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OESTROGEN AND PROGESTIN RECEPTORS IN DECIDUOMA OF THE RAT

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SUMMARY

The ability of cytoplasmic and nuclear fractions from decidual tissue to bind [^{3}H]-oestradiol-17β and the progesterone analogue [^{3}H]-R-5020 was examined during the growth phase, maintenance and regression of trauma-induced unilateral deciduoma of pseudopregnant rats and compared to that of the control uterine horn. High affinity ($K_D = 10^{-10}$ to 10^{-11} M) 8 S cytoplasmic oestrogen receptors, as well as nuclear oestrogen receptors, were detected at all stages of decidual life, but the concentration of both types of oestrogen receptors per unit DNA gradually decreased from the 6th to the 12th day of pseudopregnancy. Cytoplasmic progestin receptors ($K_D = 10^{-9}$ to 2×10^{-9} M) sedimenting at 4–5 S and nuclear progestin receptors were present at all stages of decidual development. The concentration of these receptors declined sharply towards the 12th day of pseudopregnancy in the deciduoma, without comparable changes in the non-decidualized horn. It is possible that the terminal decline in the concentration of steroid receptors in the deciduoma accounts for the inability of exogeneous steroids to prolong the life-span of the deciduoma or to prevent its regression.

INTRODUCTION

The decidua is a product of the structural and functional differentiation of endometrial stromal cells, induced in the pregnant animal following the contact of the blastocyst and endometrium. A similar endometrial transformation, referred to as "deciduoma", can be induced in the rat by artificial stimuli of the uterus on the 4th day of pseudopregnancy, or in the uterus of ovariectomized animals, after proper endocrine conditioning [1, 2, 3].

In the rat, the life history of an artificially induced deciduoma may be divided into three stages: (1) proliferation of stromal cells and their differentiation into decidual cells, which occurs between the 4th and 8th day of pseudopregnancy; (2) maintenance of the deciduoma, a stage in which there is no further increase in decidual weight, between days 9–11 of pseudopregnancy; (3) regression of the decidual tissue during days 12–16 of pseudopregnancy [3].

The hormones which are required for the sensitization of the uterus before a decidualizing stimulus are progesterone and small amounts of oestrogen [3–5]. Continuous presence of progesterone is essential and adequate for proliferation of the decidual cells and for the growth and maintenance of the deciduoma. However, the addition of small amounts of oestrogen is required to elicit a maximal growth response [3, 6].

The effects of progesterone and oestrogen on target tissues are mediated by binding of these hormones to cytoplasmic receptors and subsequent translocation of the steroid receptor complex into the nucleus [7, 8]. Since neither the continued provision of progesterone nor of oestrogen can prolong the life-span of the deciduoma [9] it is reasonable to assume that the growth and regression of decidual tissue may be reflected by changes in the amount or nature of the receptors to one or both steroids. We therefore examined some of the properties of oestrogen and progestin receptors during the various stages of development of an artificially induced deciduoma.

MATERIALS AND METHODS

Animals. 3-month-old-Wistar-derived rats of the departmental colony were used. Vaginal smears were examined daily. After cyclic regularity was recorded, pseudopregnancy was induced by mating with vasectomized males on the evening of proestrus. The day after a copulatory plug was found in the vagina, i.e., the first day on which a leucocytic vaginal smear was observed, was designated as L_1 and the subsequent days of pseudopregnancy as L_{2-n}. For induction of deciduoma, the anti-mesometrial aspect of the endometrium of one uterine horn was scratched on L_4 with a barbed gauge-18 hypodermic needle, inserted into the lumen. The second uterine horn served as a control.

Materials. Oestradiol-17β (E_2) and cortisol were purchased from Ikapharm (Ramat Gan, Israel), diethyl stilbestrol (DES) from Sigma Chemical Company, (St. Louis, MO, U.S.A.) and R-5020 (17,21-dimethyl-19-nor-pregna-4,9-diene-3,20-dione) was a generous gift from Dr. J. P. Raynaud, Centre de Recherches Roussel, UCLAF, Paris, France. [^{3}H]-R-5020 (86 Ci/mmol) and [2,4,6,7-^{3}H]-E_2, (92 Ci/mmol) were obtained from New England Nuclear, Boston, MA, U.S.A.

Buffers. Buffer A: 10 mM Tris–HCl, 1.5 mM EDTA, 20% (w/v) glycerol, 12 mM β-mercaptoethanol, pH 7.4; Buffer B: 20 mM Tris–HCl, 320 mM sucrose, 3 mM $MgCl_2$, 3 mM $CaCl_2$, 10 mM KCl, pH 7.4; Buffer C: composition as for buffer B, except that sucrose concentration was 250 mM.

Dextran coated charcoal (DCC) contained 0.05% dextran (T-70 Pharmacia, Upsala, Sweden) and 0.5% charcoal (Fisher Scientific Company, NJ, U.S.A.) in Tris–HCl buffer (10 mM, pH 7.4). Scintillation fluid contained 8 g PPO, 0.22 g POPOP (Packard Instr. Company, IL, U.S.A.) 1 l toluene and 0.5 l Triton X-100.

Tissue preparations. Animals were sacrificed by ether inhalation on the days indicated. Their uteri were removed immediately, placed in cold (0–4°C) buffer (buffer A when processed for sedimentation analysis and buffer C for other determinations), trimmed, and freed of fat. The traumatized uterine horns were slit longitudinally, and decidual tissue was separated from the myometrium by scraping with a scalpel. The uterine horn and decidual tissue were weighed and minced. All subsequent procedures were performed at 0–4°C, except when otherwise noted.

Homogenization. Tissues were homogenized in 5 vol. of buffer A when prepared for sucrose gradient sedimentation analysis, or in buffer B for other steroid receptor binding assays. Uteri were homogenized using three bursts (5 s) of an Ultra-Turax blender (Type 18/2, Janke & Kunkel) and 30 s intervals for cooling. The decidual tissue was homogenized using 10 strokes of a glass Teflon homogenizer.

Preparation of crude nuclear fraction. Homogenates in buffer B were centrifuged at 800 *g* for 10 min. The resulting pellets were suspended in buffer C, centrifuged again at 800 *g* and resuspended in 10 vol. of buffer C. The nuclear preparations were filtered through four layers of gauze (Miracloth, Chicopee Mills, Inc., NY, U.S.A.). These filtrates were used for nuclear binding assays.

Preparation of cytosol. Cytosol was obtained by centrifugation of the 800 *g* supernatant at 100,000 *g* for 60 min. The resulting supernatant was diluted 1:1 (v/v) in buffer A. The final concentration of protein in these preparations was 2–5 mg/ml. The concentration of protein was determined using the method of Lowry[10].

Assay and characterization of receptors

1. *Concentration of cytoplasmic receptors.* Each experiment was done in triplicate. Samples of 0.1 ml cytosol and 0.1 ml buffer A were placed in two series of glass tubes. To one series radioactive steroid solution was added at a final concentration of 15 nM. The second series contained 15 nM radioactive steroid and 3 μM of the corresponding non-radioactive oestrogen or progestin. [^{3}H]-E_2 and non-radioactive DES were used for determination of oestrogen receptors (R_E). [^{3}H]-R-5020 and non-radioactive R-5020 were used for determination of progestin receptors (R_P) [11]. Cortisol, 3 μM, was added to all tubes which contained [^{3}H]-R-5020 to prevent binding of this progesterone analogue to the corticosteroid receptors [12].

Following incubation at 0°C for 24 h, 0.4 ml DCC was added to each tube. The mixture was stirred, left to stand at 0°C for 10 min, and then centrifuged at 1500 *g* for 10 min. The supernatant was decanted into scintillation vials, and radioactivity was measured in a Packard 3310 liquid scintillation spectrometer. "Specific" binding was calculated by subtracting the "non specific" binding observed in the presence of excess of the non-radioactive steroid from "total" tritiated steroid bound in the absence of non-radioactive steroid. The amount of receptors was expressed as fmol bound steroid per μg DNA, using the method of Burton for DNA determination [13].

2. *Concentration of nuclear receptors.* Experiments were done in triplicates. Samples of nuclear preparations (0.1 ml) were placed in two series of glass tubes, using steroid concentrations as described in section 1.

For determination of nuclear R_E, incubations were performed for 1.5 h at 30°C. For determination of nuclear R_P, incubations were performed for 3 h at 15°C. At the end of the incubation periods, tubes were cooled, and 2 ml of cold buffer C were added. The mixtures were stirred and centrifuged for 10 min at 800 *g*. The supernatants were decanted and the pellets were washed once more with cold buffer. The final pellets were dissolved in 0.2 ml Soluene-350 (Tissue Solubilizer, Packard Instr. Company, IL, U.S.A.), decanted into scintillation vials and the radioactivity was measured. Calculation of specific binding and expression of results were as described in section 1.

3. *Affinity of cytoplasmic receptors.* For determining the affinity of R_E, duplicate samples of cytosol (0.1 ml)

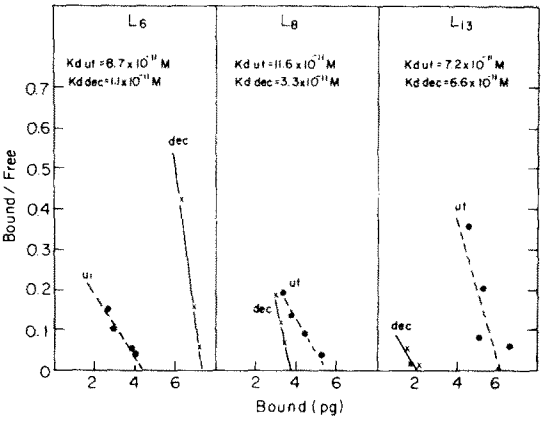

Fig. 1. Scatchard analysis of the binding of oestrogen to cytosol preparations from deciduoma (×——×) and control uterine horn (●- - -●). The cytosol prepared from deciduoma or control uterine horn at each stage of pseudopregnancy was pooled from 4–5 animals, and the assay was performed as described in Materials and Methods. K_D ut = K_D of R_E in cytosol of control uterine horn, and K_D dec = K_D of R_E in cytosol deciduoma.

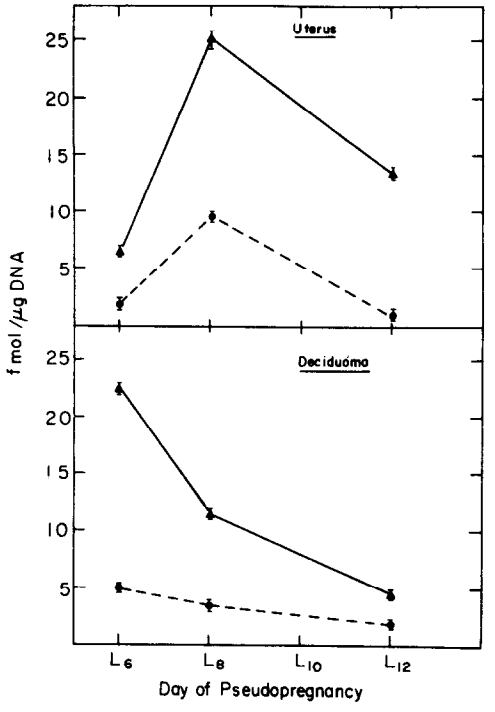

Fig. 2. Changes in the concentration of [^{3}H]-E_2 binding to cytosol and nuclear preparations from deciduoma and control uterine horn during various stages of pseudopregnancy. ●---●, nuclear binding; ▲——▲, combined cytoplasmic and nuclear binding measured as described in Materials and Methods. Vertical bars represent standard error of mean values of triplicate samples prepared from a pool of 4–5 animals.

were incubated with 0.1 ml buffer A containing 10^{-11} M [^{3}H]-E_2, and increasing amounts of non-radioactive DES, ranging from 10^{-11} to 10^{-8} M. The affinity of R_P was determined by incubating duplicate samples of 0.1 ml cytosol with 0.1 ml of buffer A containing 10^{-10} M [^{3}H]-R-5020, 2×10^{-8} M cortisol and increasing amounts of non-radioactive R-5020 (10^{-10} – 10^{-8} M).

Following incubation at 0°C for 24 h, the unbound steroid was separated from the bound fraction by DCC and specific binding was measured as described above (section 1). The affinity constants were calculated from these data, using Scatchard plot analysis [14].

4. *Sedimentation coefficient analysis.* A linear 5–20% sucrose gradient in buffer A (containing 10% glycerol) was prepared. Cytosol preparations (0.2 ml) were preincubated for 3 h with 15 nM tritiated steroid with or without 200-fold excess of the corresponding unlabelled steroid. Cortisol (3 μM) was added to all samples containing [^{3}H]-R-5020. The incubation mixtures were layered on the top of the gradient, centrifuged for 16 h at 45,000 rev./min in a SW 50.1 rotor at 1°C. Radioactivity was measured in fractions of 0.2 ml, collected from the bottom of the tube. Bovine serum albumin (BSA, fraction V, 4.5 S), and yeast alcohol dehydrogenase (ADH, 7.8 S), obtained from Sigma Chemical Company, St. Louis, Mo., USA, served as reference markers [15].

5. *Translocation of oestrogen and progestin receptors.* Female rats bearing 4-day-old deciduomata were separated into 3 groups: Group I was injected subcutaneously with 4 mg progesterone in peanut oil; Group II was injected with 25 μg E_2 in peanut oil; Group III was injected with peanut oil and served as control. Two hours after injection, the animals were killed. Nuclei and cytosols of either uteri or decidual tissues were prepared, and cytoplasmic or nuclear binding was measured as described in sections 1 and 2. Nuclear receptor concentration was expressed as fmol steroid bound per μg DNA and cytoplasmic receptor as fmol steroid bound per mg protein.

RESULTS

Cytoplasmic oestrogen receptor and nuclear translocation in decidual tissue

Using the DCC separation technique and Scatchand plot analysis, low capacity–high affinity (K_D = 1–7 × 10^{-11} M) receptors for oestrogen were found in cytosol from decidual tissue examined 2–9 days after decidual induction (Figs 1, 2). This cytoplasmic R_E sedimented at low salt concentration (buffer A) as an 8 S complex (Fig. 3).

Nuclear preparations from decidual tissue (L_6–L_{12}) also showed binding of oestrogen (Fig. 2). During the growth phase (L_6), the apparent K_D for the decidual receptor was 1.1×10^{-11} M, compared with 8.7×10^{-11} M in cytosol from the control uterine horn (Fig. 1) and the total number of oestrogen binding sites (combined cytoplasmic and nuclear receptors) was higher in the deciduoma (22 fmol/μg DNA) than in the control uterine horn (6 fmol/μg DNA) (Fig. 2). During the maintenance (L_8) and regression (L_{12}) phases, a progressive decline in apparent affinity

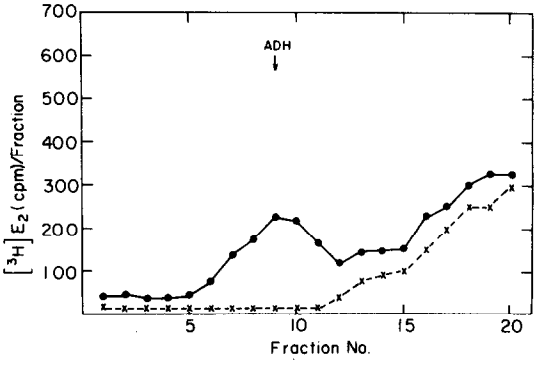

Fig. 3. Sedimentation profile (in 5–20% sucrose gradient) of the binding of [^{3}H]-E_2 to cytosol prepared from deciduoma on L_8. The assay procedure is described in Materials and Methods. ●——●, binding in the presence of 15 nM [^{3}H]-E_2; ×---×, binding in the presence of 15 nM [^{3}H]-E_2 and 3 μM DES. ADH (7.8 S) was centrifuged simultaneously in separate tubes.

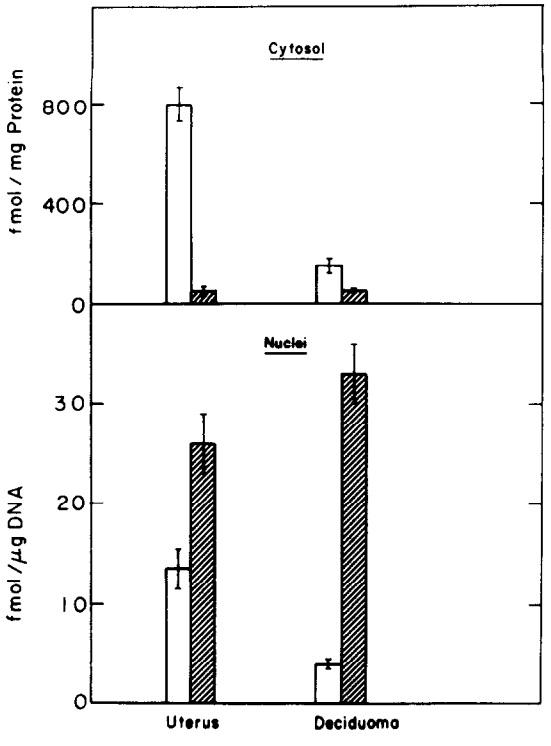

Fig. 4. Translocation of oestrogen receptors from cytosol into the nuclei of control uterine horn and deciduoma on L_8 of pseudopregnancy. Binding of [^{3}H]-E_2 to cytosol and nuclear preparations was measured: □, 2 h after injection with 0.1 ml peanut oil; ■, 2 h after injection with 25 μg E_2 in 0.1 ml peanut oil. Each column represents the mean ± S.E.M. of triplicate samples prepared from a pool of 4 animals.

(Fig. 1) and in nuclear as well as total receptor concentration (Fig. 2) in the decidua was observed. However, the inter-assay variability of K_D-determinations is considerable and the lower apparent K_D in the regressing deciduoma may merely reflect a larger contribution of non-specific binding components at low receptor concentrations. Moreover, the accuracy of K_D determination by the Scatchard plot technique was limited, since the available data points cover only a restricted segment of the saturation curve (Fig. 1). In the control uterine horns, both nuclear and total receptor concentration showed a peak on day L_8.

Injection of E_2 (25 μg/rat) into deciduoma-bearing rats on day L_8 resulted within 2 h in almost total disappearance of oestrogen receptors from uterine and decidual cytosol and in significant increase in oestradiol-binding by nuclear preparations from both the deciduoma and the control uterine horn (Fig. 4). The concentration of nuclear binding sites in the decidual tissue of oestradiol-treated animals was slightly higher than in the uterine tissue, but the difference was not statistically significant.

Cytoplasmic and nuclear progestin receptors in decidual tissue

Saturable progestin binding sites of high affinity were found in the cytoplasm of decidual tissue, with no significant difference in apparent affinity between the three developmental stages examined (L_6, L_8 and L_{13}) or between the deciduomata and the control uterine horns (K_D = 1.3–2.6 × 10^{-9} M for R-5020) (Fig. 5). This cytoplasmic R_P, whether derived from control uterine horn or deciduoma, sedimented at 4–5 S in low salt sucrose gradients (Fig. 6A); however

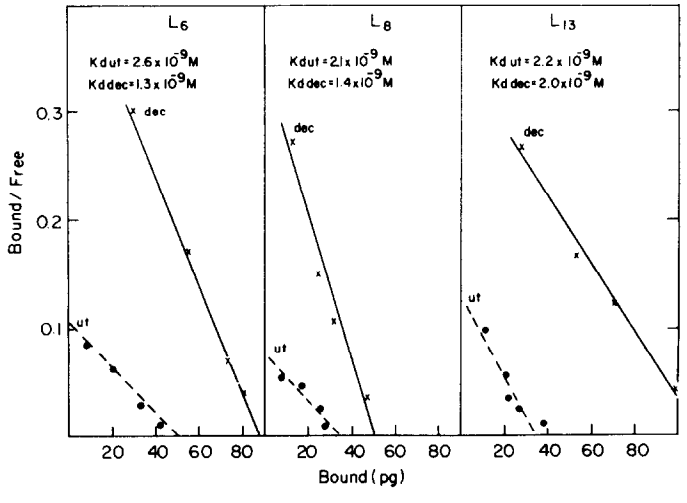

Fig. 5. Scatchard analysis of binding of R-5020 to cytosol preparations from deciduoma (×——×) and control uterine horn (●---●). The cytosol prepared from deciduoma and control uterine horn at each stage of pseudopregnancy was pooled from 4–5 animals, and the assay was performed as described in Materials and Methods. K_D ut = K_D of R_P in cytosol of control uterine horn and K_D dec = K_D of R_P in cytosol of deciduoma.

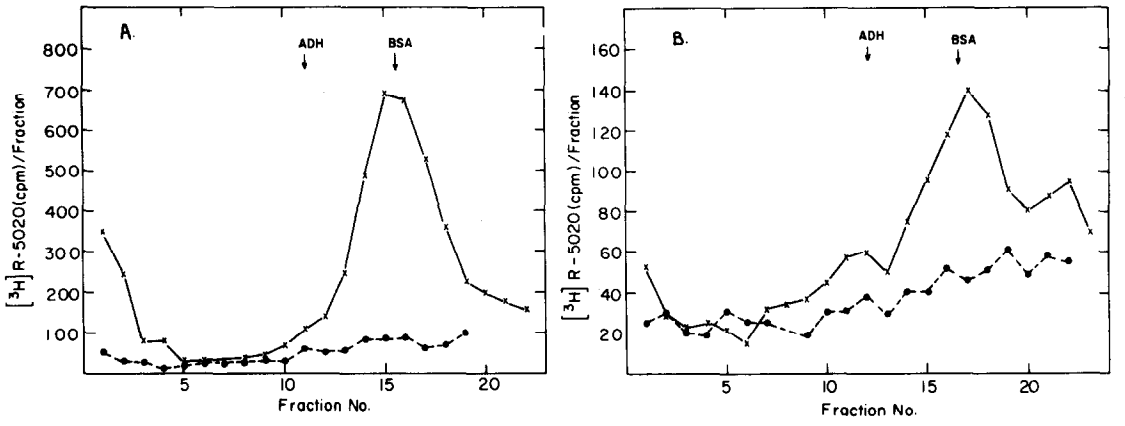

Fig. 6. Sedimentation profile (in 5–20% sucrose gradient) of the binding of [^{3}H]-R-5020 to cytosol prepared from deciduoma (A), or control uterine horn (B), on L_8. The assay procedure is described in Materials and Methods. ×——×, binding in the presence of 15 nM [^{3}H]-R-5020 and 3 μM cortisol; ●---●, binding in the presence of 15 nM [^{3}H]-R-5020, 3 μM cortisol and 3 μM non-radioactive R-5020. ADH (7.8 S) and BSA (4.5 S) were centrifuged simultaneously in separate tubes.

in uterine preparations an additional, small 8 S component could be discerned (Fig. 6B).

Total receptor concentration (cytoplasmic plus nuclear binding sites) was higher in the decidual tissue than in the control uterine horns on days L_6–L_8, but declined sharply in decidual tissue toward the end of the life-span of the deciduoma (L_{12}); no change was seen in the control uterine horns (Fig. 7). The ratio of nuclear to cytoplasmic binding was higher in decidual than in control uterine tissue at all stages of development examined.

Injection of progesterone (4 mg) into deciduoma-bearing rats on day L_8 resulted within 2 h in a 50% reduction in the concentration of cytoplasmic binding sites for R-5020 in the control uterine horns, but only a 20% decline was found in the decidual tissue. At the same time, nuclear binding of R-5020 increased in the control uterine horns, but not in the decidual tissue. Nevertheless, the concentration of nuclear binding sites in deciduoma of progesterone injected rats was higher than in nuclei from control uterine horn (Fig. 8).

DISCUSSION

The results indicate the presence of oestrogen (R_E) and progestin (R_P) receptors in deciduoma of pseudopregnant rats and describe the pattern of changes in concentration of cytoplasmic receptors to both steroids during decidual growth and regression, essentially confirming a recent report [16, 17]. The present study extends these findings by relating the changes to contemporaneous events in the non-decidualized uterine horn, and adds information on the nuclear binding of both steroids indicative of translocation of the steroid receptor from the cytoplasm into the nucleus. This process is considered essential for the biological action of steroid hormones [8].

The observed changes in the concentration of steroid receptors in the uterus and deciduoma during pseudopregnancy cannot be explained by variation in receptor occupancy for the following reasons: (i) the plasma levels of E_2 [18] and of progesterone [19] remain essentially stable during the period covered

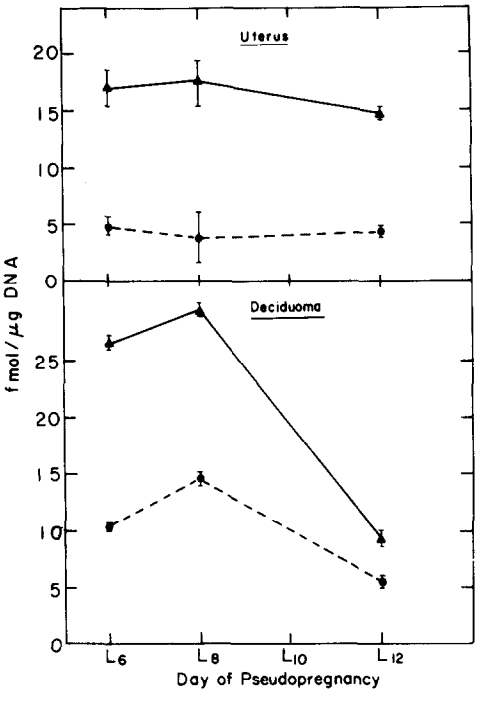

Fig. 7. Changes in the concentration of [^{3}H]-R-5020 binding to cytosol and nuclei prepared from deciduoma and control uterine horn during various stages of pseudopregnancy. ●---●, nuclear binding; ▲——▲, combined cytoplasmic and nuclear binding measured as described in Materials and Methods. Vertical bars represent standard error of mean values of triplicate samples prepared from a pool of 4–5 animals.

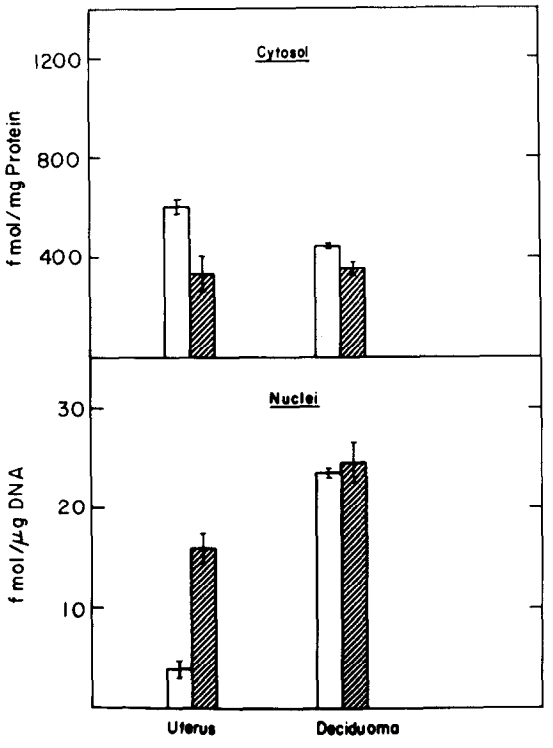

Fig. 8. Translocation of progestin receptors from cytosol into nuclei of control uterine horn and deciduoma on L_8 of pseudopregnancy. Binding of [^{3}H]-R-5020 to cytosol and nuclear preparations was measured: □, 2 h after injection with 0.1 ml peanut oil; ■, 2 h after injection with 4 mg progesterone in 0.1 ml peanut oil. Each column represents the mean ± S.E.M. of triplicate samples prepared from a pool of 4 animals.

by this study; (ii) the assay of cytoplasmic R_P employed was shown to measure the total cytoplasmic receptor concentration [20, 21]; (iii) although our assay of cytoplasmic R_E measures exclusively vacant receptors, it has been shown that only 5–10% of the oestrogen-occupied uterine receptor population remains in the cytoplasm [22]; (iv) the concentration of nuclear R_E and R_P was determined by an exchange assay and hence reflects the total receptor content of the nuclei, irrespective of occupancy.

The results obtained should be viewed in relation to existing information regarding the regulation of steroid receptor levels in the uterus. Oestrogen is believed to induce its own receptor [23–25] as well as the generation of receptors for progesterone [23, 25–27]. Progesterone on the other hand, acting in conjunction with oestrogen, has been reported to inhibit replenishment of uterine R_E [23, 25, 28]. Progesterone reduces the level of its own receptor [27, 29–31]. The concentration of R_E was high in the deciduoma during the proliferative stage (L_6) compared to the non decidualized contralateral uterine horn and declined during the maintenance phase (L_8). In contrast, the uterine R_E population (both nuclear and total) increased during this time interval, but decreased later—toward L_{12}, though both organs were exposed to the same hormonal milieu. Since the early deciduoma (until L_8) was found to contain a higher concentration of R_P, and particularly of nuclear R_P, it is possible that the earlier observed reduction in R_E concentration in the deciduoma was due to a greater sensitivity of this tissue to the putative inhibitory action of progesterone on the regeneration of the R_E [28].

During the incipient regression (L_{12}), there was a marked decline in the concentration of total and nuclear receptors for both oestrogen and progestin in the deciduoma. Armstrong *et al.*[16, 17] also reported a decline in cytoplasmic R_E and R_P (per unit protein) by L_{12}, but suggest that the decline may only be an apparent one due to an increased protein content of the tissue. However, we observed a similar decline when receptor concentration is expressed per unit DNA. It should be noted that this parameter, too, does not reflect receptor number per cell, since deciduoma consists of a heterogeneous cell population, including many multinucleated and polyploid cells [32].

The depletion of steroid receptors may be related to the limited life-span of the deciduoma, since this cannot be extended by provision of exogenous progesterone [9] and E_2 will even shorten it [5, 33]. Whether the decline in the concentration of steroid receptors and in particular of R_P is a cause or a sequel of decidual regression remains to be critically established.

The present study establishes that an efficient mechanism for nuclear translocation of R_E exists in deciduoma, as in all oestrogen responsive cells thus far examined [34]: practically the entire receptor population shifted to the nuclear fraction following administration of exogenous E_2. Nuclear binding of R-5020 was also evident in deciduoma as well as in uterine tissue, indicating that nuclear translocation must be operative in both tissues. However, in contrast to the control uterine horn, where exogenous progesterone induced an increased translocation of the cytoplasmic receptor into the nucleus, administration of exogenous progesterone failed to cause further transfer of cytoplasmic receptors into decidual nuclei. These findings suggest that the nuclear acceptor sites in the chromatin of decidual cells may be saturable and thus limit nuclear uptake.

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