Changes in the Concentration of High-Affinity Oestradiol Receptors in Rat Uterine Supernatant Preparations during the Oestrous Cycle, Pseudopregnancy, Pregnancy, Maturation and after Ovariectomy

By PATRICIA FEHERTY, D. M. ROBERTSON, H. B. WAYNFORTH AND A. E. KELLIE
Courtauld Institute of Biochemistry, The Middlesex Hospital Medical School, London W1P 5PR, U.K.

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A quantitative method was used to determine the concentration of high-affinity oestradiol-receptor sites in rat uterine supernatant preparations under various physiological conditions. Cyclic changes in concentration were observed during the oestrous cycle, with a maximum occurring in late dioestrus. The changes followed a similar pattern in endometrium and myometrium, although concentrations were higher in the former. In pseudopregnancy the concentration was initially low, rising to a maximum on the tenth day. In early pregnancy a high concentration of receptor was found to be associated with the developing placenta, but this declined in later stages of pregnancy. After ovariectomy or combined ovariectomy and adrenalectomy the receptor concentration remained at a constant low value that could be increased by treatment with oestradiol. The receptor concentration was considerably higher in immature than in adult uteri.

The presence of specific protein receptors for oestradiol in the uteri of many species is now well established (Gorski, Toft, Shyamala, Smith & Notides, 1968; Jensen, Suzuki, Numata, Smith & DeSombre, 1969). At least two distinct types of specific receptor have been identified, one in the cytoplasm and the other in the nucleus of the cell (Toft & Gorski, 1966; Shyamala & Gorski, 1967). Little is known at present about the interaction between these two or their precise role in mediating hormone action. It has been observed that the binding capacity of the cytoplasm decreases as oestradiol enters the nucleus (Jensen *et al.* 1968), which may imply a transport function for the cytoplasmic receptor.

In view of reports that oestradiol uptake and retention by the uterus *in vivo* are influenced by the hormonal state of the animal (Eisenfeld & Axelrod, 1966; McGuire & Lisk, 1968) an investigation into the effect of physiological changes on the concentration of cytoplasmic receptors in the uterus was considered to be important. This paper describes the quantitative determination of specific high-affinity oestradiol receptors in rat uterine supernatant preparations obtained during the oestrous cycle, pseudopregnancy, pregnancy, after ovariectomy or combined ovariectomy and adrenalectomy, and during the development of immature animals. An attempt has also been made to correlate some of these results with measurements

of endogenous uterine concentrations and ovarian secretion of oestradiol.

MATERIALS AND METHODS

Materials. [2,4,6,7-³H]Oestradiol (100 Ci/mmol) was obtained from The Radiochemical Centre, Amersham, Bucks., U.K. The purity was confirmed by t.l.c. on silica gel in two systems [cyclohexane-ethyl acetate (13:7, v/v) and chloroform-acetone (9:1, v/v)]. No significant contamination was detected after storage in benzene at 4°C for 7 months. The buffer solution contained 2.5 mM-EDTA and 250 mM-sucrose in 25 mM-tris-HCl buffer, pH 8.0. Dextran-charcoal suspension contained 0.0025% dextran (mol.wt. 60 000-90 000) and 0.25% Norit A in 10 mM-tris-HCl buffer, pH 8.0, containing 1 mM-EDTA and 250 mM-sucrose.

Animals. All rats used were Wistar strain virgin females obtained from the Courtauld Institute colony. Adults were aged 10-12 weeks and weighed 160-200 g. In one experiment immature rats were used, with ages varying from 1.5 to 8 weeks (20-180 g). The following methods were used to establish the physiological state of the animals.

(a) Oestrous cycle. Virgin female rats were used that had undergone at least two consecutive 4-day cycles before the experiment. The onset of oestrus was determined by microscopic examination of vaginal smears taken at 4 h intervals throughout the night following pro-oestrus. Groups of animals were killed at intervals of 0, 12, 24, 36, 54 and 78 h after the early oestrus state was first observed. Vaginal smears were again examined at the time of killing and the size and appearance of the

uterus were noted. Animals that were abnormal in either of these respects were discarded.

(b) *Pregnancy.* Vaginal smears were examined for sperm on the morning after mating, which was designated day 0. Groups of pregnant animals were then killed on successive days after this. The pregnant uterus was subdivided, as far as possible, into the component tissues. For example, on days 7–9 the 'implantation areas' including foetus, membranes, placenta and surrounding uterine wall were separated from the remainder of the uterus for the purpose of assay. In later stages of pregnancy further subdivision was possible and the foetus, membranes, foetal placenta, maternal placenta and uterine wall were dissected out and assayed separately.

(c) *Pseudopregnancy.* Pseudopregnancy was initiated by glass-rod stimulation of the uterine cervix on the day of oestrus and was confirmed by daily examination of the vaginal smear.

(d) *Ovariectomy.* Animals were ovariectomized between 14.00 and 16.00 h on the day of oestrus and groups were killed at the same time on successive days up to 4 weeks after the operation. One group underwent simultaneous ovariectomy and adrenalectomy and the animals were killed at intervals of 1 to 2 weeks later. In another group animals ovariectomized 2 weeks previously were treated with daily injections of oestradiol ($2\mu\text{g}$ in 0.2 ml of 0.9% NaCl subcutaneously) for 3 days before they were killed.

Preparation of uterine supernatant. The animals were killed by cervical dislocation and the uteri were dissected out, washed with ice-cold 250 mM-sucrose and weighed. In one experiment, the endometrium and myometrium were separated *in situ* in the anaesthetized rat by slitting each uterine horn antimesometrially along its entire length and dissecting away the endometrium from the myometrium across the mid-region, by using forceps and a fine scalpel bent at its tip. Once free of the muscle layer the endometrium was stripped anteriorly and posteriorly and cut (Waynfirth, 1965). Histological examination of sections of the tissue obtained in this way showed that the method yielded 'pure' myometrium, but the endometrium usually had a small amount of circular muscle from the myometrium adhering to it. Most of this could be removed by placing the endometrium with the luminal surface downwards on a glass plate and gently rubbing the upper surface of the endometrium with a piece of dry gauze. This procedure left only a negligible amount of myometrium attached to the endometrium. The latter was then transferred to homogenizing medium and the surface of the glass plate was rinsed with the same fluid to collect any epithelial cells that had become detached from the endometrium.

The tissue was minced and resuspended in 10 vol. of 250 mM-sucrose. Homogenization was carried out at 0°C in a tightly fitting glass-glass homogenizer by using ten 5 s strokes of the pestle after dispersal of the tissue. As an alternative a Silverson tissue disintegrator (Silverson Machine Ltd., Waterside, Bucks., U.K.) was used with three 10 s periods of homogenization at low speed interspersed with 20 s cooling intervals. The two methods gave identical recoveries. As a routine procedure homogenates were centrifuged at $800g_{av}$ for 10 min. Comparison with samples centrifuged at $105\,000g_{av}$ for 1 h showed no difference in oestradiol-binding capacity

between the low-speed and high-speed supernatants. All operations were carried out at 4°C. Supernatants and pellets were stored separately in the deep-freeze at -14°C for up to 2 weeks. No deterioration of the oestradiol binding protein was observed under these conditions.

Determination of high-affinity oestradiol receptors in uterine supernatant preparations. The establishment of conditions for the assay of high-affinity oestradiol receptors in rat uterine supernatant preparations is described in detail in the preceding paper (Méster, Robertson, Feherty & Kellie, 1970a). The routine assay procedure was as follows. Portions (0.1 ml) of low-speed uterine supernatant preparation were added to each of a series of tubes containing [^3H]oestradiol (5–100 pg) in buffer (0.2 ml). The tubes were incubated for 30 min at 30°C. Dextran-charcoal suspension (0.5 ml) was added to each tube and incubation was continued for a further 10 min at 30°C with constant shaking. The tubes were then cooled in ice and centrifuged at $800g_{av}$ for 5 min at 4°C. Portions (0.5 ml) of the resulting supernatant were transferred to counting vials containing 10 ml of scintillation liquid [0.4% 2,5-diphenyloxazole and 0.01% 1,4-bis-(5-phenyloxazol-2-yl)benzene in toluene]. Each vial was agitated for 10 s on a Whirlimixer (Fisons Scientific Apparatus Ltd., Loughborough, Leics., U.K.) and stood overnight before its radioactivity was counted. All blanks and standards were prepared and their radioactivities counted under identical conditions. The validity of using this two-phase counting system was confirmed by comparison with a dioxan-based scintillation liquid; identical results were obtained. Scintillation counting was carried out in a Packard model 3375 β -scintillation spectrometer.

The fraction of total oestradiol bound by high-affinity receptors at equilibrium was calculated from the measured radioactivity of each sample and this information was used to construct a Scatchard (1949) plot from which the molar concentration of receptor sites was determined.

Determination of uterine endogenous oestradiol. Portions (0.2 ml) of uterine supernatant preparation were heated at 60°C for 20 min to inactivate the high-affinity oestradiol receptors. The concentration of endogenous oestradiol in these samples was determined by the competitive-binding method of Méster, Robertson & Kellie (1970b).

Chemical determination of DNA and protein. The DNA content of the nuclear pellet was measured by the method of Burton (1956). The total protein concentration in the low-speed uterine supernatant preparations was determined by the method of Lowry, Rosebrough, Farr & Randall (1951).

RESULTS

Variation of oestradiol-binding capacity throughout the oestrous cycle. Table 1(a) shows the concentration of cytoplasmic high-affinity oestradiol receptors as measured in the whole uterus throughout the oestrous cycle. The concentration was low in oestrus and early dioestrus but began to rise in mid-dioestrus, reaching a peak early on the day preceding oestrus. The overall difference in terms of mol/ μg of DNA was more than tenfold. Table

Table 1. *Changes in oestradiol-binding capacity of rat uterus over the oestrous cycle*
Each value represents the mean $\pm$ S.D. of at least five individual experiments.

Stage of cycle	Tissue	No. of expts.	Tissue wt. (mg)	Oestradiol-binding capacity				$10^{10} \times K_{\text{dis.}}$ (mol/l)	
				(pmol/ μ g of DNA)	(pmol/mg of protein)	(pmol/mg of tissue)	(pmol/ uterus)		
<i>(a) Whole uterus</i>									
Early oestrus (0 h)	Whole uterus	6	368 $\pm$ 91	0.0016 $\pm$ 0.0004	0.019 $\pm$ 0.004	0.0027 $\pm$ 0.0005	1.0 $\pm$ 0.33	2.1 $\pm$ 0.3	
Oestrus (12 h)	Whole uterus	5	397 $\pm$ 33	0.0005 $\pm$ 0.0001		0.0010 $\pm$ 0.0003	0.41 $\pm$ 0.12	1.5 $\pm$ 0.2	
Metoestrus (24 h)	Whole uterus	8	261 $\pm$ 47	0.0007 $\pm$ 0.0001	0.032 $\pm$ 0.008	0.0017 $\pm$ 0.0003	0.45 $\pm$ 0.09	1.5 $\pm$ 0.2	
Dioestrus I (36 h)	Whole uterus	9	249 $\pm$ 43	0.0007 $\pm$ 0.0002		0.0018 $\pm$ 0.0002	0.42 $\pm$ 0.1	2.0 $\pm$ 0.7	
Dioestrus II (54 h)	Whole uterus	6	229 $\pm$ 34	0.0025 $\pm$ 0.0004	0.062 $\pm$ 0.010	0.0047 $\pm$ 0.0010	1.08 $\pm$ 0.27	3.1 $\pm$ 0.4	
Pro-oestrus (78 h)	Whole uterus	5	350 $\pm$ 53	0.0050 $\pm$ 0.0010	0.055 $\pm$ 0.012	0.0049 $\pm$ 0.0010	1.56 $\pm$ 0.23	3.4 $\pm$ 0.5	
<i>(b) Separated endometrium and myometrium</i>									
Oestrus	Endometrium	5	158 $\pm$ 40	0.0021 $\pm$ 0.0004		0.0058 $\pm$ 0.0023	0.91 $\pm$ 0.35	3.5 $\pm$ 0.5	
	Myometrium	5	116 $\pm$ 30	0.0014 $\pm$ 0.0003		0.0045 $\pm$ 0.0008	0.52 $\pm$ 0.15	2.5 $\pm$ 0.5	
Dioestrus I	Endometrium	5	116 $\pm$ 16	0.0007 $\pm$ 0.0004		0.0021 $\pm$ 0.0009	0.24 $\pm$ 0.10	2.4 $\pm$ 0.5	
	Myometrium	5	89 $\pm$ 8	0.0005 $\pm$ 0.0001		0.0022 $\pm$ 0.0009	0.20 $\pm$ 0.10	2.1 $\pm$ 0.4	
Dioestrus II	Endometrium	4	145 $\pm$ 7	0.0013 $\pm$ 0.0003		0.0043 $\pm$ 0.001	0.62 $\pm$ 0.15	2.8 $\pm$ 0.1	
	Myometrium	4	110 $\pm$ 9	0.0006 $\pm$ 0.0001		0.0023 $\pm$ 0.0005	0.25 $\pm$ 0.05	2.2 $\pm$ 0.2	
Pro-oestrus	Endometrium	5	183 $\pm$ 8	0.0028 $\pm$ 0.0006		0.0086 $\pm$ 0.0025	1.56 $\pm$ 0.35	2.5 $\pm$ 0.2	
	Myometrium	5	103 $\pm$ 2	0.0015 $\pm$ 0.0002		0.0053 $\pm$ 0.0004	0.55 $\pm$ 0.05	2.4 $\pm$ 0.2	

Table 2. *Changes in uterine oestradiol-binding capacity during pseudopregnancy*

Each value represents the mean of results from at least three individual experiments. Where more than five experiments were performed, the mean $\pm$ S.D. is given.

Physiological state	Tissue	No. of expts.	Tissue wet wt. (mg)	Oestradiol-binding capacity of supernatant			$10^{10} \times K_{dis.}$ (mol/l)
				(pmol/ μ g of DNA)	(pmol/mg of uterus)	(pmol/uterus)	
Pseudopregnancy							
Day 1	Whole uterus	3	280	0.0013	0.0030	0.84	2.2
Day 4	Whole uterus	3	262	0.0016	0.0050	1.35	2.1
Day 7	Whole uterus	3	175	0.0018	0.0050	0.90	1.7
Day 8	Whole uterus	6	193 $\pm$ 20	0.0024 $\pm$ 0.0006	0.0065 $\pm$ 0.0015	1.26 $\pm$ 0.10	2.1 $\pm$ 0.3
Day 9	Whole uterus	5	191 $\pm$ 15	0.0031 $\pm$ 0.0005	0.0078 $\pm$ 0.0010	1.49 $\pm$ 0.20	1.7 $\pm$ 0.3
Day 10	Whole uterus	6	259 $\pm$ 30	0.0050 $\pm$ 0.0006	0.0091 $\pm$ 0.0011	2.36 $\pm$ 0.30	2.1 $\pm$ 0.1
Day 11	Whole uterus	6	348 $\pm$ 25	0.0023 $\pm$ 0.0005	0.0040 $\pm$ 0.0010	1.39 $\pm$ 0.30	2.2 $\pm$ 0.4
Day 12 (oestrus)	Whole uterus	6	280 $\pm$ 30	0.0014 $\pm$ 0.0004	0.0034 $\pm$ 0.0009	0.95 $\pm$ 0.20	2.0 $\pm$ 0.3

Table 3. *Changes in uterine oestradiol-binding capacity after ovariectomy*

Each value represents the mean obtained from several individual animal experiments. Where more than five experiments were performed, the mean $\pm$ S.D. is given.

No. of days after ovariectomy	No. of expts.	Wet wt. of uterus (mg)	Oestradiol-binding capacity of supernatant			$10^{10} \times K_{\text{dis.}}$ (mol/l)
			(pmol/ μ g of DNA)	(pmol/mg wet wt.)	(pmol/uterus)	
(a) Ovariectomized only						
1	3	210	0.00075	0.0044	0.92	4.0
2	4	184	0.00061	0.0036	0.65	3.4
3	3	194	0.00110	0.0076	1.48	5.0
4	3	160	0.0010	0.0069	1.10	4.0
5	3	121	0.00085	0.0061	0.73	4.0
7	3	114	0.00120	0.0094	1.07	3.5
12	5	80	0.0006 ± 0.0001	0.0072	0.59 ± 0.16	3.3 ± 0.4
15	4	100	0.00086	0.0081	0.81	2.9
22	7	72	0.00070 ± 0.0002	0.0088 ± 0.0025	0.63 ± 0.20	3.3 ± 0.5
28	3	67	0.00060	0.0084	0.57	3.5
(b) Ovariectomized and adrenalectomized						
7	5	106 ± 10	0.9 ± 0.1	0.0087 ± 0.0010	0.85 ± 0.06	4.1 ± 0.8
18	4	95	0.6	0.0064	0.61	3.5
(c) Ovariectomized and injected with oestradiol						
	6	204 ± 22	3.2 ± 0.7	0.0084 ± 0.0026	1.71 ± 0.50	3.5

1(b) shows that high-affinity receptors were present in both endometrium and myometrium, although their concentration was consistently 40–100% higher in the former. A maximum receptor concentration was observed on the day before oestrus for both tissues, but the overall changes were less marked in this experiment (Table 1b) than in the previous one (Table 1a). This was probably because the stage of the cycle was less precisely defined. The dissociation constant at 30°C for the oestradiol receptor complex was approx. 2.5×10^{-10} M in both endometrium and myometrium.

Pseudopregnancy. Table 2 shows the changes in oestradiol-binding capacity during pseudopreg-

nancy. For the first week the concentration of sites/ μ g of DNA was rather low but began to rise on day 8, reaching a peak on day 10–11; it then declined rapidly as the animal came back into oestrus. The peak that is observed on the day before oestrus in the normal animal appears to be delayed until the tenth day of pseudopregnancy.

Ovariectomy. The results in Table 3 indicate that ovariectomy eliminated essentially the cyclic changes in binding capacity that were observed in the intact adult rat. The slightly higher values observed on days 3, 4 and 7 would coincide with the larger rise observed in the intact animal and may reflect a small residual cycling. However, too

Fig. 1. Concentration of cytoplasmic oestradiol receptors in rat uteri during the oestrous cycle, pseudopregnancy and after ovariectomy. ●, Oestradiol-binding capacity in the intact adult rat during the oestrous cycle (E, oestrus; DI, dioestrus I; DII, dioestrus II; P, pseudopregnancy); ○, oestradiol-binding capacity in animals made pseudopregnant by cervical stimulation during oestrus (day 0); △, oestradiol-binding capacity in animals ovariectomized during oestrus (day 0). Ranges indicate standard deviation of five or more determinations. Single points represent the means of three or four determinations.

much significance cannot be attached to these values owing to the small number of animals in each group. As the weight of the uterus decreased to about 20–30% of the original value as a result of ovariectomy, the binding capacity, when expressed in mol/mg of tissue, appeared to rise to a plateau that was rather higher than the maximum value observed in the oestrous cycle. However, the DNA content of the uterus remained virtually constant and therefore on a DNA basis the concentration of binding sites neither increased nor decreased significantly but remained at approximately the same value as in the intact animal in oestrus (Fig. 1). This concentration was maintained for at least 28 days after castration and has been observed even after several months. A similar result was obtained with animals that were simultaneously ovariectomized and adrenalectomized. Treatment of ovariectomized animals with large doses of oestradiol resulted in an approximately threefold increase of oestradiol-binding capacity. The content of total cytoplasmic protein showed a similar increase.

Distribution of oestradiol receptors in the pregnant uterus. The concentration and distribution of oestradiol receptors were investigated in the pregnant uterus from the day of mating until day 18. The results are shown in Table 4. Taking the uterus as a whole, the binding capacity/mg wet wt. of tissue was high in the first week of pregnancy compared with that of the non-pregnant animal.

However, because of the increased DNA content, the capacity/ μ g of DNA at the time of implantation was less than half the peak value observed in the oestrous cycle. In the 7–9-day-pregnant animal more than 90% of the total binding protein was found to be associated with the 'implantation areas', which included fetuses, membranes, placenta and surrounding uterine wall. The receptor concentration in these areas was five to six times that in the remaining uterine wall. Further subdivision of these 'implantation areas' was possible at later stages of pregnancy. Thus on day 10 it was found that no receptors were present in the foetus or surrounding membranes, but a relatively high concentration was found in the placenta compared with that in the uterine wall. On day 13 no oestradiol binding was detected in the amniotic fluid, amnion, foetus or foetal placenta. Receptors were present in the maternal part of the placenta but in lower concentration than on day 10. On day 18 no binding was detectable in the placenta. Thus the high oestradiol-binding capacity associated with the 'implantation areas' in early pregnancy appears to be localized in the developing placenta and to decline as pregnancy progresses. The receptor concentration in the uterine wall is consistently low throughout pregnancy.

Oestradiol-receptor concentration in the immature rat uterus. Fig. 2 shows the changes that occur in the uterine oestradiol-receptor concentration during growth of the immature rat. In the 10-day-old animal the concentration was about three times as high as the maximum value observed in the adult rat. It decreased sharply as the animal approached puberty.

DISCUSSION

The aim of this investigation was to determine the concentration of high-affinity oestradiol receptors present in uterine cytoplasmic preparations under a variety of physiological conditions. The assay technique used was rapid and simple and permitted determination of very low concentrations of high-affinity receptors in the presence of low-affinity receptors. Theoretical aspects of the assay have been discussed in some detail in the preceding paper (Mészter *et al.* 1970a). The slight instability of the oestradiol-receptor complex under the conditions used for establishing equilibrium (30 min at 30°C) has been disregarded, since the deterioration was shown to be less than 5%. The removal of [3 H]oestradiol bound to low-affinity receptors by incubating the equilibrium mixture with charcoal for 10 min at 30°C considerably improved the sensitivity of the assay. All the Scatchard plots obtained by this method were linear, indicating that the receptors measured were homogeneous in

Table 4. *Distribution of supernatant oestradiol-binding capacity in the pregnant uterus*

Each value represents the mean of at least three experiments.

Day of pregnancy	Tissue	No. of expts.	Oestradiol-binding capacity of supernatant		$10^{10} \times K_{dis.}$ at 30°C (mol/l)
			(pmol/ μ g of DNA)	(pmol/mg of tissue)	
0	Whole uterus	3	—	0.0079	3.6
1	Whole uterus	3	—	0.0079	3.0
2	Whole uterus	4	—	0.0086	3.3
3	Whole uterus	4	—	0.0070	3.3
4	Whole uterus	3	—	0.0087	4.2
5	Whole uterus	5	0.0015 ± 0.0004	0.0069 ± 0.0015	2.6
7	Conceptus	4	0.0027	0.0127	1.6
	Remaining uterine wall	4	0.0005	0.0033	2.0
9	Conceptus	3	0.0018	0.0108	2.0
	Remaining uterine wall	3	0.0003	0.0020	1.9
10	Foetus+membranes	3	0.00005	0.0005	1.7
	Placenta	3	0.0023	0.0072	1.5
	Metrial gland	3	0.0008	0.0027	1.7
	Uterine wall	3	0.0007	0.0030	1.9
13	Amniotic fluid	3	0	0	—
	Amnion	3	0	0	—
	Foetus	3	0	0	—
	Foetal placenta	3	0	0	—
	Maternal placenta	3	0.0006	0.0017	1.9
	Metrial gland	3	0.0001	0.0002	2.0
	Uterine wall	3	0.0006	0.0028	2.2
18	Placenta	2	0	0	—
	Uterine wall	2	0.0008	0.0040	2.1

Fig. 2. Changes in the concentrations of cytoplasmic oestradiol receptors in rat uterus during maturation. Each point represents a single determination. Where uteri from five or more animals were pooled for a single determination the range of weights is indicated. The range of receptor concentrations shown at 180g represents the normal range observed over the oestrous cycle (see Fig. 1).

their affinity for oestradiol. It is possible that more than one type of high-affinity receptor was present. Korenman & Rao (1968) have shown that high-affinity cytoplasmic oestradiol-receptor molecules, with a sedimentation coefficient of approx. 9S, can be reversibly dissociated into smaller subunits that have the same affinity for oestradiol. A similar dissociation may occur *in vivo*. The object of the present experiments was to determine the total concentration of high-affinity receptors and no attempt has been made to differentiate between particles of different size.

One limitation of this assay method is the exclusion of receptor sites already occupied by bound endogenous oestradiol. The low rate of dissociation of the high-affinity complex (half-life 76min) prevents occupied sites from equilibrating with added oestradiol under the conditions of the assay. The observed changes in oestradiol-binding capacity under different physiological conditions might therefore be interpreted as reflecting changes in the degree of saturation of the receptors with endogenous hormone. However, preliminary measurements of uterine endogenous oestradiol have been carried out in this department by using

a competitive-binding method (Méster *et al.* 1970b) and the results indicate that endogenous concentrations of hormone are insignificant compared with the concentration of available receptor sites. Moreover, the changes in receptor concentration over the oestrous cycle closely follow the pattern of oestradiol secretion (Hori, Ide & Miyake, 1968; Méster *et al.* 1970b) and the peak of binding capacity coincides with maximum concentration of endogenous oestradiol. Thus the observed changes in binding capacity are not due to saturation of the receptors with endogenous oestradiol.

The concentration of oestradiol receptors in the uterus appears to be influenced by the hormonal state of the animal. The observed changes do not reflect changes in the total cytoplasmic protein. The latter was found to vary by the factor 3 over the oestrous cycle, reaching a maximum during oestrus, i.e. 24 h after the maximum concentration of oestradiol receptor. The close correspondence between the changes in receptor concentration and the pattern of oestradiol secretion throughout the oestrous cycle suggests a possible activation or induction of the receptor by oestradiol. The peak in receptor concentration that normally occurs in late dioestrus is also observed on the tenth day of pseudopregnancy. After ovariectomy the peak is abolished and the receptor concentration remains at a constant low value. Treatment of the ovariectomized animal with oestradiol causes a significant increase in the concentration of oestradiol receptor. These observations are consistent with an induction or activation of the binding protein by oestradiol. However, the presence of exceptionally high concentrations of receptor sites in immature rat uteri obviously cannot be explained in these terms.

The fluctuations in cytoplasmic binding capacity may reflect changes in the distribution of receptors between cytoplasm and nucleus. This might be explained in terms of the two-step mechanism for oestrogen action proposed by Jensen *et al.* (1968), who showed that transfer of oestradiol from cytoplasm to nucleus was accompanied by a decrease in the supernatant binding capacity. Alternatively the receptors may be localized in a particular type of cell of which the number is subject to hormonal influence. The observation that receptors having a similar affinity for oestradiol are present in both endometrium and myometrium is consistent with previous studies (King & Gordon, 1966).

In the pregnant rat uterus the marked localization of oestradiol-binding capacity in the region of the implanted foetus has led to the identification of an oestradiol receptor in the placenta that appears to be identical in binding characteristics with the receptor in the non-pregnant uterus. This receptor is only found in the maternal part of the placenta, and the relatively high concentration observed in

early stages appears to decrease as pregnancy progresses. This decrease may be related to the degeneration of the maternal part of the placenta, which begins to occur on days 9–10 and continues in later stages of pregnancy. The binding capacity of the uterine wall and metrial gland remains low throughout pregnancy.

The results of studies on oestradiol uptake *in vivo* under different physiological conditions (Eisenfeld & Axelrod, 1966; McGuire & Lisk, 1968) indicate that there may be a correlation between the ability of the uterus to take up and retain injected oestradiol and the concentration of cytoplasmic receptor measured *in vitro*. Retention is reported to be greater in the intact than in the ovariectomized rat and to be increased by oestrogen pretreatment. It is also higher in dioestrus than in pro-oestrus and higher in the immature than in the adult rat.

The significance of these results in determining the role of the cytoplasmic oestradiol receptor in mediating the primary action of the hormone remains to be established. It has been demonstrated by other workers that the cytoplasmic receptor participates in the transfer of oestradiol from cytoplasm to nucleus and that a supernatant factor is required for the formation of the nuclear receptor (Jensen *et al.* 1968; Shyamala & Gorski, 1969; Rochefort & Baulieu, 1969). It has also been shown that a heat-labile cytoplasmic factor is required for oestrogen stimulation of RNA polymerase activity in isolated nuclei (Raynaud-Jammet & Baulieu, 1969). It remains uncertain whether the cytoplasmic receptor functions simply as a means of concentrating oestradiol within the cell and transferring it to a separate nuclear receptor or whether part of the oestradiol-receptor complex itself enters the nucleus. The possibility that the receptor exerts a regulatory action at the cytoplasmic level cannot be excluded. The observation that the cellular concentration of the receptor is strongly influenced by hormonal changes may contribute to an understanding of its function.

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