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A GENERAL VIEW OF THE QUANTITATIVE EVALUATION OF CYTOSOL AND NUCLEAR STEROID HORMONE RECEPTORS IN THE FETAL COMPARTMENT OF GUINEA-PIG

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SUMMARY

Comparative studies on the binding of different steroids in fetal plasma of guinea-pig (end of gestation) show very little if any binding of estradiol, estrone, testosterone and aldosterone. In contrast, a great percentage of cortisol, estrone sulphate and estradiol sulphate circulates in bound form. In a series of *in vitro* studies, the subcellular distribution of two different radioactive steroids, [^{3}H]-aldosterone and [^{3}H]-estradiol, was compared in the same fetal tissue, the kidney. The cytosol fraction contains 95 and 91%, respectively, of the total intracellular radioactivity, the nuclei 1.9 and 5.5% and the mitochondria–microsomes 3.4 and 13.3%. Furthermore, comparative studies of the distribution of [^{3}H]-estradiol in four different fetal tissues also show a significant quantity of radioactivity in the nuclei: 5.4% in the lung, 1.5% in the brain and 11.8% in the uterus. In the brain, 38.8% of the total intracellular radioactivity is associated with the mitochondria–microsomal fraction. In all four fetal tissues, from 50% to 75% of the intranuclear radioactivity resists extraction by 0.1 M Tris and 0.3 M NaCl and is extracted by 1 M NaCl, 3 M NaCl, 0.2 N HCl, 0.2 N NaOH and ethanol solutions. For the same fetal age and under the same conditions, the specific binding of [^{3}H]-estradiol in the cytosol and nuclei of fetal uterus per g of tissue (9145 fmol/g tissue) is much greater than in the other organs. Fetal lung binds 1800 fmol/g tissue, fetal kidney 530 fmol/g tissue and fetal brain 170 fmol/g tissue. The cytosol to nuclear ratio of specific estrogen receptors varies during fetal development. In the kidney, the ratio is 0.14 at 34–35 days and rises to one during fetal development. In the lung, the ratio increases from one at mid-gestation to 5 at the end of gestation and 45 in newborns (<24 h).

INTRODUCTION

It is well known that the production of various steroid hormones (e.g. estrogens, progesterone, aldosterone) increases significantly during pregnancy, particularly at the end of gestation. Corticosteroids and androgens are synthesized in the fetal compartment using mainly fetal pregnenolone or placental progesterone. The fetus synthesizes estrogen precursors (dehydroepiandrosterone, 16α-hydroxy-dehydroepiandrosterone and their sulphate esters) which are transferred to and aromatized in the placenta [1]. Most of the steroid hormones circulate in the fetal compartment as sulphate esters ar some of them (e.g. estrogen sulphates, 3β-hydroxysteroid sulphates) are greatly hydrolyzed in the placenta but others (e.g. corticosterone sulphate, testosterone sulphate) are hydrolyzed very little in this organ [2].

The guinea-pig fetus can synthesize corticosteroids at an early age of gestation (45–55 days) [3] and they circulate in the fetal compartment mainly in unconjugated form. These corticosteroids, particularly cortisol, increase significantly during fetal development of this animal species [4]. The plasma levels of estrogen in the fetal compartment of this animal are very high [5] and estrogens circulate mainly as sulphate esters [6]. In 1971 steroid hormone receptors were detected in the guinea-pig fetus for estradiol in the brain [7] and for aldosterone in the kidney [8]. In the present paper the binding of different steroid hormones in the fetal plasma of guinea-pig as well as a general view of the specific binding of these hormones in the cytosol and nuclei of different fetal tissues during fetal development are presented.

MATERIALS AND METHODS

Biological material. Hartley Albino guinea-pig fetuses, newborn females and immature females were used. Fetal age was established with an error of ±24 h since females and males were mated for only 24 h.

Radioactive material. [6,7^{3}H]-estradiol (S.A. 47.9 Ci/mmol), [6,7^{3}H]-estrone (S.A. 45 Ci/mmol), [1,2^{3}H]-testosterone (S.A. 59 Ci/mmol) [1,2-^{3}H]-aldosterone (50 Ci/mmol) and [1,2^{3}H]-cortisol (S.A. 40 Ci/mmol) were obtained from NEN Chemicals, Frankfurt, W. Germany. Purity was controlled in the appropriate paper chromatographic systems and found to be greater than 95%.

Experimental conditions. In vitro experiments were carried out by incubation of cell suspensions of each tissue in Krebs–Henseleit buffer [9]. The details of the conditions used are indicated in the Results section and in the legends of the pertinent tables.

Cell fractionation. The tissues were fractionated according to the method of Chauveau *et al.*[10] with modifications described in a previous publication

[11]. The purified nuclei obtained by this method 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 HCl, (G) 0.2 N NaOH and (H) 90% (v/v) ethanol.

Plasma binding. Plasma binding was determined either *in vivo* or *in vitro*. In the case of the estrogens, 1.25×10^{-9} mol of [^{3}H]-estradiol was injected *in situ* to each fetus for 30 min after which time the fetuses were exsanguinated and blood was collected in heparinized tubes. Undiluted fetal plasma was then chromatographed at 4° on 30 × 0.9 cm columns of Sephadex G-15 to separate macromolecule-bound from unbound radioactivity. For the other steroids, fetal plasma was incubated overnight at 4° with 1×10^{-9} M radioactive steroid before being chromatographed to determine binding.

Determination of specific binding. Specific binding was determined by subtracting from the total binding, the non-specific binding measured by parallel incubations containing a 100, 300 or 1000 fold excess of unlabelled steroid.

Analysis of radioactive material. The radioactive steroids in the macromolecule complexes of fetal plasma after *in vivo* administration of [^{3}H]-estradiol were analyzed in the following manner: The radioactive material was extracted with 80% (v/v) ethanol. The ethanol extracts were evaporated in vacuum, re-dissolved in water and extracted with dichloromethane (3 times with 1 vol.) followed by *n*-butanol (3 times with 0.5 vol). The dichloromethane extract was chromatographed on paper in the system isooctane–toluene–methanol–water (1:4:3:2 or 1:1:1:1 by vol.). The *n*-butanol extract was chromatographed in the system butyl acetate–toluene–*n*-butanol–4 N ammonium hydroxide–methanol (6:3:1:5:5 by vol.) and butyl acetate–toluene–*n*-butanol–water–acetic acid–methanol (12:6:2:9:1:10: by vol.), using the mono-sulphates and mono-glucuronides of estrone and estradiol as standards. After elution from the chromatogram, the area of the sulphate esters was submitted to solvolysis [12]. The freed radioactive material was then analysed as indicated above.

Protein and radioactivity measurement. Proteins were assayed by the method of Lowry *et al.*[13]. Radioactivity in aqueous solutions was measured in Instagel (Packard, Inc.) and radioactivity in organic solvents in a POPOP-PPO–toluene scintillation solution.

RESULTS

I. Binding of various radioactive steroids in fetal plasma

Table 1 indicates that of several steroids tested for plasma binding in the fetus either under *in vivo* or *in vitro* conditions, only [^{3}H]-cortisol, [^{3}H]-estrone sulphate and [^{3}H]-estradiol sulphate are bound to any significant extent. 59% of the [^{3}H]-cortisol is bound after incubation of fetal plasma overnight at 4°. The binding of [^{3}H]-estrone sulphate and [^{3}H]-estradiol sulphate was detected after a 30 min *in vivo* subcutaneous administration of [^{3}H]-estradiol to each fetus. Under these conditions, 21% of the total radioactivity in the fetal plasma was bound to plasma proteins of which 67% was found to be [^{3}H]-estrone sulphate and 7% [^{3}H]-estradiol sulphate [6]. As indicated in Table 1, 19% of the circulating estradiol sulphate and 32% of the estrone sulphate are bound to plasma proteins.

II. Distribution of intracellular radioactivity in fetal kidney after incubation with [^{3}H]-aldosterone and in fetal kidney, lung, brain and uterus after incubation with [^{3}H]-estradiol

Table 2 shows a comparison of the distribution of intracellular radioactivity of two different steroids ([^{3}H]-aldosterone and [^{3}H]-estradiol) in fetal kidney and of one steroid ([^{3}H]-estradiol) in four different

Table 1. *In vivo* and *in vitro* binding of different steroids in fetal guinea-pig plasma

	In vivo		*In vitro*
Steroid	% Bound*	%Bound†	% Bound‡
[^{3}H]-Estradiol	0.13	4.3	4.2
[^{3}H]-Estrone	0.11	5.0	4.6
[^{3}H]-Estradiol sulphate	1.40	19.0	—
[^{3}H]-Estrone sulphate	14.20	32.0	—
[^{3}H]-Cortisol	—		58.9
[^{3}H]-Aldosterone	—		N.D.
[^{3}H]-Testosterone	—		N.D.

* Percentage bound of the total radioactivity in the fetal plasma after the administration of 60 μCi (1.25×10^{-9} mol) of [^{3}H]-estradiol to the fetus for 30 min. † Percentage of each steroid which is bound in fetal plasma under the above conditions. ‡ Percentage bound of the total radioactivity after incubation overnight of fetal plasma *in vitro* at 4°C with 1×10^{-9} M radioactive steroid. N.D. = not detectable. The values are the average of two experiments.

Table 2. Distribution of intracellular radioactivity in fetal guinea-pig kidney after *in vitro* incubation with [^{3}H]-aldosterone and in fetal kidney, lung, brain and uterus after incubation with [^{3}H]-estradiol

	[^{3}H]-Aldosterone	[^{3}H]-Estradiol			
	Kidney	Kidney	Lung	Brain	Uterus
Cytosol	94.7	91.3	72.9	59.8	79.9
Nuclear extracts					
(A) 0.1 M Tris	0.4	1.7	1.1	0.5	1.2
(B) 0.3 M NaCl	0.3	1.0	0.8	0.3	1.6
(C) 1 M NaCl	0.9	1.9	2.0	0.4	3.8
(D) 3 M NaCl pH 7.4	0.1	0.2	0.2	0.1	0.5
(E) 3 M NaCl pH 8.4	—	0.1	0.1	0.03	0.3
(F) 0.2 N HCl	—	0.2	0.3	0.03	—
(G) 0.2 N NaOH	—	0.3	0.7	0.06	3.0
(H) Ethanol	0.2	0.1	0.2	0.06	1.4
Mitochondria–microsomes	3.4	13.3	21.7	38.8	7.1

1.0 g of fetal kidney was incubated with 5×10^{-8} M [^{3}H]-aldosterone or [^{3}H]-estradiol, 1.0 g of fetal lung and 2.0 g of brain were incubated with 5×10^{-8} M [^{3}H]-estradiol and 0.15 g of uterus was incubated with 8×10^{-8} M [^{3}H]-estradiol at 37° for 15 min. The values are the average of 3 experiments.

fetal tissues (kidney, lung, brain and uterus). In the fetal kidney, there is relatively more [^{3}H]-estradiol in the nuclear extracts and mitochondria–microsomal fraction than [^{3}H]-aldosterone. However, the extraction of both [^{3}H]-aldosterone and [^{3}H]-estradiol from the nucleus by the different salt solutions is similar. About 40% of the intranuclear [^{3}H]-aldosterone or [^{3}H]-estradiol is extracted by the 1 M NaCl solution.

The distribution of intracellular [^{3}H]-estradiol is similar in kidney and lung but in the brain 38.8% of the [^{3}H]-estradiol is in the mitochondria–microsomal pellet. In both kidney and lung approximately 40% of the intranuclear radioactivity is extracted by 1 M NaCl while slighly less (27%) is extracted from nuclei of fetal brain. Furthermore, a significant quantity of the nuclear radioactivity is still extracted by the 3 M NaCl solutions, as well as by 0.2 N HCl and 0.2 N NaOH which represent 15–24% of the total nuclear radioactivity.

Comparative experiments under similar conditions show that in the fetal uterus 11.8% of the total intracellular radioactivity is localized in the nucleus of which 32% is extracted by the 1 M NaCl solution. It is interesting to note that in this tissue a great percentage of the nuclear radioactivity is extracted by the 0.2 N NaOH solution.

III. Comparison of the specific binding of [^{3}H]*-aldosterone in fetal kidney and* [^{3}H]*-estradiol in fetal kidney, lung, brain and uterus*

Numerous studies in this laboratory have led to the observation that specific receptors of several steroid hormones are present simultaneously in various fetal tissues [6–8, 11]. Fetal guinea-pig kidney contains receptors for both [^{3}H]-aldosterone and [^{3}H]-estradiol (Tables 3 and 4). There is 4.5 times more specific binding of [^{3}H]-aldosterone per mg of DNA than [^{3}H]-estradiol in the cytosol but the specific binding in the different nuclear extracts is similar for the two hormones.

Fetal lung has a greater concentration of estrogen receptors than the kidney (1354 fmol/g tissue in lung cytosol versus 309 fmol/g tissue in kidney cytosol) (Tables 4 and 5). There is a larger proportion of binding in lung cytosol than in the nucleus. Also the specific binding of estradiol in the nuclei of the fetal lung is higher than in the kidney nuclei. In the fetal brain the specific [^{3}H]-estradiol binding (per mg DNA) in the cytosol is higher than in the kidney but less than

Table 3. Cytosol and nuclear specific binding of [^{3}H]-aldosterone in the fetal kidney of guinea-pig

	fmol/mg protein	fmol/g nuclear DNA	fmol/g tissue
Cytosol	65.8	160	934
Nuclear extracts			
(A) 0.1 M Tris	14.1	4.0	23.5
(B) 0.3 M NaCl	21.0	5.9	34.7
(C) 1 M NaCl	6.6	13.1	76.7

1 g of fetal kidney was incubated with 5.5×10^{-8} M [^{3}H]-aldosterone or with the same quantity of radioactivity plus a 1000-fold excess of unlabelled *d*-aldosterone in 4 ml Krebs–Henseleit buffer at 37° for 16 min. Fetuses were from 40–60 days of gestation. The results are the averages of 3 experiments.

Table 4. Cytosol and nuclear specific binding of [^{3}H]-estradiol in the fetal kidney of guinea-pig

	fmol/mg protein	fmol/mg nuclear DNA	fmol/g tissue	fmol/ kidney
Cytosol	18.5	53.0	309.4	55.4
Nuclear extracts				
(A) 0.1 M Tris	27.5	11.6	67.4	12.1
(B) 0.3 M NaCl	15.5	9.9	57.8	10.4
(C) 1 M NaCl	8.7	17.5	102.1	18.3

1 g of fetal kidney 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 4 ml Krebs–Henseleit buffer at 37° for 15 min. The results are the average of 34 fetuses at 50 days of gestation (3 experiments).

Table 5. Cytosol and nuclear specific binding of [^{3}H]-estradiol in the fetal lung of guinea-pig

	fmol/mg protein	fmol/mg nuclear DNA	fmol/g tissue	fmol/ lung
Cytosol	120.5	209.7	1354.2	621.6
Nuclear extracts				
(A) 0.1 M Tris	164.7	14.2	159.5	73.2
(B) 0.3 M NaCl	70.5	9.3	74.1	34.0
(C) 1 M NaCl	21.8	20.7	217.0	99.6

1 g of fetal lung 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 4 ml Krebs–Henseleit buffer at 37° for 15 min. The results are the average of 8 fetuses at 50 days of gestation (3 experiments).

Table 6. Cytosol and nuclear specific binding of [^{3}H]-estradiol in the fetal brain of guinea-pig

	fmol/mg protein	fmol/mg nuclear DNA	fmol/g tissue	fmol/ brain
Cytosol	15.6	110.2	159.8	236.5
Nuclear extracts				
(A) 0.1 M Tris	9.3	2.1	3.1	4.6
(B) 0.3 M NaCl	9.4	0.8	1.1	1.6
(C) 1 M NaCl	3.6	3.7	5.3	7.8

2 g of fetal brain were 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 4 ml Krebs–Henseleit buffer at 37° for 15 min. The results are the average of 6 fetuses at 50 days of gestation (3 experiments)

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

	fmol/mg protein	fmol/g tissue	fmol/ uterus
Cytosol	630	5450	218
Nuclear extracts			
(A) 0.1 M Tris	300	270	11
(B) 0.3 M NaCl	560	835	33
(C) 1 M NaCl	670	2590	104

100–150 mg of fetal uterus was incubated with 5.3×10^{-8} M [^{3}H]-estradiol or with the same quantity of radioactivity plus a 100-fold excess of unlabelled extradiol in 3 ml of Krebs–Henseleit buffer at 37° for 15 min. The data represent the average of 15 fetuses at 49–50 days of gestation (2 experiments).

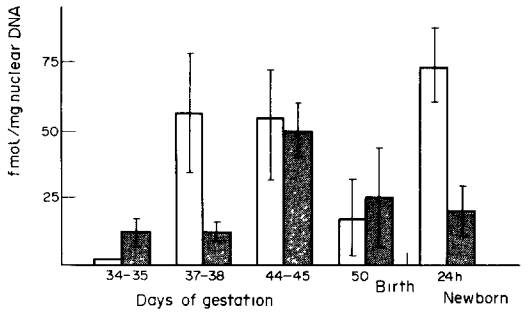

Fig. 1. Specific [^{3}H]-estradiol binding in the cytosol (open columns) and nuclei (dark columns) of fetal guinea-pig kidney during fetal development. 1 g of kidney was incubated with 5.2×10^{-8} M [^{3}H]-estradiol in Krebs–Henseleit buffer at 37° for 15 min. The quantity of specifically bound [^{3}H]-estradiol in the cytosol and in the combined 0.1 M Tris, 0.3 M NaCl and 1 M NaCl nuclear extracts is expressed as fmol per mg of nuclear DNA. The bars represent the average plus the extreme values of 2–3 experiments with 36 fetuses at 34–35 days, 65 at 37–38 days, 17 at 44–45 days, 9 at 50 days and 2 newborns.

in the lung (Table 6). In the nuclei these values are significantly less than in lung or kidney. In the fetal uterus specific binding of [^{3}H]-estradiol in both the cytosol and nuclear extracts is many times that in the other fetal tissues (Table 7).

IV. Cytosol and nuclear relationship of [^{3}H]*-estradiol specific binding throughout fetal development in the kidney and lung*

Figure 1 gives the [^{3}H]-estradiol binding in the cytosol and nuclei in the fetal kidney during fetal development. It is observed that at an early age of gestation (34–35 days) the estradiol receptors are found mainly in the nuclei with a ratio of cytosol estradiol receptor–nuclear estradiol receptor of 0.14 which remains close to unity throughout fetal evolution and reaches 3.80 in newborns. Similar studies are presented in Fig. 2 for [^{3}H]-estradiol receptors in fetal lung. In this tissue this ratio is approximately one at an early stage of development but increases significantly at the end of gestation: cytosol extradiol receptor–nuclear estradiol receptor = 5 at 50 days and 45 in newborns.

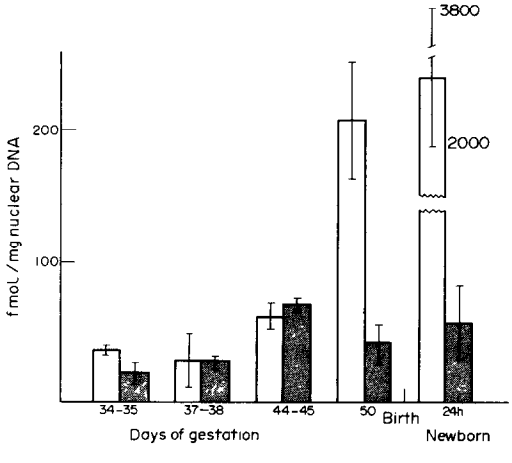

Fig. 2. Specific [^{3}H]-estradiol binding in the cytosol (open columns) and nuclei (dark columns) of fetal guinea-pig lung during fetal development. Same conditions as in Fig. 1. The bars represent the average plus the extreme values of 2–3 experiments with 36 fetuses at 34–35 days, 31 at 37–38 days, 11 at 44–45 days, 5 at 50 days and 2 newborns.

DISCUSSION

The plasma binding of several steroid hormones to fetal plasma proteins of guinea-pig was studied in fetuses at the end of gestation both *in vivo* and *in vitro*. As is indicated in Table 1, there are significant differences in plasma binding: estradiol, estrone, aldosterone and testosterone are bound to only a very small extent while a great proportion of cortisol, estrone sulphate and estradiol sulphate is bound.

In what concerns cortisol, it is well known that this hormone increases significantly during fetal development of the guinea-pig and that most of it is bound to corticosteroid binding globulin (CBG) [14]. It has also been demonstrated that CBG is produced in the fetal compartment but the plasma concentration of this protein in the fetus is 3–4 times less than in the mother [15].

As indicated in Table 1, a large percentage of the estrogens bound to plasma proteins is estrone sulphate and estradiol sulphate in the fetal compartment of guinea-pig while there is very little binding of unconjugated estradiol and estrone. Although it is well known that estrogens circulate in the fetal compartment as sulphate esters (e.g. human fetus [1, 2]) little information is available at the present on how these sulphate esters are transported during fetal development. In this connection, it is interesting to note that estrone sulphate binds to human serum albumin with two sets of binding sites: $K_{A1} = 1 \times 10^5\ M^{-1}$ and $K_{A2} = 0.2 \times 10^4\ M^{-1}$ [16].

The present studies are part of a continuing project to investigate plasma binding of different steroid hormones throughout fetal development. Furthermore, the method of separating bound and unbound steroid by Sephadex G-15 column chromatography which is currently being used will be compared with other methods such as equilibrium dialysis.

When the intracellular distribution of two different radioactive steroids ([^{3}H]-aldosterone and [^{3}H]-estradiol) are compared in the same tissue (fetal kidney), it can be observed that more [^{3}H]-estradiol is associated with the mitochondria–microsomal fraction than [^{3}H]-aldosterone. This significant quantity of [^{3}H]-estradiol in the mitochondria–microsomal fraction was also found in other fetal tissues, particularly in the brain (35–40%, see Table 2). It is interesting to note that in all of the tissues studied either with [^{3}H]-aldosterone or [^{3}H]-estradiol, 50–75% of the nuclear radioactivity resists extraction by 0.1 M Tris and 0.3 M NaCl and is extracted by the 1 M

NaCl, 3M NaCl, 0.2 N HCl, 0.2 N NaOH and ethanol solutions, which confirms previous results from this laboratory [6, 11]. Similar findings of "resistant" nuclear radioactivity have been obtained for other steroid hormones in other tissues [17, 18]. It is noteworthy that in the fetal uterus, 25% of the nuclear radioactivity is extracted by the 0.2 N NaOH solution (see Table 2).

Comparative studies on the specific binding of [^{3}H]-aldosterone and [^{3}H]-estradiol in the fetal kidney indicate that there is 3 times more specific binding per mg DNA of [^{3}H]-aldosterone than [^{3}H]-estradiol. Moreover, most of the [^{3}H]-aldosterone receptors is localized in the cytosol fraction. Important differences in [^{3}H]-estradiol binding were also observed between different fetal tissues. The specific cytosol and nuclear binding of [^{3}H]-estradiol in the fetal uterus (9145 fmol/g tissue) under the same conditions and for the same age was much greater than in the other organs, followed by fetal lung (1800 fmol/g tissue), fetal kidney (530 fmol/g tissue) and brain (170 fmol/g tissue).

The ratio of cytosol binding to nuclear binding also varies with the steroid and the tissue studied. In the same tissue (fetal kidney), the cytosol to nuclear binding ratio of [^{3}H]-aldosterone per mg DNA is 7 while that of [^{3}H]-estradiol is 1.5. For the same steroid ([^{3}H]-estradiol) in different fetal tissues, the ratio is 1.5 in the uterus, 4.7 in the lung, 1.5 in the kidney and 16.7 in the brain. Throughout fetal development, the cytosol to nuclear binding ratio in fetal kidney is about one while in the lung this ratio increases with fetal age and particularly in newborns (<24 h) (Fig. 2).

The biological significance of the presence of these steroid hormone receptors or the activity of these hormones in the fetal guinea-pig tissues has not yet been elucidated although a series of studies is in progress. For the purposes of comparison, the biological effect of cortisol in fetal rabbit lung on cell maturation [19] and on the induction of choline phosphotransferase [20] has been demonstrated.

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DISCUSSION

McEwen. I would like to make two comments with respect to your interesting results. The first is that in the rat fetus the brain does not appear to have estrogen receptors until after birth, but after birth these receptors may well function in the process of sexual differentiation which is largely a postnatal phenomenon in the rat. The second is that the α-fetoprotein, which binds estradiol in the rat, is present in late prenatal life and in early postnatal life; and its ability to bind estradiol in large amounts makes difficult the detection of true receptors unless one uses synthetic estrogens such as RU 2858 or diethylstilbestrol which do not bind to alpha-fetoprotein.

Pasqualini. Concerning your first problem, it seems that it is difficult to compare two different species (rat and guinea pig) in particular during fetal life because it is well known that many biological functions of the fetal guinea pig appear before those in fetal rats. This is perhaps the reason that estrogen receptors are present in the fetal brain of guinea pig but not in the fetal brain of rats. Concerning the second problem, at the present it is well known that α-feto protein exists in human and rats and in rats it binds significantly to estradiol but in the fetus of guinea pig very little (if any) of the circulating estradiol is bound to fetal plasma proteins (Acta endocr., Copenh. **83** (1976) 811–828). If α-feto protein exists in fetal guinea pig, it is perhaps in another form of configuration which does not bind estradiol. This is also another example of the differences in binding in different species.

McEwen. I would like to support your comments about the guinea pig. Dr. Linda Plapinger has looked very carefully at the guinea pig fetus, and has not found estrogen-binding activity in blood or in brain tissue which is in any way comparable to the α-fetoprotein in the rat. What we do not know, however, is whether the guinea pig brain in fetal life is susceptible to estrogens causing sexual differentiation, as is the case in the rat. Even if it were, it is conceivable that there may be other protection mechanisms besides a blood binding protein, such as metabolism of estrogens to render them less effective in the fetus.

Pasqualini. In relation to the protection mechanism of the biological action of estradiol in the fetal compartment of guinea pig, it seems in agreement with the data that we obtained that one mechanism of deactivating estradiol is its conversion to its sulfate esters as 30 min after injection of [^{3}H]-estradiol to the fetus more than 80% of the circulating radioactivity is made up of [^{3}H]-estradiol sulfate and [^{3}H]-estrone sulfate, (Acta endocr., Copenh. **83** (1976) 811–828). In what concerns the fetal brain, a great percentage of estradiol is also converted to estrone.

Naftolin. I would like to point out something that is often overlooked. The important thing in many of the considerations regarding brain imprinting is the time from conception not whether the animal is pre- or postnatally exposed. Where this imprinting occurs the critical interval is between 20 and 60 days after conception. This applies to the dog if one uses urination behaviour patterns; to guinea pigs which have to be treated *in utero*; to rats, mice monkeys, sheep and whatever we have in other primates. So there is not that much difference between species when one looks at timing of general imprinting mechanisms.

Sonnenschein. Could you comment on the significance of estrogen receptors in "non-target cells" of guinea pigs.

Pasqualini. The significance of the biological activity of estradiol in non target tissues is an interesting and basic problem. Studies are being carried out in our laboratory to establish a possible correlation of the presence of estradiol receptors and the biological activity of the hormone.