

Changes in Cytosolic and Nuclear Progesterone Receptors During Pregnancy in Rat Placenta¹

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ABSTRACT

These studies examine changes in placental growth and the abundance of progesterone receptors (Rp) in whole placentas between Days 9 and 22 of pregnancy. In addition, some placentas were dissected into decidual basalis, junctional zone, and labyrinth zone before assay of Rp. High affinity binding of ³H-progesterone to Rp was detected at all stages of pregnancy in whole placentas and in decidual basalis and the junctional zone of the placenta. Cytosolic and nuclear receptors exhibited similar affinity for progesterone in both tissues ($K_d = 3.1 \pm 0.3$ and 4.4 ± 0.7 nM, respectively). Receptor binding in whole placentas increased from Day 9 to Day 12 ($p < 0.05$), declined markedly at Day 16 ($p < 0.05$), and returned to former levels on Days 19 and 22 ($p < 0.05$). Decidual basalis contained 84% of total Rp on Day 14, which declined to 67% on Day 17 ($p < 0.05$). The junctional zone contained 16% of total Rp on Day 14 and 33% on Day 17. After Day 17, junctional zone was the only source of Rp. The decline in Rp on Day 16 followed regression of decidual basalis; recovery of Rp thereafter was due to growth of the junctional zone. The labyrinth zone did not express significant amounts of Rp at any stage despite a 4-fold increase in growth in late pregnancy. Although the biologic role of the Rp in maintenance of pregnancy is poorly understood, these studies suggest that the maternal decidual basalis and fetal junctional zone are targets of progesterone action.

INTRODUCTION

Little is known about the action of progesterone (P) in the placenta. Such studies are very difficult to design because placental structure and function change continually with the advance of pregnancy (Faber and Thornburg, 1983). Indeed, the effects of P probably also change with stage of placental development. Nevertheless, many studies have shown that the placenta atrophies and pregnancy is terminated when the supply of P is interrupted (Dickman and Hart, 1972; Cheese and Chatterton, 1982; Phillips et al., 1988). We have previously used the rat placenta as a model to study P action and found that it contains progesterone receptors (Rp) and nuclear acceptor sites that appear to mediate action

of the hormone (Ogle, 1980, 1986a,b, 1987; Ogle et al., 1987).

The present study examines changes in the concentration of cytosolic and nuclear Rp throughout pregnancy in whole placentas and in decidual and dissected regions of the chorioallantoic placenta. These changes are examined with regard to tissue levels of P and the capacity of the placenta to synthesize proteins in an effort to better understand the role of this hormone in the regulation of placental function.

MATERIALS AND METHODS

Animals

Female Long-Evans rats (225–250 gms) from Blue Spruce Farms (Altamont, NY) were maintained under a 12L:12D photoperiod and a temperature of 22°C. Day 1 of pregnancy was determined by appearance of vaginal sperm on the morning following overnight exposure to

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fertile males. Rats were killed at 0800–0830 h between Days 9 and 22 of pregnancy; whole placental tissue was then prepared for study as described below. Observations taken on Day 22 were within 8–12 h of parturition for animals in our colony.

Placental Dissection and Tissue Preparation

Uteri were rapidly excised and placed in ice-cold TG-30% buffer (0.01 M Tris-HCl, [Tris(hydroxymethyl)aminomethane hydrochloride], Sigma Chemical Co., St. Louis, MO, and 30% glycerol, Fisher Scientific, Fairlawn, NJ, vol/vol, pH 7.8 at 0°C). Uteri were sliced longitudinally and each placenta was carefully separated from amniotic membranes, the embryo, and maternal tissue by gentle teasing with needles and fine forceps (Ogle, 1980, 1986a). The procedure for dissecting the chorioallantoic placenta into decidua basalis, junctional, labyrinth, and choriovitelline zones has been described previously (Soares et al., 1985; Soares, 1987). For these experiments only, pregnant Holtzman rats (Harlan Sprague-Dawley, Indianapolis, IN) were used. The embryos with their encapsulating decidual tissues were dissected from the uteri on Days 14, 17, and 20 of gestation, under a dissecting microscope (10–20× magnification). The junctional zone, identified by its pale appearance due to the absence of fetal blood, was separated from the labyrinth zone, a richly vascularized tissue, and from the decidua basalis with fine forceps and 23-gauge needles. The overlying decidual tissue and the underlying yolk sac/umbilical insertion were removed as completely as possible with fine forceps and iridectomy scissors. The choriovitelline placenta was dissected on Day 14 and assayed for Rp content. The completeness of the dissection procedure has been verified by histological examination. The tissues were immediately frozen in liquid nitrogen and stored frozen at –80°C in TDGL buffer (TG buffer containing 1 mM dithiothreitol and 0.2 mM leupeptin) until assayed for Rp. Preliminary experiments showed no loss of receptor compared to fresh tissue when frozen in this manner.

Assay of Rp

Whole placentas or dissected tissues were pooled and homogenized at 0°C in a hand-driven glass homogenizer. Ice-cold conditions were maintained throughout subsequent procedures. The Rp assays have been validated

and are well characterized (Ogle, 1980, 1981, 1982, 1986a,b). A brief description of the procedures is given below.

Cytosol receptor (Rpc) exchange assay. The placental homogenate was filtered through two layers of nylon gauze, then centrifuged at 800 × g for 15 min. The supernatant, containing Rpc, was decanted, leaving the pellet containing the nuclear receptor (Rpn) fraction. Endogenous P was removed from supernatant by treatment with dextran-coated charcoal and the cytosol was prepared by centrifugation at 157,000 × g for 50 min.

The Rpc was partially purified by differential precipitation with ammonium sulfate (Ogle, 1981). One hundred microliters of the Rp preparation (0.1–0.2 mg protein) were incubated with 200 μl TDGL or TDGF (TG buffer containing 1.0 mM phenylmethylsulfonyl-fluoride) containing four or six concentrations of ³H-P (1.0–17 nM) in the presence or absence of a 100-fold molar excess of unlabeled P at 0–4°C for 18–20 h (Ogle, 1980, 1983). The results of these assays were identical with TDGL or TDGF buffer.

Rpn exchange assay. The nuclear pellet was rinsed twice with fresh TG buffer after the 800 × g centrifugation and finally suspended in TDGL or TDGF buffer. Aliquots (100 μl) containing 0.1–0.2 mg DNA were incubated 22–24 h at 4°C with 5–6 concentrations of ³H-P with or without 100-fold molar excess of unlabeled P (Ogle, 1986a).

Protein Synthesis by Placentas, in Vitro

Protein synthesis was measured in whole placental tissues taken from rats on Days 9, 12, 14, 16, 19, and 22 of pregnancy. Placental minces were incubated with 2 μCi of [4,5-³H(n)]-L-leucine (60.0 Ci/mmol, New England Nuclear, Boston, MA) in Eagle's basal medium Sigma Chemical Co.) with Earle's salts, L-glutamine (2 nM), leucine (108 mg/ml), and 25 mM HEPES buffer (N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid) at 37°C for 1.5 h with shaking in a humidified atmosphere of 93% oxygen and 7% carbon dioxide at a final pH 7.2–7.4. The minces were homogenized in 0.5 M perchloric acid (PCA) at 0°C. Precipitable material was resuspended and rinsed 3 times with PCA. The pellet was solubilized in 1 ml of 0.3 M NaOH and aliquots were taken for protein determination (Lowry et al., 1951). An aliquot was emulsified in ScintiVerse II (Fisher Scientific) and the amount of labeled protein

was determined by liquid scintillation counting after correcting for quenching.

This assay was validated by the criteria of Rannels and coworkers (1982). Additionally, the volume of distribution of ^{14}C -sorbitol, an extracellular marker, was less than 3% of the ^3H -leucine values, indicating that cellular integrity was maintained throughout the incubation period. The rate of ^3H -leucine incorporation into placental protein was linear with time and proportional to the amount of tissue under these conditions (data not shown).

Preparation and Assay of Uterus, Decidua, and Placenta for P Content

Tissues for determination of P content were collected from rats on Days 9, 12, 14, 16, 19, and 22 of pregnancy ($n = 7\text{--}11$ rats/group). The uterus was rapidly excised, placed in ice-cold saline, and cut longitudinally. Segments of uterus containing the implantation sites were carefully separated and the placentas were carefully dissected from the embryo, amniotic membranes, and maternal tissue. The dissected tissues from each animal were grouped into uterine segments, decidua, and placentas, and then repeatedly rinsed in saline to remove blood. The tissues were blotted, weighed, minced, and rinsed twice more in ice-cold saline to remove remaining blood before being homogenized. After homogenization, aliquots were taken for protein (Lowry, et al., 1951) and DNA determinations (Hill and Whatley, 1975). The tissues were stored frozen at -20°C until assayed for P. Serum was also collected and stored similarly until assayed for P.

P was measured in uterine tissues and serum by an established radioimmunoassay procedure (Mills and Osteen, 1977). P was extracted from tissue homogenates with diethyl ether and redissolved in ethanol and aqueous buffer before exposure to P antibody. The P antibody exhibited cross-reactivity of less than 1% for androgens, estrogens, and progestins (including 20 α -dihydroprogesterone and tetrahydroprogesterone).

Calculation of Binding Parameters

Equilibrium binding parameters were calculated by the direct linear plot (Eisenthal and Cornish-Bowden, 1974) and by a nonlinear regression data analysis program. The nonlinear regression program (ENZFITTER, Elsevier-BIOSOFT, Cambridge, U.K.) fits experimental

data to the Michaelis-Menten equation by using the enhanced algorithm of Marquart to determine best fit by repeated iterations. Double reciprocal plots were also analyzed by this procedure. Binding parameters determined by these methods were in essential agreement.

Expression of Data and Statistical Analysis

Specific activity of binding or synthesis is expressed per unit protein or DNA. Total activity is used to express the capacity of the entire tissue for binding or synthesis (i.e. per tissue). All values are reported as means \pm SEM. Comparisons between means were made by ANOVA and the Student-Newman-Keul's multirange test.

Chemicals

Unlabeled P, Tris-HCl, dithiothreitol, leupeptin, sucrose, Eagle's basal medium with Earle's salts, leucine, HEPES buffer, and PCA were purchased from Sigma Chemical Co. Radiolabeled P ($[1,2,6,7\text{-}^3\text{H}]\text{P}$; sp. act., 90–114 Ci/mmol) and ^3H -leucine were purchased from New England Nuclear, Boston, MA. The labeled steroid was purified before use on Sephadex LH-20 column (0.5×16 cm) in a solvent system of benzene/methanol (85:15, vol/vol).

RESULTS

Concentration of R_{pc} and R_{pn} Sites in Whole Placentas (Table 1)

The specific binding activity of R_{pc} sites (pmols/mg protein) was maximum on Day 9 and decreased about 66% by Day 12 ($p < 0.05$), falling more gradually thereafter. R_{pn} sites were in greatest concentration in early pregnancy (Days 9–14) and exhibited a 4-fold decline at Day 16, which was maintained for the remainder of pregnancy ($p < 0.05$). R_{pc} content remained relatively constant throughout pregnancy but decreased from the highest levels on Day 12 to minimal values on Days 16 and 22 ($p < 0.05$). Placental content of R_{pn} was plentiful throughout pregnancy and exhibited no significant change as pregnancy progressed. Total receptor content of the placenta (pmols R_{pc}/placenta + pmols R_{pn}/

TABLE 1. Changes in the progesterone receptor in rat placenta during pregnancy.^a

Day of pregnancy	K _d (nM)	Cytosolic receptor		K _d (nM)	Nuclear receptor	
		pmols/mg protein	pmols/placenta		pmols/mg DNA	pmols/placenta
9	1.5 ± 0.2	1.0 ± 0.1	0.06 ± 0.01	4.2 ± 0.8	13.7 ± 1.3	0.09 ± 0.01
12	2.5 ± 0.2	0.32 ± 0.08*	0.21 ± 0.06	3.2 ± 0.8	16.2 ± 2.8	0.24 ± 0.04
14	2.4 ± 0.3	0.11 ± 0.01**†	0.06 ± 0.01	5.8 ± 0.7	12.1 ± 2.4	0.23 ± 0.02
16	3.4 ± 2.4	0.03 ± 0.01**†	0.02 ± 0.01 ⁺	4.0 ± 0.7	3.2 ± 0.6**	0.08 ± 0.02
19	4.8 ± 0.5*	0.08 ± 0.01**†	0.10 ± 0.02	7.5 ± 0.8	4.4 ± 0.7**	0.22 ± 0.04
22	4.0 ± 1.3*	0.03 ± 0.01**†	0.03 ± 0.01 ⁺	9.1 ± 2.4	4.6 ± 1.4**	0.22 ± 0.03

^aValues are means ± SEM. Means represent determinations made on placentas from 4–6 rats for each day of pregnancy. Statistical analysis by ANOVA and Student-Newman-Keul's multirange test.

*Values differ from Day 9; *p* < 0.05.

†Values differ from Day 12; *p* < 0.05.

**Values differ from Day 9, Day 12, Day 14; *p* < 0.05.

SATURATION ANALYSIS OF THE NUCLEAR PROGESTERONE RECEPTOR IN PLACENTA

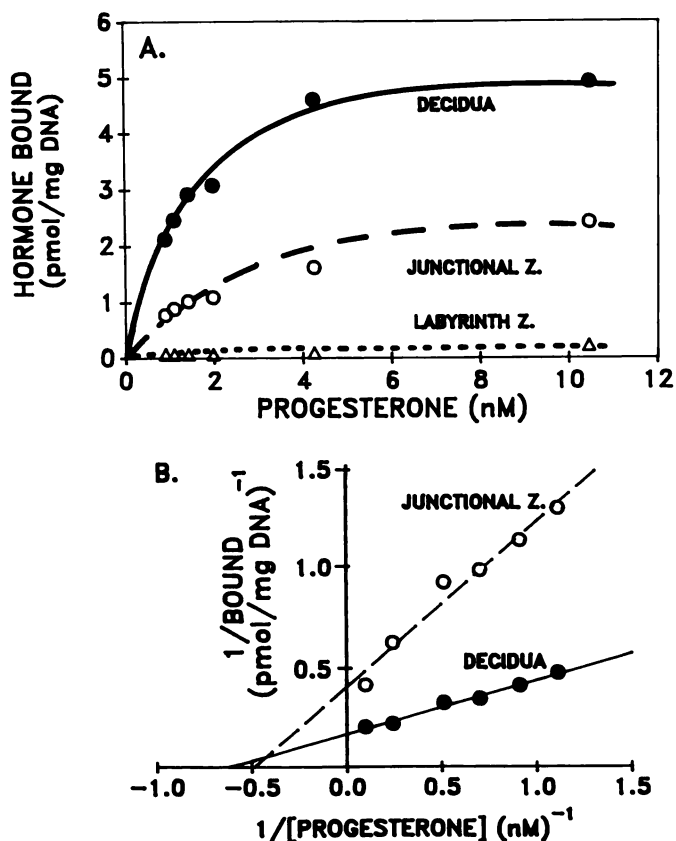


FIG. 1. Saturation analyses (A) and double reciprocal plots (B) of ³H-P binding to the nuclear progesterone receptor in the decidua basalis, junctional zone, and labyrinth zone of rat placenta. Binding parameters: Decidual basalis (Day 17), K_d = 1.6 nM; 5.9 pmol binding sites/mg DNA. Junctional zone (Day 20), K_d = 2.2 nM; 2.0 pmol binding sites/mg DNA. Labyrinth zone (Day 17), K_d = 98.3 nM; 0.08 pmol binding sites/mg DNA (the values for the double reciprocal plot were off scale and could not be presented on these axes). Representative binding parameters are depicted except for labyrinth zone, which usually exhibited no specific binding. See text for explanation. All values corrected for nonspecific binding.

placenta) increased from Day 9 to Day 12 (*p* < 0.05), declined markedly at Day 16 (*p* < 0.05), and was restored to former levels on Days 19 and 22 (*p* < 0.05).

Concentration of R_{pc} and R_{pn} Sites in Decidua, Junctional Zone, and Labyrinth Zone

To better understand the distribution of the Rp within the placenta, we dissected the decidua basalis, junctional zone, labyrinth zone, and choriovitelline zone on Days 14, 17, and 20. The choriovitelline zone was assayed only on Day 14 since it regresses thereafter (Davies and Glasser, 1968); however, no Rp was detected (data not shown). Only the decidua basalis and the junctional zone exhibited high affinity and saturable binding as expected of the Rp (Fig. 1). The K_d for the binding reaction as identical for both tissues (K_d ranged from 1.3 to 3.9 nM for R_{pc} and R_{pn}) and equal to that determined in whole placentas (Table 1), suggesting that the same Rp molecule was present in these tissues. The decidua basalis showed greatest concentration and content of Rp on Day 14 from all other regions of the placenta and time periods (*p* < 0.05), but the level decreased by Day 17 (*p* < 0.05) (Fig. 2). The decidua basalis atrophies after Day 17 and could not be dissected at more advanced stages (Davies and Glasser, 1968). Rp moieties were also expressed by cells of the junctional zone on Day 14, significantly increasing between Days 17 and 20 (*p* < 0.05). Although the labyrinth zone grew throughout gestation, it did not demonstrate the presence of Rp (Figs. 1 and 3). However, several preparations of labyrinth zone exhibited very low levels of saturable binding (10–17% of junctional zone values) and low affinity for P, K_d ~100 nM (Fig.

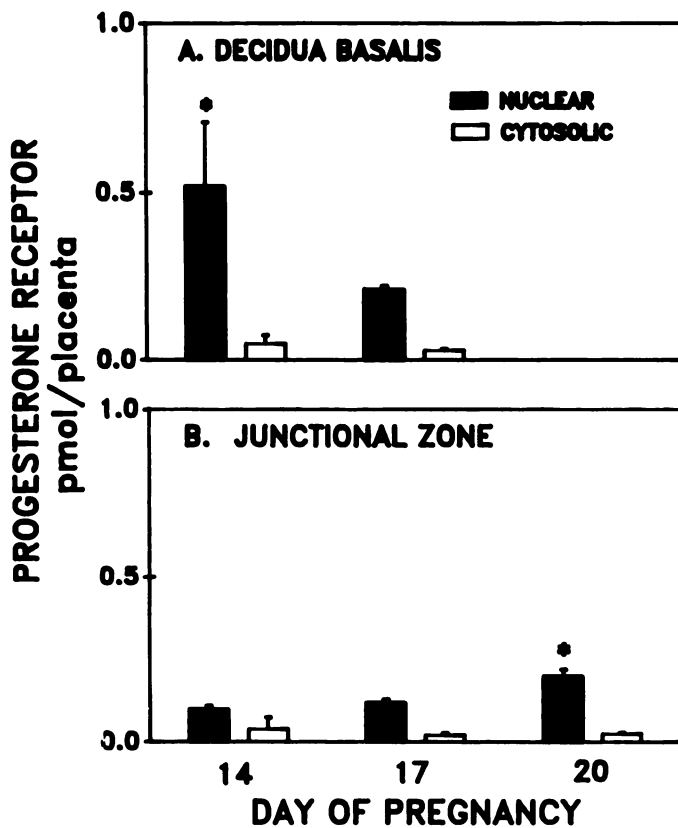


FIG. 2. Changes in the number of progesterone receptor binding sites in the decidua basalis and junctional zone of the rat placenta at Days 14, 17, and 20 of pregnancy. *Solid bars*: nuclear progesterone receptor; *open bars*: cytosolic progesterone receptor. *A*) *Mean greater than all others, $p < 0.05$. *B*) *Mean greater than Days 14 and 17, $p < 0.05$. Values are means \pm SEM of 3–6 assays.

1). These results could represent a nonreceptor moiety that binds P with low affinity and is obscured easily by high levels of nonspecific binding that characterize labyrinth preparations.

Uterine P Content

Serum P increased from Day 9 to a peak at Day 16 and declined very rapidly between Days 19 and 22 (Fig. 4). During the first two weeks of pregnancy, uterine P was distributed rather uniformly between placental tissue, decidual tissue, and interplacental segments of the uterus. P content roughly paralleled increases in serum P and ranged between 300 and 1400 ng P/mg DNA. However, placental concentration of P on Day 14 was higher than at any other time in pregnancy (2100 ± 457 ng P/mg DNA) and significantly higher than the Day 14 levels in decidua (1411 ± 223 ng P/mg DNA) and uterus (1072 ± 269 ng P/mg DNA)

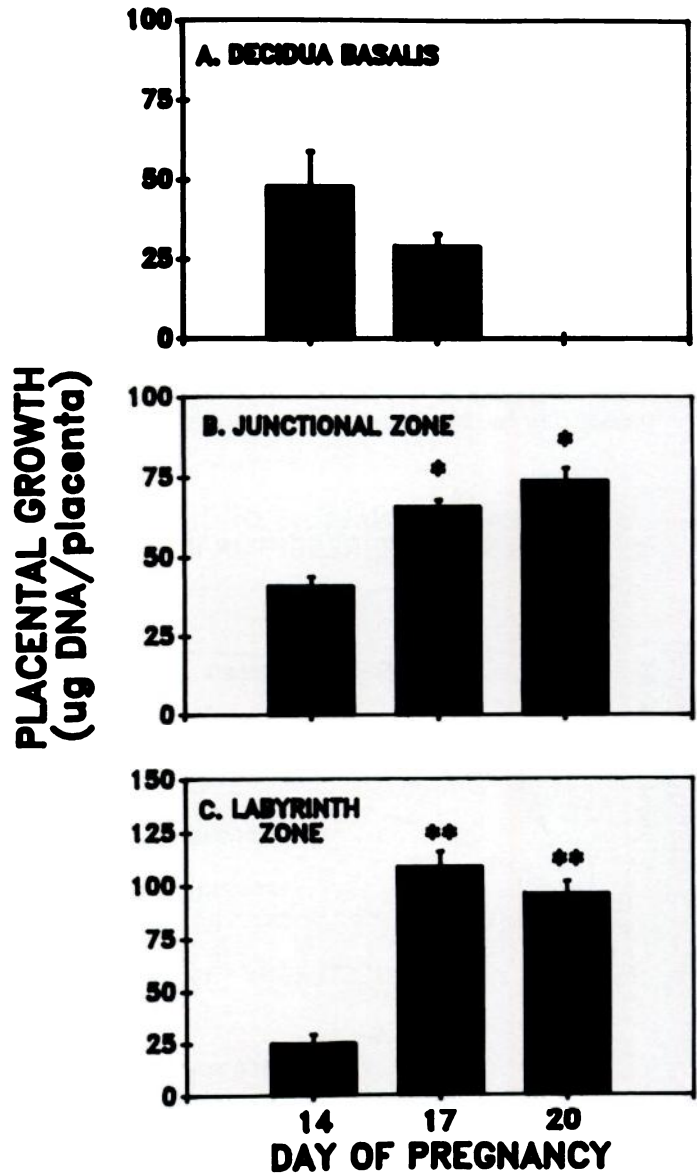


FIG. 3. Growth of the decidua basalis (*A*), junctional zone (*B*), and labyrinth zone (*C*) of rat placenta on Days 14, 17, and 20 of pregnancy. Values are means \pm SEM of 3–6 determinations. **Means greater than all others, $p < 0.05$. *Mean greater on Day 17 than Day 14 and greater on Day 20 than Day 17 or Day 14 in junctional zone, $p < 0.05$.

($p < 0.05$). By Day 19, placental tissue exhibited a 61% decrease in P concentration ($p < 0.05$), whereas decidua showed a lesser decline of 29%. By the morning of Day 22, 8–12 h prior to parturition, serum P (5 ± 1 ng P/ml) decreased 10-fold from Day 19 (54 ± 10 ng P/ml) levels, resulting in a 70% reduction of P in the placenta (610 ± 137 ng P/mg DNA), an 81% reduction in the uterus (231 ± 63 ng P/mg DNA), and 78% in decidua (411 ± 82 ng P/mg DNA).

TABLE 2. Placental growth and protein synthesis during pregnancy.^a

Day of pregnancy	Growth		Protein synthesis	
	Wet weight mg/placenta	DNA content µg/placenta	nmol ³ H-Leucine/mg protein/h	nmol ³ H-Leucine/placenta/h
9	16.7 ± 0.8*	7.2 ± 2.0*	9.8 ± 1.7 [†]	5.0 ± 0.6*
12	46.7 ± 1.7*	10.0 ± 1.8*	9.2 ± 0.7 [†]	13.8 ± 2.4*
14	77.5 ± 3.7*	14.0 ± 2.6*	5.6 ± 0.6	8.9 ± 1.6*
16	202.0 ± 6.5	26.0 ± 3.4	5.5 ± 0.3	24.8 ± 3.2
19	350.0 ± 1.3	33.0 ± 3.4	3.5 ± 0.1	34.3 ± 0.9
22	460.0 ± 1.9	23.3 ± 3.0	2.3 ± 0.3	33.9 ± 5.0

^aValues are means ± SEM. Placentas from 6–11 rats comprise each mean. Statistical analysis by ANOVA and Student-Newman-Keul's multirange test.

[†]Values differ from Day 14, Day 16, Day 19, Day 22; *p*<0.05.

*Values differ from Day 16, Day 19, Day 22; *p*<0.05.

Placental Growth and Protein Synthesis

The placenta grew most rapidly in the first 2 wk of pregnancy; this rapid growth was correlated with active protein synthesis and rapid increases in DNA (Table 2).

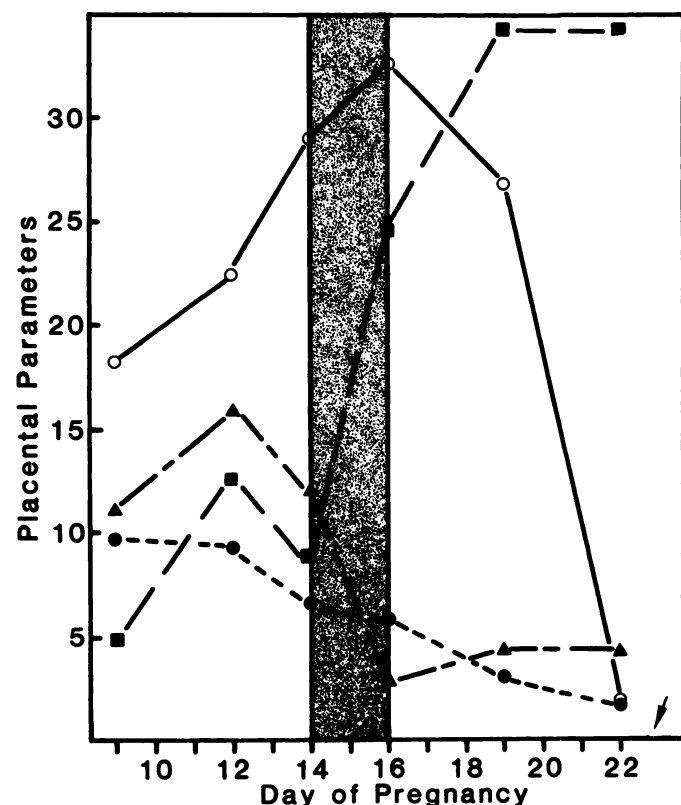


FIG. 4. A summary of changing activities in whole placenta during pregnancy with regard to the proposed "critical period." Some parameters exhibit a sharp increase and others a decrease during this interval. Refer to the text for a more detailed explanation. Values taken from Tables 1 and 2. Open circles: serum concentration of progesterone, ng/0.5 ml. closed circles: specific rate of protein synthesis, nmol leucine incorp./mg protein/h. Closed triangles: nuclear progesterone receptor, pmols/mg DNA. Closed squares: total protein synthesis, nmol leucine incorp./placenta/h. Arrow: Expected time of parturition.

The specific rate of protein synthesis (expressed per mg protein) was greatest on Days 9 and 12 and decreased continually thereafter (*p*<0.05). The total capacity of the placenta for protein synthesis slowly increased from Day 9 to Day 14. Between Days 14 and 16, a rapid increase occurred (*p*<0.05), which was maintained until term. These observations show that the placenta grew throughout pregnancy and expressed a high capacity for protein synthesis. Placental growth in the last third of pregnancy was characterized by low specific rates of protein synthesis. These findings suggest that early placental growth was due primarily to cell proliferation (increasing DNA content/mg protein) that slowed after Day 14 (*p*<0.05), and cellular hypertrophy (decreasing DNA content/mg protein) became increasingly more important thereafter.

DISCUSSION

Placental development has been characterized in this study by changes in the abundance of R_{pc} and R_{pn} and in the protein-synthesizing capacity of placental tissues. The findings suggest that the interval between Days 14 and 17 represents a critical time in the biology of the rat placenta (Fig. 4). Before this period, specific activity of P binding to the R_{pn} was greatest (*p*<0.05) as was the specific rate of protein synthesis (*p*<0.05). During this early period, the placenta grew by cell proliferation (reflected by increases in DNA) and was active in the synthesis of proteins. During the Day 14–17 transition period, the placenta became differentiated into well-defined zones and entered a hypertrophic growth phase (DNA content stabilized while protein synthesis continued, Table 2). The Day 14–17 transition period heralds the appearance of cells containing large stores of glycogen in the junctional zone (Jollie, 1964).

The labyrinth zone also undergoes considerable growth and maturation, gaining the capacity to produce placental lactogens (Soares, 1987); in late pregnancy it develops the ability to transfer nutrients and wastes between maternal and fetal compartments (Rosso, 1975). Some of these changes in placental structure and function may be induced by P.

The biologic role of the Rp in the regulation of placental protein synthesis and growth is not fully understood. However, these activities appear to be P dependent because they are blocked by ovariectomy and restored by exogenous P (Ogle et al., 1987). Furthermore, protein synthesis is blocked in the placenta by the antiprogesterone, RU-38486, which competes with P for receptor binding sites (Ogle et al., 1987; Okulicz, 1987; Ogle, 1988).

Our studies suggest that Rp in the rat placenta are restricted to cells of the decidual basalis and junctional zone. Rp are not reliably found in the labyrinth zone. The decidual basalis, the major source of Rp in the midpregnant conceptus, regresses after Day 14 and disappears after Day 17 (Davies and Glasser, 1968). On the other hand, the junctional zone continues to grow in late pregnancy and the number of Rpn increases, becoming maximal on Day 20.

The possibility exists for an interactive paracrine relationship between decidual tissue and trophoblast cells. Vicovac and Genbacev (1988) have shown that cocultured explants of human decidua and syncytial trophoblast reciprocally inhibit protein synthesis and hormone production. Bell et al. (1985) found that specific proteins induced by P action in human decidual cells also foster decidual-trophoblast interactions.

Estradiol (E) and P represent another interactive agonist/antagonist system that must be held in delicate balance for the successful completion of pregnancy. E is required for P production by the rat corpus luteum (Gibori and Keys, 1978) and stimulates the number of Rp binding sites in the placenta (Ogle, 1980, 1986b) and uterus (Luu Thi et al., 1975; Isomaa et al., 1979). The distribution of E receptors (Re) and Rp in the developing conceptus is similar; both are restricted to the decidual basalis and junctional zone (McCormack and Glasser, 1976, 1978; present study). The placental concentration of Re is high early in pregnancy (McCormack and Glasser, 1978). E, acting through the Re, up-regulates the abundance of Rp (Jordan and Dix, 1979; Horwitz et al., 1985). However, by Day 16, Re is

undetectable (McCormack and Glasser, 1978) and probably contributes to the down-regulation of Rp and Rpn that we describe at Day 16 (Fig. 4). The fall in Re and Rp may result, at least in part, from the atrophy of decidual tissue (Davies and Glasser, 1968). The increase in Rp observed at Day 20 results from growth of the junctional zone and becomes the only source of Rp. It is unknown whether estrogen induction of junctional zone Rp is direct or indirect via actions on the decidua. Decidual cells may have a role in maintaining steroid hormone responsiveness of trophoblast cells in much the same way as has been proposed for stromal-epithelial interactions in other steroid-responsive tissues (Cunha et al., 1983, 1985).

In many tissues, P inhibits replenishment of the Re and thus curtails E action and often down-regulates the Rp (Hsueh et al., 1976; McCormack and Glasser, 1978; Leavitt et al., 1983; Horwitz et al., 1985; Okulicz, 1986; West et al., 1986). However, exogenous P maintains its own receptor in the placenta after ovariectomy (Ogle, 1986b; Ogle et al., 1987). Rapid and reversible nuclear processing of receptor, which we have described previously, may allow the placenta to remain in a constantly responsive state in spite of prolonged exposure to very high concentrations of P, as occur in pregnancy (Ogle, 1986b). The placenta cannot be refractory to P or withdrawn from P support for even a few hours in early pregnancy without irreversible deleterious effects (Ogle et al., 1987). Thus, special strategies for the regulation of hormone action appear to have developed to facilitate maintenance of pregnancy.

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