINDING PROTEINS OF THE RAT UTERUS*

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SUMMARY

DEAE-cellulose filtration was used to measure high affinity binding sites in uteri of rats during the estrus cycle. The cytosol concentrations (pmol/mg protein ± S.E.M.) of progesterone-specific (R) sites were: proestrus, 0.9 ± 0.07; estrus, 0.4 ± 0.04; metestrus, 0.4 ± 0.04; diestrus-1, 0.2 ± 0.04; and diestrus-2, 0.6 ± 0.03. This pattern is consistent with the concept that the concentration of R sites is increased by estrogens, known to rise in rat serum on the evening of diestrus-1 and to remain high at proestrus. The respective values for corticosteroid-binding globulin-like (G) sites were 12 ± 0.2, 8 ± 0.1, 13 ± 0.2, 12 ± 1.1 and 16 ± 0.2. That the lowest number of R sites was at diestrus-1 and of G sites at estrus may indicate different half-lives or regulatory mechanisms for the two proteins. Preliminary data showed that nuclear progesterone binding was largest at proestrus and metestrus.

The equilibrium association constant of the R protein for progesterone was measured in preparations purified by spheroidal hydroxylapatite chromatography. The estimates were in the range $2\text{–}8 \times 10^8\,M^{-1}$. Of several steroids tested, deoxycorticosterone and 20α-dihydroprogesterone competed for G sites while dexamethasone and 5α-dihydroprogesterone did not. Only the latter steroid and deoxycorticosterone showed some affinity for R sites. When uterine nuclei from adrenalectomized-ovariectomized rats injected with either progesterone or corticosterone were added to incubation mixtures containing [^{3}H]-progesterone or [^{3}H]-corticosterone, significant exchange occurred only between nuclear progesterone and [^{3}H]-progesterone, suggesting that nuclei take up only progesterone bound to R sites.

INTRODUCTION

Limited and conflicting information has been published on the identity and properties of progesterone binding proteins (putative receptors) in the rat uterus. Various studies have shown at least two high-affinity progesterone-binding proteins in rat uterine cytosol preparations. One of these proteins (R) is progesterone specific and does not bind corticosteroids [1–4]; the other protein (G) is similar to plasma corticosteroid-binding globulin (CBG-like) and binds both progesterone and corticosteroids. If the R protein is a steroid receptor, changes in its concentration may modulate the actions of progesterone on cellular processes. In addition, changes in the levels of other high-affinity progesterone-binding proteins will affect the partitioning of steroid between these proteins.

The concentration of the progesterone-binding proteins in the uterus and in the oviduct is increased by estrogen priming [5–7]. Since estrogen levels change during the estrous cycle, physiological fluctuations in the concentrations of rat uterine progesterone-binding proteins may also be expected. Variations in progesterone binding have been described during the estrous cycle and during pregnancy in several species [3, 8–11]. In some of these studies no distinction was made between progesterone-specific and total high-affinity binding sites; in other studies only relative amounts, rather than actual concentrations of binding sites, were measured. Therefore, we were interested in determining whether the concentrations of both types of high-affinity progesterone-binding sites changed with the stage of the estrous cycle and whether the changes followed the same or a different pattern. We measured the concentrations of high-affinity progesterone-binding sites using a DEAE-cellulose filtration assay [12]. The results indicated that the concentrations of R and G sites change with the day of the cycle.

Preparations of the R protein, partially purified by chromatography on columns of spheroidal hydroxylapatite, were used to measure the equilibrium association constant (K_A). In order to compare the ligand specificities of the two proteins, we tested the effectiveness of several steroids as competitors for either the G or the R sites.

Most of the work on nuclear uptake of progesterone–receptor complexes has been done by O'Malley and his coworkers using the chick oviduct [13]. They showed that isolated nuclei take up and retain steroid–receptor complexes to a greater extent than either free steroid or receptor. In contrast, there are very few studies on nuclear uptake of progesterone using mammalian uteri. Smith *et al.*[14] briefly stated that

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they had demonstrated nuclear entry of progesterone–receptor complexes from human oviduct but full details of their results have not yet been published. Saffran *et al.*[15] recently demonstrated *in vitro* nuclear uptake and retention of progesterone–receptor complexes using rabbit and guinea pig uteri. The nuclear exchange assays that we present in this article have confirmed and extended the work of Hsueh *et al.*[16]. Both progesterone and corticosterone were used in nuclear exchange experiments. In a preliminary experiment we also measured nuclear-bound progesterone during the estrous cycle.

EXPERIMENTAL

Reagents, animals, and cytosol preparation. Radioactive steroids, [1,2-^{3}H]-progesterone, [1,2-^{3}H]-cortisol, [1,2-^{3}H]-corticosterone (approximately 50 Ci/mmol), were obtained from New England Nuclear Corporation (Boston, MA) and were purified by t.l.c. The following unlabeled steroids were purchased from Schwarz/Mann (Orangeburg, NY): progesterone, 5α-dihydroprogesterone, 3β-hydroxy-5α-pregnan-20-one, 20α-dihydroprogesterone, deoxycorticosterone, corticosterone, and 5α-dihydrotestosterone. Testosterone and cortisol were obtained from Sigma Chemical Company (St. Louis, MO). Dexamethasone (9α-fluoro-11β,17α,21-trihydroxy-16α-methyl-1,4-pregnadiene-3,20-dione) and 3α-hydroxy-5α-pregnan-20-one were purchased from Research Plus Steroids (Denville, NJ). Estradiol-benzoate (17β-hydroxy-1,3,5(10)-estratriene-3-benzoate) was obtained from Steraloids (Queens, NY). All unlabeled steroids were used without further purification.

Adult female rats (200–260 g, Holtzman Company, Madison, WI) were used in all experiments. Cytosol was prepared as previously described [4] except that a Polytron PT-10 (Brinkmann Instruments, Switzerland) was used for tissue homogenization. Three bursts of 5–10 s duration were used at slightly above the minimum rheostat setting needed for the instrument to run. At least 30 s were allowed between bursts for cooling on ice.

TEG buffer (Tris 0.01 M, Na_2EDTA 0.001 M, 20% glycerol, pH 7.4) was used for homogenization. A number of sodium phosphate buffers, pH 7.4, of different molarity but all containing 20% glycerol (NaPG) were used for hydroxylapatite chromatography.

Spheroidal hydroxylapatite (BDH) was obtained from Gallard-Schlesinger (Carle Place, NY). Short columns, 4.3 cm, of spheroidal hydroxylapatite were used to separate *R* from *G* sites [17].

Protein was measured by the Lowry method [18]; DNA was measured by the diphenylamine reaction [19].

Equilibrium dialysis experiments. Equilibrium dialysis was performed as previously described [4]. Single bags containing 1-ml samples of the hydroxylapatite fraction containing the *R* protein (*R* pool) were placed in 10 ml of steroid solution at varying progesterone concentrations (0.7–250 pmol/flask). Unlabeled cortisol (200 pmol/flask) was used to offset the effect of any residual CBG-like contamination. Dialysis was stopped after 24 h at 4°C. A bag which did not contain protein was included as a check that equilibrium had been reached.

Competition experiments. The ability of several unlabeled steroids to compete for *G* sites was tested with a single cytosol preparation. Incubation mixtures were prepared containing 200 nM [^{3}H]-cortisol. For each unlabeled steroid an incubation mixture containing [^{3}H]-cortisol and an excess of unlabeled steroid was prepared. Nonspecific cortisol binding was measured using an excess of unlabeled cortisol. Aliquots of a benzene–ethanol solution containing the unlabeled steroid were dried in culture tubes and the tritiated cortisol solution was added. The concentration ratio of unlabeled steroid to [^{3}H]-cortisol was 500:1.

A similar competition experiment was performed to test the ability of the same steroids to compete for the *R* sites. The unlabeled steroids were used at 500 times the concentration of [^{3}H]-progesterone (20 nM); excess unlabeled cortisol was also used in each incubation [12]. Nonspecific progesterone binding was measured using an excess of unlabeled progesterone.

In a second type of competition experiment, we measured the relative affinities of several steroids for *R* sites. Some of these experiments used cytosol and some used the *R* pool. The concentrations of competing steroids used were 1–100 times that of [^{3}H]-progesterone. Since the *G* protein was present in the cytosol preparations, the steroid solution used for these experiments contained excess unlabeled cortisol. When the *R* pool was used, progesterone binding was initially measured both in the presence and absence of excess cortisol as a check for CBG-like contamination; the percentages of progesterone bound in the two incubation mixtures were not significantly different. Specific binding was determined by subtracting nonspecific binding (binding in the presence of excess unlabeled progesterone) from total binding.

The ratio of the percentages of [^{3}H]-progesterone specifically bound in the presence and in the absence of competitor (as numerator and denominator, respectively) was defined as Z. The relative affinity was defined as the P/F ratio which resulted in 50% reduction of the binding (where P = progesterone concentration and F = competitor concentration). The calculations were performed after fitting a linear regression to the binding data plotted as logit Z versus log (F/P). Since P is constant in a given experiment, this plot has the same justification as the logit-log transformation [20] commonly used in competitive protein-binding assays.

Cycle experiments. Rats (approximately 200 g upon receipt) were housed, 2/cage, with access to food and water *ad libitum*. The lights were on from 6:00 AM

until 6:00 PM CST and the ambient temperature was 22–24°C.

Vaginal smears were obtained daily for at least a month between 7:30 and 8:30 AM CST. The estrous cycle of Holtzmann rats varies in length even for individual animals which will, on occasion, shift from a 5-day cycle to a 4-day cycle or vice versa. Rats with the most consistent cycles were chosen for the experiments. For each day of the cycle, 1–4 rats were killed by decapitation between 7:30 AM and 9:00 AM CST. The uteri were removed, pooled to obtain sufficient tissue, and cytosol was prepared.

Cytosol, 90 μl, was mixed with 45 μl of various steroid solutions; after 75 min at 4°C, 50 μl aliquots were filtered on DEAE-cellulose discs [12]. Progesterone-specific binding was measured at a [^{3}H]-progesterone concentration of approximately 20 nM in the presence of a 500-fold excess of unlabeled cortisol. Nonspecific progesterone binding was measured in the presence of a 500-fold excess of unlabeled progesterone. CBG-like binding was measured at approximately 200 nM [^{3}H]-cortisol; nonspecific cortisol binding was measured in the presence of excess unlabeled cortisol. The protein concentration in the incubations was between 1.9 and 4.5 mg/ml.

The concentrations of R and G sites (N_R and N_G) were calculated, as previously described [12], from the differences between the percentages of steroid bound in the two types of incubations (total and nonspecific). In one experiment the endogenous progesterone concentration in the cytosol preparations was measured by radioimmunoassay [21] and the filtration data were corrected for the endogenous steroid.

Nuclear exchange assays. The nuclear exchange assays were performed as described by Hsueh *et al.* [16]. The rats used in these studies were adrenalectomized-ovariectomized. At least 6 days after surgery they were injected with 400 μg estradiol-benzoate (0.4 ml in corn oil, s.c.). One week after estrogen injection the rats were injected with 4 mg of progesterone or corticosterone dissolved in 0.4 ml dimethylsulfoxide (DMSO). One group of rats received only DMSO. The rats were decapitated 30 min after injection; the uteri were removed, rinsed, weighed, and a homogenate was prepared in Tris–glycerol buffer (0.01 M, 10%, pH 7.4.). After centrifugation at 800 g for 10 min, the pellets were washed three times with the same buffer and resuspended to yield an equivalent of approximately 80 mg of tissue per ml.

Aliquots (0.5 ml) of the nuclear suspension were incubated with tritiated progesterone or corticosterone (S.A. approximately 22 Ci/mmol). "Total" retention was measured using 74 nM labeled steroid; "nonspecific" retention was measured using the same amount of labeled steroid and a 250-fold excess of the unlabeled steroid. After incubation at 15°C for 5 h the tubes were centrifuged and the pellets were washed three times with 1.5 ml buffer. The pellets were extracted twice with 3 ml of ethanol and aliquots of the extracts were counted. Nuclear exchange was defined as the d.p.m. in the "total" extract minus the d.p.m. in the "nonspecific" extract.

Nuclear exchange was also measured during the rat estrous cycle. The rats were not injected with steroid. The nuclear fraction was prepared and the exchange assays were performed as described except that TEG buffer was used throughout the procedure.

RESULTS

Equilibrium dialysis

Equilibrium dialysis was used to measure the equilibrium association constant of the R protein for progesterone. The experiment was performed several times using different preparations of the R pool. Based on a three-parameter model [4], which provided the best fits, the range of K_A values was $2\text{–}8 \times 10^8\,M^{-1}$.

Competition experiments

The competition of several steroids for G sites in a cytosol preparation was tested at a concentration 500 times greater than that of the labeled cortisol. The high-affinity binding of cortisol was reduced $84 \pm 6\%$ ($\pm$S.D.) by deoxycorticosterone and $82 \pm 8\%$ by 20α-dihydroprogesterone. The reduction in binding by dexamethasone was not significant ($20 \pm 26\%$); 5α-dihydroprogesterone, 3α-hydroxy-5α-pregnan-20-one, and 3β-hydroxy-5α-pregnan-20-one did not cause any decrease in cortisol binding.

Using a different cytosol preparation, the same steroids were tested as competitors of progesterone for R sites at the same concentration ratio. Deoxycorticosterone eliminated the high-affinity, progesterone-specific binding while 5α-dihydroprogesterone reduced it by $95 \pm 12\%$. The binding was reduced $87 \pm 11\%$ by 20α-dihydroprogesterone, $52 \pm 8\%$ by 3β-hydroxy-5α-pregnan-20-one, $35 \pm 10\%$ dexamethasone, and $25 \pm 10\%$ by 3α-hydroxy-5α-pregnan-20-one.

The relative affinities of several steroids for the R sites were measured and the results are shown in Table 1. Only two of the steroids tested, 5α-dihydroprogesterone and deoxycorticosterone, showed much affinity for the R sites.

Progesterone binding during the estrous cycle

Progesterone-specific (R) and CBG-like (G) binding sites were measured during the estrous cycle in two separate experiments. The results of one experiment are shown in Fig. 1. Both R and G sites varied with the stage of the cycle and their patterns of variation were not identical. While the G sites were lowest at estrus, the R sites peaked at proestrus and were lowest at diestrus-1.

It was conceivable that the apparent variations in progesterone-binding sites were not due to variation in the actual number of sites, but to differences in the levels of endogenous progesterone. Since the data

Table 1. Relative affinities for progesterone-specific binding sites

Steroid	Relative affinity	Preparation
Progesterone	(1.00)	—
Corticosterone	0.01	cytosol
Deoxycorticosterone	0.2	cytosol
20α-Dihydroprogesterone	0.004	cytosol
5α-Dihydroprogesterone	0.3	*R* pool
5α-Dihydrotestosterone	0.02	*R* pool
Testosterone	0.01	*R* pool

of Feil *et al.*[3] indicate that the time for 50% dissociation of the progesterone-*R* complexes is between 5 and 34 min. (in 10 or 30% glycerol, respectively) any endogenous progesterone will equilibrate with, and dilute, the added steroid. Therefore, progesterone concentrations were measured in cytosol samples from this experiment. Table 2 shows the endogenous steroid levels present in the incubations and also the endogenous progesterone levels in the cytosol, expressed per g of tissue. Figure 2 illustrates that, when the data are corrected for endogenous progesterone levels, the cyclic fluctuations in the steroid binding sites remain.

Table 3 gives the results of a second experiment in which endogenous progesterone levels were not measured. The pattern of the *R* sites is about the same as in the first experiment; the pattern of the *G* sites is also approximately the same as in the first experiment although the values are about 1.5 times higher.

Nuclear exchange assays

Table 4 shows the results of a nuclear exchange experiment using [^{3}H]-progesterone and [^{3}H]-corticosterone. Nuclear exchange was defined as specific retention of labeled steroid which was calculated as

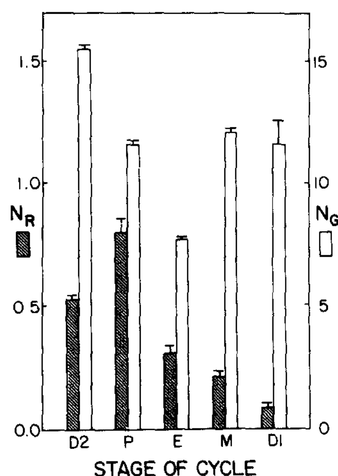

Fig. 1. Progesterone-binding sites were measured using the filtration assay [12] as described in the text. N_R and N_G are expressed as pmol sites/mg cytosol protein. The bars represent standard error. The stages of the cycle are: diestrus-2 (D2), proestrus (P), estrus (E), metestrus (M), and diestrus-1 (D1).

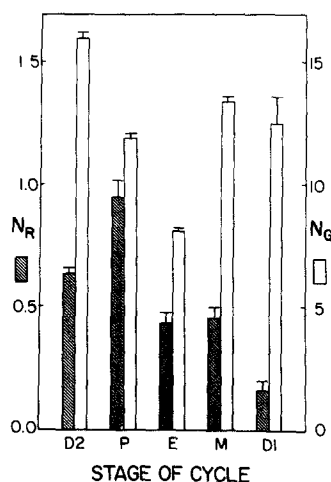

Fig. 2. The progesterone-binding sites shown in Fig. 1 were corrected for the endogenous progesterone concentrations (Table 2) in the filtration assay incubations. N_G and N_R are expressed as pmol sites/mg cytosol protein. The bars represent standard error.

Table 2. Endogenous progesterone levels during the estrous cycle*

Stage of cycle	In Incubation mixtures* (nM)	In cytosol* (pmol/g tissue)	Plasma progesterone† (nM)
Diestrus-2	3.2	16	19
Proestrus	3.3	18	32
Estrus	6.8	44	35
Metestrus	14	73	64
Diestrus-1	13	71	110

* Measured by radioimmunoassay [21]. † Estimated from the data of Nequin *et al.*[22].

Table 3. Progesterone-binding proteins during the estrous cycle: experiment 2. The number of rats from which uteri were pooled is in parentheses

Stage of cycle	Length of cycle (days)	pmol sites/mg protein (± S.E.; $n = 5$)	
		N_R	N_G
Diestrus-2 (2)	5	0.64 ± 0.06	21.4 ± 0.6
Proestrus (3)	5	1.64 ± 0.10	21.0 ± 0.6
Proestrus (2)	4	1.06 ± 0.13	23.5 ± 0.8
Estrus (2)	5	0.41 ± 0.03	12.3 ± 1.0
Estrus (1)	4	0.49 ± 0.10	15.3 ± 0.6
Metestrus (2)	4–5*	0.26 ± 0.06	18.9 ± 0.8
Diestrus-1 (3)	4	0.25 ± 0.04	19.6 ± 0.6

* One rat had a 4-day cycle and the other a 5-day cycle.

Table 4. Nuclear exchange of [^{3}H]-progesterone or [^{3}H]-corticosterone

Steroid injected (unlabeled)	Radioactive steroid	Nuclear exchange	
		d.p.m./mg tissue	d.p.m./μg DNA
Progesterone	Progesterone	4152 ± 1854	1692 ± 756
Corticosterone	Progesterone	1548 ± 450	806 ± 234
None	Progesterone	1387 ± 425	613 ± 188
Progesterone	Corticosterone	90 ± 17	37 ± 7
Corticosterone	Corticosterone	124 ± 20	65 ± 10
None	Corticosterone	66 ± 14	29 ± 6

Adrenalectomized-ovariectomized rats were injected with 400 μg estradiol-benzoate one week before the day of the experiment. Six rats were used in this experiment; two were injected with 4 mg progesterone and two with 4 mg corticosterone. After 30 min the rats were decapitated and the uteri were removed. Each nuclear fraction was prepared from two uteri corresponding to the proper injection category. Aliquots of each nuclear preparation were incubated with labeled progesterone or labeled corticosterone. The nuclear preparation and exchange assay are described in the text. Values are mean ± S.D. ($n = 3$).

the difference between "total" and "nonspecific" retention of labeled steroid. Statistical analysis showed that there was a significant difference between "total" and "nonspecific" retention.

The progesterone exchange was greatest in the nuclei prepared from rats which were progesterone injected. There was no difference in progesterone exchange between nuclei prepared from rats which were injected with corticosterone or DMSO alone. Under all conditions, the exchange of progesterone was greater than the exchange of corticosterone.

The results of a preliminary experiment using uterine nuclei prepared from rats at different stages of the estrous cycle are shown in Fig. 3. The greatest nuclear exchange of [^{3}H]-progesterone was in proesstrus and metestrus. Nuclear exchange of [^{3}H]-corticosterone was measured on three of these days (proestrus, metestrus, diestrus-1) and was minimal (≤ 0.2 pmol/40 mg tissue).

DISCUSSION

Our estimates of the equilibrium association constant of the R protein of the rat uterus for progesterone are probably the best currently available. Other investigators [3] have obtained lower estimates, as we did, when a significant amount of endogenous progesterone was present in the preparations. In addition, CBG-like binding did not interfere with the measurements we made.

Several steroids were tested for their ability to compete for each type of progesterone-binding site. Of the steroids tested, the only ones which competed to a significant extent for R sites were deoxycorticoster-

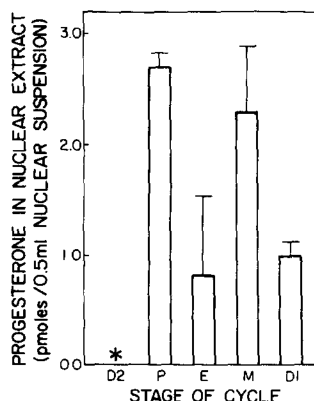

Fig. 3. Rats were killed at various stages of the estrous cycle and nuclear exchange assays were performed as described in the text. The ordinate, "progesterone in nuclear extract," refers to the exchanged progesterone. Error bars are standard deviation.

one and 5α-dihydroprogesterone. Deoxycorticosterone, which binds to plasma CBG, also competed for G sites. Although 5α-dihydroprogesterone did not compete for G sites, its relative affinity was not high enough for it to be a useful probe for the study of progesterone-specific binding. Both deoxycorticosterone and 5α-dihydroprogesterone have also been found to compete for the "progesterone receptor" in the pregnant rat [9] and in several other species [11, 23–25].

Testosterone binding has been studied in the uteri of several species [26–29], including the rat [26, 27]. The results of some of these studies indicated that progesterone competed for testosterone-binding sites. When we did the reverse experiment, testosterone did not compete to any significant extent for R sites; 5α-dihydrotestosterone competed to a slightly greater extent but the relative affinity was low. Therefore, the uterine testosterone-binding protein does not appear to be the same as the progesterone-specific binding protein.

Serum estradiol levels begin to rise on the evening of diestrus-1 and remain high through the day of proestrus [22], and our results show maximum progesterone-specific binding in the uteri of proestrus rats. These results, in combination with those which show that estrogen injection increases progesterone binding [5–7], confirm that estrogen is important in the regulation of the concentration of progesterone-binding sites. It is interesting to note, however, that while the R sites are lowest in diestrus-1, the G sites are lowest in estrus. Therefore, there may be differences in the half-lives or the regulation of these proteins.

Studies of the regulation of progesterone-binding proteins in the guinea pig uterus have shown that the amount of protein-bound progesterone sedimenting at 4.5 S increase 6 h after injecting a single dose of estradiol-17β, with a maximum of binding at about 20 h, when there is a 6.7 S peak with a 4.5 S shoulder. There is a gradual falling-off in bound progesterone which remains elevated 7 days after estrogen treatment, with approximately equal peaks of radioactivity sedimenting at 4.5 and 6.7 S [6]. It has also been shown [30] that there is virtually no corticosterone binding in uteri from castrated guinea pigs unless they are estrogen primed, suggesting that the CBG-like binding is also under estrogen control. Progesterone injection causes a rapid decrease in progesterone-binding proteins in the estrogen-primed uteri of guinea pigs with a slow return to basal levels. Milgrom and Baulieu [6] suggest that, in the guinea pig, the proestrus increase in estrogen causes an increase in the progesterone receptor concentration while the secretion of progesterone at ovulation, and later by the corpus luteum, causes a decrease.

Since progesterone-binding sites were measured at only one time of day during the cycle, it is possible that we have not measured the sites at their true highest and lowest points. Fluctuations in progesterone-binding sites during the estrous cycle and during pregnancy have been studied in several species [3, 8–11]. Feil *et al.* [3] studied the relative amounts of 6–7 S progesterone binding during the estrous cycle of the mouse by sucrose gradient ultracentrifugation. They found the greatest 6–7 S binding during proestrus; there was a slight indication of 6–7 S binding during diestrus. Progesterone-binding sites were measured by Sephadex G-25 chromatography during the estrous cycle of the guinea pig [10]. The greatest concentration of sites was found in proestrus and the lowest in mid-diestrus; cortisol was not used to block any CBG-like binding which might have been present. Cortisol was used when progesterone-binding sites were measured in the hamster uterus [11]; the greatest number of binding sites was again found in proestrus and the lowest in diestrus. Thus, the fluctuations we observed in progesterone-binding proteins in the rat uterus are similar to fluctuations which have been seen in other species. This suggests that there may be common mechanisms for the regulation of progesterone-binding sites in the uterus.

Cytosol levels of progesterone were measured in order to correct our estimates of the concentrations of binding sites for endogenous progesterone levels and to make preliminary comparisons between serum and cytosol progesterone levels. Serum progesterone levels show a sharp peak on the evening of proestrus and are low again by the morning of estrus. A smaller, but longer lasting, increase in progesterone levels begins on the morning of metestrus and peaks, in rats which have a 5-day cycle, on the morning of diestrus-1 [22]. The cytosol levels of progesterone were elevated on the morning of estrus and were still higher on the mornings of metestrus and diestrus-1. Since these are one-point measurements on each day, we do not know whether the estrus increase in cytosol progesterone is due to tissue retention of progesterone produced in proestrus or to an increase in tissue levels prior to the rise in serum progesterone which begins on the morning of metestrus.

The protein which we call the G protein was first detected in preparations of rat uterine cytosol by Milgrom and Baulieu [2]. They called it a CBG-like protein on the basis of the observed intrinsic equilibrium association constant, electrophoretic mobility, steroid specificity, and elution volume by gel filtration. Its origin and function remain to be determined. CBG-like proteins are also found in uterine cytosol preparations from other species. Several experiments indicate that the presence of this protein is not due to contamination with blood. On the basis of indirect evidence Milgrom and Baulieu[31] suggested that it is intracellular. The changes in N_G during the estrous cycle do not cast additional light on the identity, origin or location of the G or CBG-like binding sites, particularly since simultaneous determinations of plasma CBG were not made.

The nuclear exchange assays using progesterone and corticosterone confirmed and extended the work of Hsueh *et al.*[16]. Progesterone exchange was great-

est in uterine nuclei prepared from rats which were progesterone injected; corticosterone exchange was extremely low even in rats which were corticosterone injected. These experiments provided indirect evidence that the progesterone-specific binding protein was taken up into the nucleus. The nuclear exchange of progesterone seen in rats which were not progesterone injected may be due to residual endogenous progesterone; a cytosol sample contained a low but significant amount of progesterone.

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REFERENCES

1. Baulieu E.-E., Atger M., Best-Belpomme M., Corvol P., Courvalin J.-C., Mester J., Milgrom E., Robel P., Rochefort H. and de Catalogne D.: *Vitams Horm.* **33** (1975) 649–736.
2. Milgrom E. and Baulieu E.-E.: *Endocrinology* **87** (1970) 276–287.
3. Feil P. D., Glasser S. R., Toft D. O. and O'Malley B. W.: *Endocrinology* **91** (1972) 738–746.
4. Booth B. A. and Colás A. E.: *Gynec. Invest.* **6** (1975) 272–284.
5. Toft D. O. and O'Malley B. W.: *Endocrinology* **90** (1972) 1041–1045.
6. Milgrom E., Thi L., Atger M. and Baulieu E.-E.: *J. biol. Chem.* **248** (1973) 6366–6374.
7. Corvol P., Falk R., Freifeld M. and Bardin C. W.: *Endocrinology* **90** (1972) 1464–1469.
8. Davies I. J. and Ryan K. J.: *Endocrinology* **92** (1973) 394–401.
9. Haukkamaa M.: *J. steroid Biochem.* **5** (1974) 73–79.
10. Milgrom E., Atger M., Perrot E. and Baulieu E.-E.: *Endocrinology* **90** (1972) 1071–1078.
11. Leavitt W. W., Toft D. O., Strott C. A. and O'Malley B. W.: *Endocrinology* **94** (1974) 1041–1053.
12. Booth B. A. and Colás A. E.: *Analyt. Biochem.* **76** (1976) 74–82.
13. Buller R. E., Toft D. O., Schrader W. T. and O'Malley B. W.: *J. biol. Chem.* **250** (1975) 801–808.
14. Smith R. G., Iramain C. A., Buttram V. C., Jr. and O'Malley B. W.: *Nature* **253** (1975) 271–272.
15. Saffran J., Loeser B. K., Bohnett S. A. and Faber L. E.: *J. biol. Chem.* **251** (1976) 5607–5613.
16. Hsueh A. J. W., Peck E. J. and Clark J. M.: *Steroids* **24** (1974) 599–611.
17. Booth B. A., Dyer R. D. and Colás A. E.: *J. steroid Biochem.* **8** (1977) 1179–1182.
18. Lowry O. H., Rosenbrough N. J., Farr A. L. and Randall R. J.: *J. biol. Chem.* **193** (1951) 265–275.
19. Burton K.: *Biochem. J.* **62** (1956) 315–323.
20. Rodbard D., Bridson W. and Rayford P. L.: *J. lab. clin. Med.* **74** (1969) 770–781.
21. Clark J., Dierschke D. and Wolf R.: *Biol. Reprod.* (1977) submitted for publication.
22. Nequin L. G., Alvarez J. and Schwartz N. B.: *J. steroid Biochem.* **6** (1975) 1007–1012.
23. Kontula K., Jänne O., Vihko R., de Jager E., de Visser J. and Zeelen F.: *Acta endocr., Copenh.* **78** (1975) 574–592.
24. Feil P. D. and Bardin C. W.: *Endocrinology* **97** (1975) 1398–1407.
25. Verma U. and Laumas K. R.: *Biochim. biophys. Acta* **317** (1973) 403–419.
26. Heyns W., Verhoeven W. and De Moor P.: *J. steroid Biochem.* **7** (1976) 335–343.
27. Giannopoulos G.: *J. biol. Chem.* **248** (1973) 1004–1010.
28. Harrison R. W. III and McKenna T. J.: *J. steroid Biochem.* **7** (1976) 521–526.
29. Wagner R. K., Gorlich L. and Jungblut P. W.: *Hoppe-Seylers Z. physiol. Chem.* **353** (1972) 1654–1656.
30. Faber L. E., Sandmann M. L. and Stavely H. E.: *J. biol. Chem.* **247** (1972) 8000–8004.
31. Milgrom E., Baulieu E.-E.: *Biochem. biophys. Res. Commun.* **40** (1970) 723–730.