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SPECIFIC [^{3}H]-ESTRADIOL BINDING IN THE FETAL UTERUS AND TESTIS OF GUINEA PIG. QUANTITATIVE EVOLUTION OF [^{3}H]-ESTRADIOL RECEPTORS IN THE DIFFERENT FETAL TISSUES (KIDNEY, LUNG, UTERUS AND TESTIS) DURING FETAL DEVELOPMENT

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SUMMARY

Specific estradiol receptors have been found in fetal uterus in the cytosol fraction and in the nuclear extracts obtained by successive extractions with (A) 0.1 M Tris–HCl–0.0015 M EDTA, (B) 0.3 M NaCl–0.01 M Tris–HCl, and (C) 1 M NaCl–0.01 M Tris–HCl. In uterine cytosol, an estradiol-specific component with a sedimentation coefficient of 8.5–9 *S* in sucrose density gradient was detected. The K_D of the binding of estradiol in fetal uterine cytosol is 4×10^{-10} M. Similar [^{3}H]-estradiol receptors are present in fetal testis.

A systematic study of the evolution of specific [^{3}H]-estradiol binding during fetal development has shown the following results. The specific binding of [^{3}H]-estradiol per mg protein in fetal kidney cytosol increased from no detectable binding at 34–35 days of gestation to 35 fmol/mg protein at 59 days with a subsequent decrease to 22 at 24 h after birth. Specific binding in the three nuclear extracts studied (0.1 M Tris, 0.3 M NaCl and 1 M NaCl) also increased from 11 fmol/mg protein at 34–35 days of gestation to 147 fmol/mg protein at 59 days and decreased to 50 at 24 h after birth. In fetal lung cytosol, specific [^{3}H]-estradiol binding increased dramatically from 24 fmol/mg protein at 34–35 days to 434 at 59 days, decreasing to 345 fmol/mg protein at 24 h after birth. Nuclear binding also increased from 27 fmol/mg protein at 34–35 days to 329 at 59 days and decreased to 114 at 24 h after birth. In the fetal uterus, specific binding in the cytosol fraction is 900 fmol/mg protein at 34–35 days of gestation and increases to 500 fmol/mg protein at the end of gestation (55–60 days). In contrast, fetal heart showed very little or no specific binding of [^{3}H]-estradiol.

INTRODUCTION

During human pregnancy, the placenta synthesizes huge quantities of estrogens and progesterone; consequently, the production rates of these hormones are significantly increased during this period [1, 2]. The concentration of estrogens in the maternal and fetal compartments also increases, particularly at the end of pregnancy [3].

Quantitative values of estradiol in fetal plasma of guinea pig show that in the early stage of gestation (30–35 days), the concentration of this hormone is 160–200 pg/ml and these high values are maintained during fetal development [4].

In previous studies in this laboratory, the presence of estrogen receptors has been shown in the brain [5], kidney [6, 7] and lung [8] of the fetal guinea pig. Recently, the existence of estrogen receptors was also demonstrated in the fetal uterus of this species [9].

In this paper, further studies on [^{3}H]-estradiol binding in fetal uterus as well as the quantitative evolution of the estradiol receptors in the different tissues (kidney, lung, uterus and testis) during fetal development are described.

MATERIALS AND METHODS

Biological material

Fetuses of Hartley Albino guinea pigs from 34–35 days to the end of gestation were used. Females and males were mated for 24 h; consequently, the days of gestation were established with an error of ±24 h.

Radioactive material

[6,7-^{3}H]-estradiol (S.A. 47.9 Ci/mmol) and [1,2-^{3}H]-testosterone (S.A. 59 Ci/mmol) were purchased from NEN Chemicals, GmbH, Frankfurt, W. Germany. Their purity was controlled by paper chromatography in the system: isooctane–toluene–methanol–water (1:4:3:2 by vol.) and, after acetylation, in the system: isooctane–methanol–water (5:3:2 by vol.). Purity was >98%.

Experimental conditions

The studies were carried out *in vivo* after subcutaneous and *in situ* administration of [^{3}H]-estradiol to the fetus or *in vitro* after incubation of the different fetal tissues in Krebs–Henseleit [10] buffer (pH 7.4). The details of the experimental conditions of each study are indicated in the results section.

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Cell fractionation

In the *in vivo* or *in vitro* experiments, different tissues (kidney, lung, uterus, testis, and heart) were fractionated according to the method of Chauveau *et al.*[11], with modifications indicated previously [12].

Tissues were homogenized in 0.25 M sucrose–0.01 M Tris–HCl–0.003 M $CaCl_2$ (pH 7.4) and centrifuged at 900 *g*. The supernatant was centrifuged at 250,000 *g* to obtain the cytosol fraction and the mitochondria–microsomal pellet. The 900 *g* pellet was washed with 0.4 M sucrose–0.01 M Tris–HCl–0.003 M $CaCl_2$ solution and centrifuged at 900 *g*. This pellet was homogenized in 2.0 M sucrose–0.01 M Tris–HCl–0.003 M $CaCl_2$ solution, layered on an equal vol. of the same solution and centrifuged at 250,000 *g* to obtain purified nuclei. Purified nuclei were extracted successively with the following solutions at pH 7.4: (a) 0.1 M Tris–HCl–0.0015 M EDTA (*0.1 M Tris*), (b) 0.3 M NaCl–0.01 M Tris–HCl (*0.3 M NaCl*), (c) 1 M NaCl–0.01 M Tris–HCl (*1 M NaCl*) (d) 3 M NaCl–0.01 M Tris–HCl (*3 M NaCl pH 7.4*), (e) 3 M NaCl–0.01 M Tris–HCl, pH 8.4 (*3 M NaCl pH 8.4*) (f) 0.2 N NaOH and (g) 90% (v/v) ethanol.

Sucrose density gradients

The gradients were prepared with 5–20% (w/v) sucrose solutions containing thioglycerol (0.012 M) and EDTA (0.001 M) with or without 0.5 M NaCl.

Protein and DNA evaluation

Proteins were measured according to the method described by Lowry *et al.*[13] including the modification suggested for very dilute protein solutions. DNA was determined by the procedure of Burton [14].

Determination of specific binding

The individual tissues were incubated either with [^{3}H]-estradiol alone (5.2×10^{-8} M) or with a 100–300-fold excess of unlabeled estradiol. Specific binding was calculated by the difference in radioactive hormone bound per mg protein after Sephadex G-15 column chromatography at 4°C. Equilibrium constants were determined by application of the Scatchard method [15] and the specific binding was subsequently calculated by applying the graphical correction of Rosenthal [16].

Measurement of radioactivity

Radioactivity in aqueous solutions was measured in Instagel (Packard, Inc.) and radioactivity in organic solvents was counted in a POPOP–PPO–toluene scintillation solution.

RESULTS

(1) *Specific binding in fetal uterus of guinea pig*

(a) "In vivo", *and* "in vitro", *experiments with the total cell.* Table 1 indicates the binding of [^{3}H]-estradiol in the different subcellular fractions after administration of [^{3}H]-estradiol (8×10^{-10} mol) to the fetus (55–60 days of gestation) or after administration of the same quantity of radioactivity plus a 100-fold excess (mol/mol) of unlabeled estradiol. Table 2 shows the data obtained after incubation of the fetal uterus with [^{3}H]-estradiol. In both cases, a significant quantity of specific [^{3}H]-estradiol binding was observed in the cytosol and in the nuclear extracts. It is interesting to note that 12.2–13.2% is localized in the nuclei of which 34.4–36.3% can be extracted by a 1 M NaCl solution. Furthermore, 31.8–42.6% of the nuclear radioactivity can still be extracted after the 1 M NaCl solution.

(b) *Effect of different steroids on the formation of* [^{3}H]*-estradiol complexes in the cytosol fraction of fetal uterus.* As indicated in Fig. 1, estradiol competes significantly in the formation of [^{3}H]-estradiol complexes. Estrone and estriol also compete but less intensely. Very little or no effect was observed with testosterone, progesterone, aldosterone or cortisol.

(c) *Affinity of* [^{3}H]*-estradiol binding to the cytosol receptor.* The application of the Scatchard method [15] showed the presence of binding sites with high affinity ($K_D = 4 \times 10^{-10}$ M) and a number of sites = 7×10^{-14} mol/mg protein.

Table 1. Subcellular distribution of radioactivity and quantity bound in the fetal uterus of guinea pig 30 min after "*in vivo*" and "*in situ*" [^{3}H]-estradiol (^{3}H–E_2) administration to the fetus

Subcellular fractions	Experiment 1 [^{3}H]-E_2(8×10^{-10} mol) % in cell	Experiment 1 [^{3}H]-E_2(8×10^{-10} mol) Bound d.p.m./mg protein	Experiment 2 [^{3}H]-E_2(8×10^{-10} mol) + E_2(×100 mol/mol) % in cell	Experiment 2 [^{3}H]-E_2(8×10^{-10} mol) + E_2(×100 mol/mol) Bound d.p.m./mg protein
Cytosol	83	18,700	78	620
Nuclear extracts				
(a) 0.1 M Tris	1.6	9,800	5.0	720
(b) 0.3 M NaCl	2.6	15,360	0.6	1000
(c) 1 M NaCl	4.8	6,650	6	620
(d) 3 M NaCl (pH: 7.4)	0.3	—	—	—
(e) 3 M NaCl (pH: 8,4)	0.2	—	N.D.	—
(f) NaOH 0.2 M	3.2	—	N.D.	—
(g) Ethanol	0.5	—	N.D.	—

The data represent the average of 2 experiments (8 fetuses).

Table 2. Subcellular distribution of radioactivity and quantity bound in the fetal uterus of guinea pig after incubation with [^{3}H]-estradiol (^{3}H-E_2)

Subcellular fractions	Experiment 1 [^{3}H]-E_2(8×10^{-8} M)			Experiment 2 [^{3}H]-E_2(8×10^{-8} M) + E_2($\times$ 100 mol/mol)		
	%* in cell	Bound fmol/mg protein	pmol/g tissue	%* in cell	Bound fmol/mg protein	pmol/g tissue
Cytosol	80.3	575	17.50	93.5	90	2.80
Nuclear extracts						
(a) 0.1 M Tris	1.2	720	0.34	0.30	105	0.04
(b) 0.3 M NaCl	1.6	815	0.58	0.20	75	0.05
(c) 1 M NaCl	4.2	950	3.90	0.40	45	0.17
(d) 3 M NaCl (pH: 7.4)	0.5	—	—	0.03	—	—
(e) 3 M NaCl (pH: 8.4)	0.3	—	—	0.02	—	—
(ff) NaOH 0.2 M	3.0	—	—	0.08	—	—
(e) Ethanol	1.4	—	—	0.08	—	—

* Percentage of the total radioactivity in the cell. The incubations of the cell suspensions of the fetal uterus were carried out in Krebs–Henseleit buffer for 15 min at 37°C in the proportion of 100 mg of tissue per 1 ml of buffer. The values represent the average of two experiments. In each of the experiments the uteri of 4–5 fetuses of 55–60 days of gestation were used.

(d) *Sucrose density gradients.* In the cytosol fraction, a component with a coefficient of sedimentation of 8.5–9 *S* was detected after ultracentrifugation in a sucrose density gradient. The addition of a 100-fold excess of unlabelled estradiol showed a significant effect on this 8.5–9 *S* component (Fig. 2).

(e) *Specific [^{3}H]-estradiol complexes in the nuclei of fetal uterus.* The incubation of the purified nuclei of the uterus with [^{3}H]-estradiol showed the presence of specific estradiol receptors (Table 3). Specific estradiol receptors were also found after incubation of the 1 M NaCl nuclear extract with [^{3}H]-estradiol (Fig. 3).

(2) [^{3}H]-*Estradiol receptors in the different fetal tissues during the course of fetal development*

A series of studies established the curves of the weight and length of the fetus at an early stage and their variation during fetal development. Table 4 indicates the weight of the fetus, placenta and sack from 13 to 26 days of gestation and Figs. 4 and 5 show the weights and lengths from 30–35 days to the end of gestation. As can be noted, there is a great increase in weight between 30–40 days of gestation.

Fetal kidney, lung, uterus and testis from 34–35 days to the end of gestation and those of newborns were incubated with [^{3}H]-estradiol (5×10^{-8} M) or with the same quantity of radioactivity plus a 100–300-fold excess of unlabeled hormone.

(a) *Specific estradiol binding in the fetal kidney.* In order to correlate the growth of the fetal kidney and the presence of specific estradiol receptors, the weight of the kidney during the evolution of the fetus was measured as indicated in Fig. 6. Table 5 shows the quantitative values of specific estradiol receptors in the cytosol and in the different nuclear extracts. It is observed that the specific binding per unit of protein or g of tissue increases during fetal evolution in both the cytosol and nuclear extracts. After birth, these values are still high in the cytosol fraction but decrease very significantly in the different nuclear fractions.

(b) *Specific estradiol binding in the fetal lung.* Similar studies were carried out with the fetal lung; Fig. 7 shows the increase in weight of this tissue with fetal age. In Table 6 is presented the quantitative evolution of the cytosol and nuclear receptors in fetal lung. Specific [^{3}H]-estradiol binding is present from at least 34–35 days of gestation. As was the case for the kid-

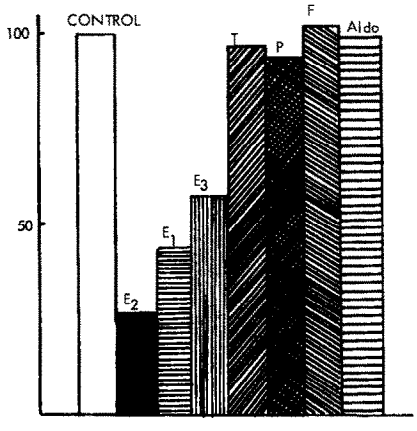

Fig. 1. Effect of estradiol (E_2), estrone (E_1), estriol (E_3), testosterone (T), progesterone (P), cortisol (F) and aldosterone (Aldo) on the cytosol [^{3}H]-estradiol complexes of the fetal uterus. The cytosol fraction was incubated for 18 h at 0°C with 2.7×10^{-9} M [^{3}H]-estradiol (control) or with the same quantity of [^{3}H]-estradiol + 5.40×10^{-8} M (20-fold excess mol/mol) of estradiol (E_2) ■, estrone (E_1) ▤, estriol (E_3) ▥, testosterone (T) ▨, progesterone (P) ▩, cortisol (F) ■ or aldosterone (aldo) ▤. The percentage of the effect was calculated by considering the specific activity (d.p.m./mg protein) in the [^{3}H]-estradiol complexes after chromatography on columns of Sephadex G-15. The bars represent the average of 2 experiments.

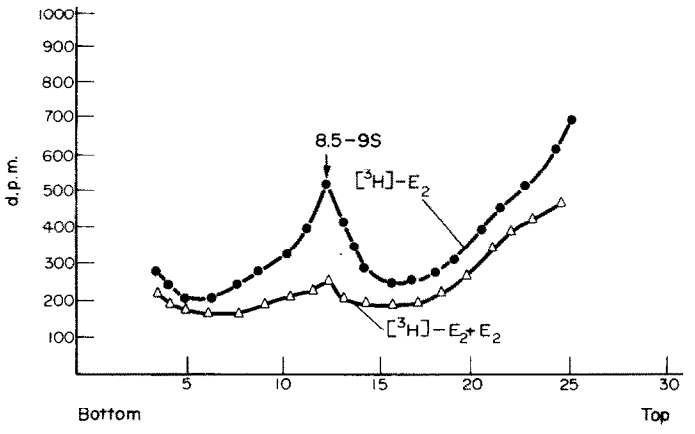

Fig. 2. Sucrose density gradient of the cytosol fraction of the fetal uterus of guinea pig. [^{3}H]-estradiol (8×10^{-8} M) (1) or the same quantity of [^{3}H]-estradiol + 100 fold excess (mol/mol) of estradiol (11) were incubated for 15 min at 37°C with the cell suspensions of the fetal uterus. The cytosol fractions of (1) —●—●— or of (11) —△— were chromatographed on Sephadex G-15. 0.2 ml of the [^{3}H]-estradiol complexes were layered on a sucrose gradient (5–20% w/v) containing 0.012 M of thioglycerol and 0.01 M EDTA. Centrifugation at 250,000 × *g* was carried out for 18 h at 2°C. The sedimentation coefficients (*S*) were calculated using as standards bovine serum albumin (4.3 S) and γ globulin (7 *S*).

Table 3. [^{3}H]-Estradiol binding in the nuclear extracts of fetal guinea pig uterus after incubation of the purified nuclei

Nuclear extracts	[^{3}H]-estradiol (4.4×10^{-8} M) d.p.m./mg prot.	[^{3}H]-E_2 + 20 times mol/mol of E_2 d.p.m./mg prot.
0.1 M Tris	48,400	35,000
0.3 M NaCl	63,400	51,800
1 M NaCl	36,000	18,900

Purified nuclei from 0.75 g of fetal uterus were incubated in Krebs–Henseleit buffer for 15 min at 37°C with [^{3}H]-estradiol (2.5×10^{-8} M) or with the same quantity of [^{3}H]-estradiol plus a 20-fold excess (mol/mol) of estradiol. The specific activities (d.p.m./protein) in the different nuclear extracts were calculated after Sephadex G-15 chromatography. The values represent the average obtained with the uteri of 15 fetuses of 50–60 days of gestation.

Table 4. Weight of guinea pig fetuses during fetal evolution (early gestation)

Days of gestation	Fetus + placenta + sack (mg)	Fetus (mg)
13	150	—
18	890	18
22	—	90
26	—	415

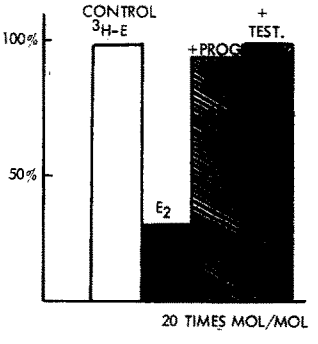

Fig. 3. Incubation of the 1 M NaCl nuclear extract of fetal uterus of guinea pig with [^{3}H]-estradiol (9.4×10^{-10} M). The purified nuclei of fetal uterus (300–400 mg) were incubated with [^{3}H]-estradiol (9.4×10^{-10} M), Control □, or with the same quantity of [^{3}H]-estradiol plus a 20-fold excess of estradiol ■, progesterone ■ or testosterone ▩. Incubation was carried out at 37°C for 5 min. The columns represent the effect in the [^{3}H]-estradiol-macromolecule complexes as d.p.m./mg protein, obtained after chromatography in Sephadex G-15.

ney, the quantity of estradiol receptors in the nucleus decreases after birth.

(c) *Specific estradiol binding in the fetal uterus and testis.* Quantitative determination of the cytosol [^{3}H]-estradiol receptors in the fetal uterus showed their presence at an early stage of gestation and that the values (per unit of protein) increase many times at the end of gestation (Table 7). The values of specific binding are very high in the different nuclear fractions, in particular in the fraction extracted by 1 M NaCl (Table 8). As in the other tissues, these nuclear receptors diminished after birth.

Parallel studies on [^{3}H]-estradiol binding in fetal testis also showed specific binding but many times less than in the fetal uterus (Table 9). The application of the Scatchard method to the binding of [^{3}H]-estra-

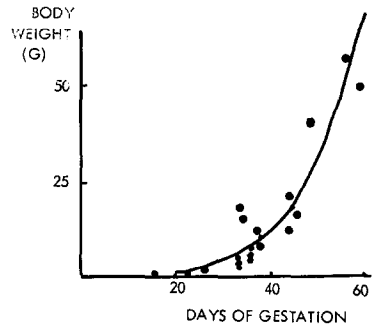

Fig. 4. Weight of guinea pig fetuses.

diol to specific macromolecules of the cytosol fraction of the fetal testis showed sites of great affinity with a dissociation constant of 6×10^{-10} M.

(d) *Binding of* [^{3}H]*-estradiol in fetal heart.* To compare the specific binding of estradiol in other fetal tissues, similar studies were performed using the fetal heart. As is shown in Table 10, very little or no specific binding was detected in this tissue during fetal evolution.

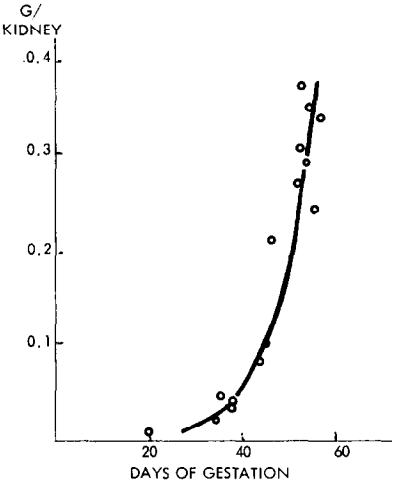

Fig. 6. Weight of kidney of guinea pig during fetal evolution.

(e) *Binding of* [^{3}H]*-testosterone in the fetal testis and uterus.* In order to compare estradiol binding with the binding of androgens in fetal gonads, [^{3}H]-testosterone (8×10^{-8} M) or the same quantity

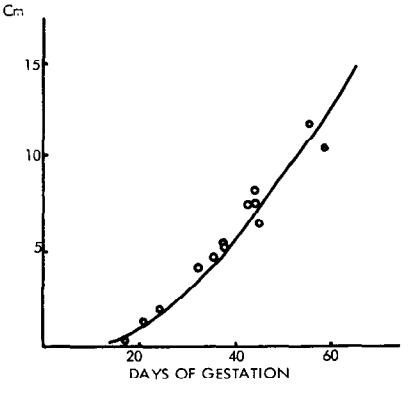

Fig. 5. Length of guinea pig fetuses.

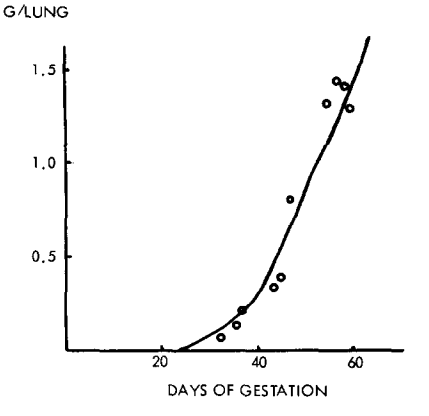

Fig. 7. Weight of lung of guinea pig during fetal evolution.

Table 5. Specific [^{3}H]-estradiol binding in the kidney of guinea pig after "*in vitro*" incubation

	Cytosol		Nuclear extracts					
			0.1 M Tris		0.3 M NaCl		1 M NaCl	
Days of gestation	fmol/mg protein	fmol/g tissue	fmol/mg protein	fmol/g tissue	fmol/mg protein	fmol/g tissue	fmol/mg protein	fmol/g tissue
34–35	0	6	9	22	0	0	2	23
37–38	19	297	16	10	11	8	5	43
45	19	263	24	49	20	48	15	152
50	19	289	23	38	24	56	11	69
59	35	610	75	131	52	85	20	255
Newborns ♀ <24 h	22	329	16	24	25	33	9	77
Immature ♀ 4 weeks	18	171	16	20	21	18	12	114

1 g of tissue was incubated with 5.2×10^{-8} M [^{3}H]-estradiol or with the same quantity of radioactivity plus a 300-fold excess of unlabelled estradiol in Krebs–Henseleit buffer at 37°C for 15 min. The values represent the average of two experiments with 36 fetuses at 34–35 days, with 42 at 37–38 days, with 10 at 45 days, with 6 at 50 days, with 2 at 59 days and with 2 newborns and 1 immature female.

Table 6. Specific [^{3}H]-estradiol binding in the lung of guinea pig after "*in vitro*" incubation

Days of gestation	Cytosol		Nuclear extracts					
			0.1 M Tris		0.3 M NaCl		1 M NaCl	
	fmol/mg protein	fmol/g tissue	fmol/mg protein	fmol/g tissue	fmol/mg protein	fmol/g tissue	fmol/mg protein	fmol/g tissue
34–35	24	173	9	7	15	12	3	65
37–38	26	158	57	55	32	36	9	74
45	65	384	132	114	68	107	19	208
50	122	1481	113	99	57	63	14	142
59	434	2479	210	120	98	62	21	151
Newborns ♀								
< 24 h	345	8034	71	46	36	30	7	68
Immature ♀								
4 Weeks	439	13836	31	15	6	4	6	36

The conditions of incubation are the same as those indicated on Table 5. The values represent the average of two experiments with 36 fetuses at 34–35 days, with 21 at 37–38 days, with 7 at 45 days, with 3 at 50 days, with 1 at 59 days and with 2 newborns and 1 immature female.

of radioactivity plus a 100-fold excess (mol/mol) of unlabeled testosterone was incubated with the fetal testis or uterus. As is indicated in Table 11, very little specific binding was found in these tissues during the period studied (36 50 days of gestation).

DISCUSSION

The present data show the existence of specific estradiol receptors in the fetal uterus and testis. In the fetal uterus, the binding of [^{3}H]-estradiol to receptors in the cytosol fraction shows a high affinity with a dissociation constant of 4×10^{-10} M which is of the same order of magnitude as that found in the uterus of immature rats [17]. A specific component with a sedimentation coefficient of 8.5–9 *S* was detected after ultracentrifugation in a sucrose density gradient (Fig. 2). These values are the same as those found in the uterus of immature or adult rats [18, 19]. It is noteworthy that a very high percentage of the radioactivity is localized in the nucleus, particularly in the fraction extracted by a 1 M NaCl solution which contains 90–95% of the nuclear DNA [12]. Also, a significant quantity of the radioactivity can still be extracted by 3 M NaCl and by the alkaline solutions (Tables 1 and 2). This finding of nuclear resistant sites could be related to the interaction of other steroid hormones in the nuclei of other fetal tissues which give similar findings (e.g. aldosterone in kidney [12], estradiol in kidney [6, 7] and brain [5]). Similar results of resistant sites were recently found in other tissues, e.g. for androgen receptors in the nuclei of epididymis of castrated rabbits [20], for estradiol receptors in the nuclei of the uterus of immature rats [21]. The biological significance of this residual nuclear binding is to be explored.

The binding of [^{3}H]-estradiol in the fetal testis is of high affinity which is similar to that in the fetal

Table 7. Cytosol specific [^{3}H]-estradiol binding in the fetal uterus of guinea pig

Days of gestation	fmol/mg protein	fmol/g tissue
34–35	90	840
36–37	175	1420
44–45	395	3250
49–50	530	4850
3 days after birth	560	6250

0.1 g of tissue/ml buffer was incubated with [^{3}H]-estradiol (3.3×10^{-9} M) or with the same quantity of radioactivity plus a 100-fold excess of unlabeled estradiol in Krebs–Henseleit buffer at 37°C for 15 min. The values represent the average of two experiments. 0.1 g of fetal uterus was obtained with 14–16 fetuses at 34–35 days of gestation, with 11–13 at 36–37 days with 8–10 at 44–45 days, with 4–6 at 49–50 days and with 2–4 newborns 3 days after birth.

Table 8. [^{3}H]-Estradiol specific binding in the cytosol and in the nuclear extracts of guinea pig uterus (fmol/mg protein)

	Cytosol	Nuclear extracts		
		0.1 M Tris	0.3 M NaCl	1 M NaCl
55–60 days of gestation	572	721	810	950
5 days after birth	480	80	300	370

0.15 g of tissue/ml buffer was incubated with [^{3}H]-estradiol (8×10^{-8} M) or with the same quantity of radioactivity plus a 100-fold excess of unlabelled estradiol in Krebs–Henseleit buffer at 37°C for 15 min. The values represent the average of two experiments.

Table 9. Cytosol specific [^{3}H]-estradiol binding in the fetal testis of guinea pig (fmol/mg protein)

Days of gestation	Specific binding fmol/mg protein	fmol/g tissue
34–35	38	370
36–37	37	462
44–45	30	402
49–50	56	676

0.15 g of tissue/ml of buffer was incubated with [^{3}H]-estradiol (3.3×10^{-8} M) or with the same quantity of radioactivity plus a 100-fold excess of unlabeled estradiol in Krebs–Henseleit buffer at 37°C for 15 min. The values represent the average of 2 experiments at 34–35 days of gestation, the tissues of 18 fetuses were used at 36–37 days, 37 fetuses at 44–45 days, and at 49–50 days, 12 fetuses.

Table 11. Cytosol specific [^{3}H]-testosterone binding in the fetal testis and uterus of guinea pig during fetal evolution

Days of gestation	Specific binding Fetal testis fmol/mg protein	Fetal testis fmol/g tissue	Fetal uterus fmol/mg protein	Fetal uterus fmol/g tissue
36–37	9	111	16	125
44–45	4	74	22	172
49–50	16	180	24	210

0.1–0.15 g of tissue/ml of buffer was incubated with [^{3}H]-Testosterone (3.3×10^{-8} M) or with the same quantity of radioactivity plus a 100-fold excess of unlabeled testosterone in Krebs–Henseleit buffer at 37° for 15 min. The values represent the average of 2 experiments.

Table 10. Specific [^{3}H]-estradiol binding in the heart of fetal guinea pig (fmol/mg protein)

Days of gestation	Cytosol	Nuclear extracts 0.1 M Tris	0.3 M NaCl	1M NaCl
34–35	0	0	0	0
37–38	21	0	0	0
44–45	16	0	0	0
59	28	0	0	0

1 g of tissue was incubated with 5.2×10^{-8} M [^{3}H]-estradiol or with the same quantity of radioactivity plus a 300-fold excess of unlabeled estradiol in Krebs–Henseleit buffer at 37°C for 15 min. The values represent the average of 20 fetuses at 34–35 days, 14 fetuses at 37–38 days, 3 fetuses at 44–45 days and 2 fetuses at 59 days.

uterus. It is interesting to note that in adult tissues, the presence of estradiol receptors in male gonads has also been observed [22]. On the other hand, very little or no testosterone receptors are found in the fetal testis or uterus. Studies are in progress to investigate these receptors in the gonads during the early stages of fetal development.

In the studies on the evolution of estradiol receptors during fetal development in the kidney, lung, uterus and testis, it was observed in all of these tissues that quantitative values of estradiol receptors (per mg protein or g tissue) in the cytosol fraction and the nuclear extracts increased during development and maximum values were found at the end of gestation. After birth, the cytosol receptors remained high, but nuclear binding decreased significantly. One explanation could be that due to a decrease in the production of endogenous estrogens after birth, the receptors remained in the cytosol fraction. The observation that at the end of gestation there are high values of receptors in both the cytosol and nuclei despite the high concentration of circulating estrogens suggests a considerable production of receptors in the fetal compartment. The biological significance of these findings remains to be investigated. It is interesting to note that in ontogenic studies of glucocorticoid receptors in the fetal lung of rabbit, the specific binding of [^{3}H]-dexamethasone in the cytosol fraction increases from 21 to 28–30 days of gestation. These values decrease significantly after birth or in the adult organism [23].

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DISCUSSION

Lippman. I think the demonstration of evolution of sex hormone receptors is extremely interesting. But some care has to be employed in assuming that the generation of receptor necessarily is correlated with the appearance of functional response. About 2 years ago, we published data in which we examined the evolution of glucocorticoid receptor in chick neural retina, and in that tissue we were able to demonstrate that approximately 5 days before one can induce the precocious induction of glutamine synthetase by glucocorticoid one could see the induction of perfectly normal amounts of glucocorticoid receptor in the immature neural retina and that this glucocorticoid receptor was fully capable of nuclear transfer at least by *in vitro* kind of assay. And yet the tissue was wholly unresponsive to glucocorticoid and remained so, for several more days of development. So I think what you are describing as the evolution of a specific part of the steroid hormone response mechanism is extremely interesting but I don't think one can prematurely correlate that with induction by those hormones of specific products in those tissue.

Pasqualini. This is an interesting comment. I agree with you that to look at the correlation of the presence of the hormone receptor and a particular biological effect is a basic problem. Studies are in progress in our laboratory to correlate the presence of the fetal receptor with its effect on a specific protein. But in any case the biological significance of the presence of specific steroid receptors at an early stage of fetal development is an intriguing and unresolved problem at the moment.

Mousseron Canet. Listening to your results, I suppose you assume estradiol of the fetus is maternal in origin since it decreases so rapidly after birth. It does not depend on sex too.

Pasqualini. Most of the estradiol is of placental origin and the values of maternal plasma concentration are similar to those found in the fetal compartment. The determination of plasma estradiol concentration after birth is in progress and we do not know yet if there are differences in estrogen concentration between females and males.

Hamilton. Have you examined the mullerian ducts of male embryos before and during regression.

Pasqualini. This is being studied. The earliest that we have studied is 30 days of gestation.

Bergink. I was a little bit surprised that there was such a high concentration of specific binding sites in the lung tissue of these foetuses. If I remember correctly the concentration was about 200 fmol/mg protein. My question is: Is this high concentration also observed in the adult animal?

Pasqualini. We have not yet compared with the adult animal but we have some complementary information that there is a large transformation, especially in the lung cytosol of estradiol to estrone. Moreover, in other studies we have observed that estrone is bound in fetal-lung cytosol with a high affinity ($K_D = 6 \times 10^{-10}$ M).

Clark. There is one interesting piece of data that I would like to quote from the work of Drs. Glasser and McCormick at Baylor. They have also measured a specific estrogen binding molecule in the fetal membranes. They find rather large quantities of this estrogen binding substance in the rat and we have discussed the possibility that it is not a receptor at all, instead some kind of binding molecule that protects the fetus from estrogens. Just recently, a very nice paper was presented in New York on a progesterone binding molecule which appeared to perform the same sort of function. This kind of progesterone binder could be very important in parturition. Toward the end of pregnancy, progesterone is high in the blood stream but in the tissues it is tied up by this protector molecule which permits estrogen to dominate the uterus and promotes excitability. The binder which you described may also be in this family.

Crabbe. Concerning the binding of estradiol to fetal kidney tissue: Is there a sex linked difference? I have in mind what has been reported by Fanestil's group that demonstrated the presence of such receptors in kidney tissue of rats. My second point is you have briefly alluded to aldosterone binding to fetal kidney tissue; Is there any other tissue you have looked at? Again, Fanestil reported (*Endocrinology* **98** (1976) 676) that aldosterone 'receptors' are present in the central nervous system tissue.

Pasqualini. The data we have now for aldosterone receptors is in the fetal kidney but in preliminary studies some years ago, we also found specific binding in fetal skin. Concerning the sex difference, we do not have any data at the moment.