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STEROID HORMONE RECEPTORS IN FETAL GUINEA-PIG KIDNEY

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SUMMARY

Cytosol and nuclear subcellular fractions isolated from fetal kidney of guinea pigs (35–55 days of gestation) form steroid macromolecule complexes both "*in vivo*" and "*in vitro*" with [^{3}H]-aldosterone or [^{3}H]-estradiol but not with [^{3}H]-progesterone. *d*-Aldosterone competes in the formation of the cytosol and nuclear [^{3}H]-aldosterone complexes but estradiol has no effect. Estradiol competes in the formation of the [^{3}H]-estradiol complexes of both the cytosol and the nuclear extracts but *d*-aldosterone has no effect, suggesting the presence of two different receptors for these hormones in the fetal kidney during this period of development. In the [^{3}H]-estradiol experiments 30 min after administration of this hormone to the fetus or 15 min after incubation at 37°C with the cell suspensions of the fetal kidney, the bulk of the [^{3}H]-complexes (60–70%) is located in the nucleus; of this, 60% is found in the chromatin fraction. 62–95% of the radioactivity in the complexes consists of non-metabolized estradiol. In addition to estradiol, estrone and estriol were found to compete for binding sites albeit less intensively, whereas testosterone and cortisol had no effect. Ultracentrifugation in a sucrose density gradient (in 0·5 M NaCl) of the 0·3 M NaCl and 1 M NaCl nuclear extracts yielded a component with a sedimentation coefficient of 3·7 S in which estradiol had a significant competitive effect. Experiments carried out with the [^{3}H]-estradiol cytosol-complex obtained at 4°C and then reincubated with the purified nuclei at 4° or 37°C show that the quantity of [^{3}H]-estradiol found in the different nuclear extracts was 2·5–3 times greater at 37°C than at 4°C. By the application of the Scatchard method, the presence of two binding sites was established, the first with a dissociation constant of $2{\cdot}5 \times 10^{-10}$ M and the second with a K_d of $7{\cdot}7 \times 10^{-9}$ M. When [^{3}H]-estrone was administered subcutaneously "*in situ*" to the fetus, it was noted that most of the radioactive material in the ^{3}H-macromolecule complexes of the cytosol and nuclear extracts of the kidney is [^{3}H]-estradiol. It is concluded that during this period of fetal development (35–55 days of gestation): estradiol receptors are present in the fetal kidney; in the interconversion of estrone ⇄ estradiol in the same fetal tissue the reaction proceeds predominantly in the formation of estradiol.

INTRODUCTION

It is well established that steroid hormones are biosynthesized in the fetal compartments in man and in various animal species[1–4]. In the guinea pig fetal compartment it has been demonstrated that after subcutaneous administration of [^{3}H]-progesterone to the fetus, a significant part of the radioactivity is converted into [^{3}H]-corticosterone and [^{3}H]-cortisol in the fetal adrenals. Furthermore, incubation of [^{3}H]-corticosterone with fetal guinea pig adrenals leads to the synthesis of [^{3}H]-aldosterone[5]. Steroid hormones such as progesterone, aldosterone and estradiol circulate in the fetal compartment of the guinea pig in relatively high concentrations[6, 7].

Receptors of steroid hormones in adult animals have been well demonstrated for different target organs: estradiol in rat uterus[8], cortisol in rat thymus[9], testosterone and 5α-dihydrotestosterone in rat prostate [10], progesterone in the chick oviduct[11], aldosterone in rat kidney[12], etc. Previous studies in our laboratory have shown the presence of several steroid hormone receptors in tissues of the guinea-pig fetus: for aldosterone in the kidney[13, 14], for estradiol in the kidney and brain[15, 16] and for cortisol in the brain[17]. The present paper describes a comparative study on the formation of estradiol, aldosterone and progesterone macromolecule complexes in the cytosol and nuclear subcellular fractions of the fetal kidney of guinea pigs and presents further studies on the physicochemical properties as well as the mechanism of formation of estradiol receptors in this tissue.

EXPERIMENTAL

Biological material:

Fetuses of 35–55 days of gestation of Hartley Albino guinea pigs were used.

Radioactive material:

[6, 7-^{3}H]-Estradiol (s.a.: 46 Ci/mmol), [1, 2-^{3}H]-aldosterone (s.a.: 54 Ci/mmol), [1, 2-^{3}H]-progesterone (s.a.:

47·6 Ci/mmol) and [6, 7-^{3}H]-estrone (S.A.: 54·3 Ci/mmol) were purchased from NEN Chemicals, GmbH, Frankfurt, W. Germany.

The purity of [^{3}H]-estradiol and [^{3}H]-estrone was tested by paper chromatography in the system: isooctane : toluene : methanol : water (1 : 4 : 3 : 2 by vol), [^{3}H]-progesterone in the system: ligroine/propanediol and [^{3}H]-aldosterone in the system: chloroform/formamide. In all cases, the purity was >98%.

Methods:

Experiments were carried out "*in vivo*" and "*in situ*" after subcutaneous administration of the hormone to the fetus or *in vitro* after incubation of cell suspensions, the cytosol fraction or the purified nuclei of the fetal kidneys. In the *in vivo* experiments the radioactive hormones were injected into the fetuses of a lightly ether-anesthesized mother, and the fetuses were removed 30 min. after injection. In the *in vitro* experiments, the incubations were carried out in Krebs–Henseleit buffer (pH: 7·4)[18]. (Alternative experimental conditions are indicated in the results section).

Cell fractionation. In the *in vitro* or *in vivo* experiments, the kidney tissues were fractionated using the method of Chauveau *et al.*[19], with modifications described previously[13]. The tissue was first homogenized in 0·25 M sucrose–0·01 M Tris–HCl–0·003 M $CaCl_2$ (pH: 7·4) solution and centrifuged at 900*g* for 10 min at 2°C. The supernatant was centrifuged again at 250,000*g* for 30 min at 2°C to obtain the cytosol fraction. The pellet of this centrifugation consists of a mixture of mitochondria and microsomes. The pellet from the first 900*g* centrifugation was washed with a 0·4 M sucrose–0·01 M Tris–HCl–0·003 M $CaCl_2$ solution and centrifuged at 900*g*. The resulting pellet was homogenized in a 2·0 M sucrose–0·01 M Tris–HCl–0·003 M $CaCl_2$ solution, layered on an equal volume of the same solution and centrifuged for 60 min at 250,000*g* to obtain purified nuclei. The purified nuclei were extracted successively with the following solutions: (*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). The radioactive macromolecule complexes of the cytosol and the different nuclear extracts were obtained from the excluded volume after chromatography on columns of Sephadex G-15.

Sucrose density gradients were carried out by ultracentrifugation in a solution of sucrose 5–20% w/v containing thioglycerol (0·012 M) and EDTA (0·001 M), with or without 0·5 M NaCl. The equilibrium constant of the cytosol binding of [^{3}H]-estradiol was calculated using the method of Scatchard[20] after incubation of the cytosol for 18 h at 2°C.

Proteins were evaluated using the method of Lowry [21]. Radioactivity in aqueous solution was measured in Instagel (Packard Inc.) and radioactivity in organic solvents was measured in a POPOP-PPO-Toluene scintillation solution. The radioactive material in the macromolecule complexes or in the total extracts was analysed after isolation of the different radioactive steroids by paper chromatography and identification by chemical transformation or crystallization with the non-labelled steroid to constant specific activity.

RESULTS

1. *Comparative studies on the formation of* [^{3}H]-Estradiol, [^{3}H]-Aldosterone *and* [^{3}H]-Progesterone *macromolecule complexes in the fetal kidney of guinea pigs*

Table 1 indicates the percentage of the administered

Table 1. Relative distribution of total radioactivity in the fetal kidney of guinea pig 30 min after administration of [^{3}H]-estradiol; [^{3}H]-aldosterone or [^{3}H]-progesterone to the fetus

Steroid administered	Exp. I* [3H]-estradiol (7.7×10^{-10} moles per fetus)	Exp. II* [3H]-aldosterone (4.4×10^{-10} moles per fetus)	Exp. III* [^{3}H]-progesterone (9×10^{-10} moles per fetus)
% of the administered dose in kidney	0·52	0·42	0·10
	% in cell†	% in cell†	% in cell†
I Cytosol	83	89·0	65·6
II Nuclear extracts			
(A) 0·1 M Tris	1·4	0·55	3·4
(B) 0·3 M NaCl	2·0	0·40	2·3
(C) 1 M NaCl	6·5	2·60	3·5
III Mitochondria-microsomes	4·5	0·37	20·2

The data represent the average values in the different experiments.
* Number of fetuses: Exp. I: $n = 11$; Exp. II: $n = 7$; Exp. III: $n = 5$.
† These values represent the percentage of the total intra-cellular radioactivity in each of the fractions.

Table 2. Percentage of the radioactivity bound in the different subcellular fractions of the fetal kidney 30 min after administration of [^{3}H]-estradiol, [^{3}H]-aldosterone or [^{3}H]-progesterone to the fetus

Steroid administered	Exp. I [^{3}H]-estradiol (7.7×10^{-10} moles per fetus)	Exp. II [^{3}H]-aldosterone (4.4×10^{-10} moles per fetus)	Exp. III [^{3}H]-progesterone (9×10^{-10} moles per fetus)
	% Bound*	% Bound*	% Bound*
Cytosol	2–18	0·60–0·66	0
Nuclear extracts			
(A) 0·1 M Tris	45–50	35–90	0
(B) 0·3 M NaCl	64–75	—	—
(C) 1 M NaCl	70–75	68–70	0

* Percentage bound in each subcellular fraction. The number of fetuses is the same as that indicated in Table 1. The data of Exp. I and II are in references 15 and 13 respectively.

dose found in fetal kidneys 30 min after administration of [^{3}H]-estradiol, [^{3}H]-aldosterone or [^{3}H]-progesterone to the fetus. This table also shows the distribution of radioactivity in the different subcellular fractions. It will be noted that for the different steroids injected, most of the radioactivity is in the cytosol fraction, but a significant percentage is found in the nuclear extracts, particularly in the chromatin fraction extracted by 1 M NaCl. A significant percentage is also associated with the mitochondrial-microsomal fraction after [^{3}H]-estradiol or [^{3}H]-progesterone administration to the fetus.

Table 2 shows a comparison of the percentage of bound radioactivity in the cytosol and in the different nuclear extracts after administration of [^{3}H]-estradiol, [^{3}H]-aldosterone or [^{3}H]-progesterone to the fetus. After administration of [^{3}H]-estradiol or [^{3}H]-aldosterone, a large percentage of estradiol was bound in the cytosol fraction while there was less binding of aldosterone. On the other hand, for both steroids, the radioactivity associated with macromolecules was very high in the different nuclear extracts. Following [^{3}H]-progesterone administration, no radioactivity was found in the bound fractions of either the cytosol or the nuclear extracts. The percentages of non-metabolized hormone in the bound fractions of the cytosol and the different nuclear extracts after [^{3}H]-estradiol or [^{3}H]-aldosterone administration are shown in Table 3. Since no bound radioactivity was found following [^{3}H]-progesterone administration (Table 2), the data for progesterone in Table 3 represent unbound, non-metabolized progesterone in the different subcellular fractions. It is observed that in the [^{3}H]-estradiol and [^{3}H]-aldosterone experiments, most of the radioactivity associated with the macromolecules is the non-metabolized hormones. It is interesting to note that in the [^{3}H]-progesterone experiments, despite the fact that no [^{3}H]-progesterone complexes were

Table 3. Percentage of the non-metabolized hormones in the macromolecule complexes of the different subcellular fractions of the fetal kidney 30 min after administration of [^{3}H]-estradiol, [^{3}H]-aldosterone or [^{3}H]-progesterone to the fetus

Steroid administered	Exp. I [^{3}H]-estradiol (7.7×10^{-10} moles per fetus)		Exp. II [^{3}H]-aldosterone (4.4×10^{-10} moles per fetus)	Exp. III [^{3}H]-progesterone* (9×10^{-10} moles per fetus)
	In the complex		In the complex	In the total extract
	$\%E_2$†	$\%E_1$†	% aldosterone	% progesterone
Cytosol	65	6	50	40
Nuclear extracts				
(A) 0·1 M Tris	62	—	80	80
(B) 0·3 M NaCl	90	5	—	85
(C) 1 M NaCl	93	6	85	90

* Since no bound [^{3}H]-progesterone was present in the different subcellular fractions (see Table 2) these data correspond to the non-metabolized progesterone in the total extracts.

† E_2 = Estradiol, E_1 = Estrone.

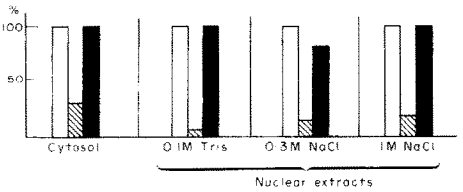

Figure 1

Effect of estradiol and d-aldosterone *in vivo* on the formation of ^{3}H-estradiol complexes in the cytosol and nuclear extracts in the fetal kidney 30 min after ^{3}H-estradoil administration to the fetus ($7{\cdot}7 \times 10^{-10}$ moles per fetus).

The percentage of the effect is calculated by considering the specific activity (DPM/mg of protein) in the ^{3}H-estradiol macromolecule complexes after Sephadex G-15 chromatography. □ Control, ▨ + E_2 (Estradiol) (15 fold excess mol/mol), ■ + d-aldo (Aldosterone) (15 fold excess mol/mol). Values represent the average of two experiments.

formed, in the total extracts, most of the radioactivity in the different subcellular fractions is non-metabolized progesterone.

2. *Effect of estradiol and other steroids on the formation of* [^{3}H]-*estradiol–macromolecule complexes*

In a series of experiments, either [^{3}H]-estradiol alone or a mixture of [^{3}H]-estradiol and non-labelled estradiol (15-fold excess mol/mol) or non-labelled *d*-aldosterone (15-fold excess mol/mol) was injected into the fetus. As indicated in Fig. 1 estradiol caused a significant competitive effect on the formation of the [^{3}H]-estradiol complexes of the cytosol and particularly of the nuclear extracts, whereas aldosterone had no effect.

In a series of *in vitro* experiments, it was also shown that estradiol competes in the formation of [^{3}H]-estradiol complexes of the cytosol and nuclear extracts (Fig. 2). Estrone and estriol were also found to exhibit a competitive effect, while testosterone and cortisol had no influence on the formation of [^{3}H]-estradiol complexes.

3. *Determination of the equilibrium constant and number of sites of the binding of* [^{3}H]-*estradiol in fetal kidney cytosol.*

Figure 3 indicates the Scatchard plot of the specific binding of various concentrations of [^{3}H]-estradiol in the cytosol fraction of the fetal kidney.

As is observed, two sets of binding sites were found, the first component with a $K_d = 2{\cdot}5 \times 10^{-10}$ M and a number of sites of: $4{\cdot}5 \times 10^{-14}$ mol/mg protein and the second with a $K_d = 7{\cdot}7 \times 10^{-9}$ M and a number of sites of: $1{\cdot}4 \times 10^{-13}$ mol/mg protein.

4. *Coefficient of sedimentation in a sucrose gradient of the* [^{3}H]-*estradiol complexes of the nuclear extracts*

After incubation of [^{3}H]-estradiol or [^{3}H]-estradiol with either unlabelled estradiol or cortisol with cell suspensions of the fetal guinea pig kidney, the 0·3 M NaCl or 1 M NaCl nuclear extracts were layered on a sucrose density gradient (5–20% w/v), containing 0·5 M NaCl, 0·012 M thioglycerol and 0·001 M EDTA, and centrifuged at 250,000 **g** for 18 h at 2°C. The results

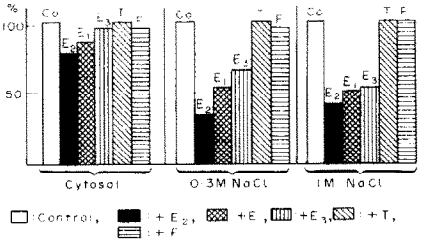

Figure 2

Effect of estradiol, estrone, estriol, testosterone and cortisol on the formation of ^{3}H-estradiol complexes in the cytosol and nuclear extracts after incubation of the cell suspensions of fetal kidney of guinea pig with ^{3}H-estradiol.

In each experiment, 1 g of fetal kidney was incubated with 10 μCi of ^{3}H-estradiol (5.5×10^{-8} M) control □, or with the same quantity of ^{3}H-estradiol + 8×10^{-7} M (15 fold excess, mol/mol) of estradiol (E_2) ■, estrone (E_1) ▨, estriol (E_3) ▥, testosterone (T) ▧ or cortisol (F) ▤. Incubations were carried out for 15 min at 37°C. The percentage effect is calculated by considering the specific activity (DPM/mg of protein) in the ^{3}H-estradiol macromolecule complexes after Sephadex G-15 chromatography. Values represent the average effect of two experiments.

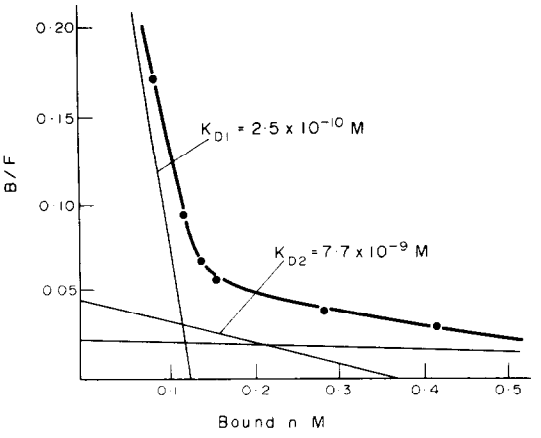

Figure 3
Scatchard plot of the ^{3}H-estradiol apecific binding in the cytosol fraction of the foetal kidney of guinea pig.

incubated with 6 × 10^{-10} M to 1·4 × 10^{-8} M ^{3}H-estradiol. Specific binding was determined by applying the Rosenthal (22) correction. The "background" value was measured by parallel incubations containing a 100–2000 fold excess of estradiol-17β.

1·2 ml of the cytosol (containing 5 mg of protein) were obtained with the 0·3 M NaCl and 1 M NaCl nuclear extracts are shown in Figs. 4 and 5, respectively. In both nuclear extracts, a component with a sedimentation coefficient of 3·7 S was observed. This 3·7 S peak did not appear when unlabelled estradiol (× 15 fold excess mol/mol) was added to the incubation. Addition of cortisol (× 15 fold excess mol/mol) had no effect on the displacement of [^{3}H]-estradiol from the 3·7 S [^{3}H]-estradiol component.

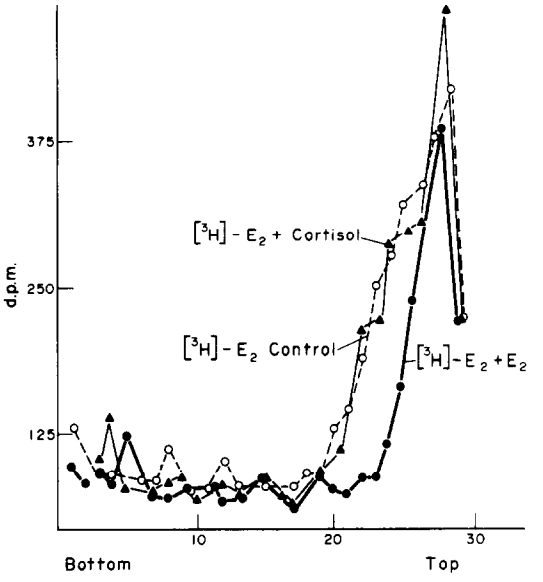

Figure 4
Sucrose density gradient of the 0·3 M NaCl nuclear extract after incubation of the cell suspension of fetal kidney with ^{3}H-estradiol.

10 μCi of ^{3}H-estradiol or 10 μCi of ^{3}H-estradiol + estradiol or cortisol were incubated with 1 g of fetal kidney and 0·2 ml of the 0·3 M NaCl nuclear extracts were layered on a sucrose gradient (5–20%) containing 0·5 M NaCl, 0·012 M thioglycerol and 0·001 M EDTA. The tubes were centrifuged for 18 h at 2°C at 250,000 × g. The sedimentation coefficient was calculated using as standard: Bovine serum albumin (4·3S) and γ-globulin (7S). -----○----- ^{3}H-estradiol; ——●—— ^{3}H-estradiol + estradiol (15 fold excess mol/mol); ——▲—— ^{3}H-estradiol + cortisol (15 fold excess mol/mol).

Table 4. Percentage of the radioactivity in the nuclear extracts after incubation of the cytosol [^{3}H]-estradiol macromolecule complexes with purified nuclei of the fetal kidney of guinea pig at different temperatures

	Exp. I				Exp. II	
	A		B			
DPM of the cytosol ^{3}H-estradiol complexes incubated	79,800	79,800	190,000	190,000	190,000 at 30°C ↓	190,000 at 30°C ↓
Incubation temperature	4°C	37°C	4°C	37°C	4°C	37°C
Nuclear extracts	%*	%	%	%	%	%
(A) 0·1 M Tris	1·4	4·6	1·6	3·6	2·5	3·9
(B) 0·3 M NaCl	1·3	3·4	1·5	2·6	1·9	2·7
(C) 1 M NaCl	2·4	7·7	3·0	5·8	4·8	8·1
Krebs buffer	84·4	71·9	83·8	76·3	81·0	73·1

* Percentage of total radioactivity incubated.

Exp. I: 10 ml of cytosol were incubated with 25 μCi of [^{3}H]-estradiol (5·4 × 10^{-8} M) for 30 min at 4°C. The [^{3}H]-estradiol complexes were isolated by chromatography in Sephadex G-15. 4 ml of the column eluate containing the cytosol [^{3}H]-estradiol macromolecule complexes were reincubated with the purified nuclei from 1 g of fetal kidney at 4°C for 30 min or at 37°C for 15 min and the nuclei were extracted as indicated in the Methods Section.

Exp. II: Same as Exp. I except that the cytosol [^{3}H]-estradiol macromolecule complexes were first pre-incubated at 30°C for 15 min before being incubated with the nuclei at 4°C for 30 min or at 37°C for 15 min.

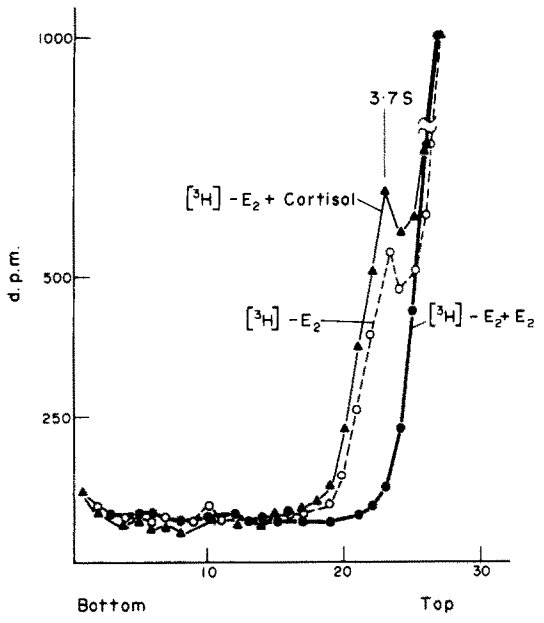

Figure 5
Sucrose density gradient of the 1 M NaCl nuclear extract after incubation of the cell suspension of fetal kidney with ^{3}H-estradiol*.

Experimental conditions are the same as is indicated in the legend of Figure 4.

5. *Effect of temperature on the transfer of cytosol* [^{3}H]-*estradiol complexes into the nucleus*

In order to establish the influence of temperature on the transfer of the cytosol complexes into the nucleus, the cytosol [^{3}H]-estradiol complexes were prepared after incubation of [^{3}H]-estradiol with the cytosol fraction for 30 min at 2°C. The [^{3}H]-estradiol complexes were obtained after chromatography in Sephadex G-15 and re-incubated with the purified nuclei at 4°C or at 37°C. As is indicated in Table 4 a significant part of the radioactivity was found in the different nuclear extracts and was 2–3 times higher in the experiments carried out at 37°C than at 4°C. It was also observed that pre-incubation of the [^{3}H]-estradiol complexes at 30°C increased the radioactivity in the nuclear extracts as compared with pre-incubation at 4°C.

6. *Studies with* [^{3}H]-*estrone in guinea pig fetuses*

As is indicated in Fig. 2, estrone exerts a competitive effect on the formation of cytosol and nuclear [^{3}H]-estradiol complexes following incubation of [^{3}H]-estradiol with cell suspensions obtained from fetal kidneys. In order to explore the possibility that estrone is converted to estradiol in the kidney of the fetus, a series of *in vitro* and *in vivo* experiments was carried out.

(a) "*In vivo*" *administration of* [^{3}H] *estrone to the fetus.* Table 5 indicates the percentage of the administered dose of [^{3}H]-estrone as well as the percentage of [^{3}H]-estrone and [^{3}H]-estradiol in the different fetal tissues and the placenta. Significant conversion of [^{3}H]-estrone to [^{3}H]-estradiol was found in the different tissues studied, particularly in the fetal kidney where 60% of the radioactive material was found to be [^{3}H]-estradiol. Table 6 shows the distribution of radioactivity in the different subcellular fractions, the percentage bound and the percentage of [^{3}H]-estrone and [^{3}H]-estradiol in each fraction. The percentage of the radioactivity in the nuclear extracts as well as the percentage bound were less than after [^{3}H]-estradiol administration (see Tables 1 and 2). In addition, the data in Table 6 show that the conversion of estrone to estradiol in the different subcellular fractions was high.

Table 5. Relative percentage of [^{3}H]-estrone and [^{3}H]-estradiol in the different fetal tissues and in the placenta of the guinea pig 30 min after administration of [^{3}H]-estrone subcutaneously to the fetus

	% of the administered dose	[^{3}H]-Estrone %*	[^{3}H]-Estradiol %*
Fetal tissues			
Lungs	0·6	72	21
Liver	2·8	60	11
Intestine	1·3	73	13
Kidney	0·3	24	59
Residual fetal tissues	46·0	83	11
Placenta	1·2	54	18

Values represent the average of the combined tissues of seven fetuses after administration of 31 μCi of [^{3}H-estrone ($7{\cdot}6 \times 10^{-10}$ moles) to each fetus, *in situ* and subcutaneously.

* The percentages of [^{3}H]-estrone and [^{3}H]-estradiol correspond to the percentage of the total radioactivity in the dichloromethane extract which contains the unconjugated material. Analytical studies of the radioactive material in the aqueous phase are in progress and will be published in a subsequent article.

Table 6. Distribution of radioactivity in the different subcellular fractions of the fetal kidney of guinea pig 30 min after [^{3}H]-estrone administration to the fetus

Subcellular fractions	% of the total cell	Bound* %	Estrone† %	Estradiol† %
Cytosol	89	1·6	25	42
Nuclear extracts				
(A) 0·1 M Tris	0·7	12·0	38	55
(B) 0·3 M NaCl	1·3	31·7	8	89
(C) 1 M NaCl	3·1	33·5	26	60

Percentage of the administered dose in kidneys: 0·3%.
The data represent the average of 4 fetuses which received 41,6 μCi (7.6×10^{-10} moles) of [^{3}H]-estrone per fetus.
* Percentage of each fraction.
† The percentages of [^{3}H]-estrone and [^{3}H]-estradiol correspond to the percentage of the total radioactivity in the dichloromethane extract which contains the unconjugated material.

(b) *"In vitro" conversion of* [^{3}H]-*estrone to* [^{3}H]-*estradiol.* The conversion of [^{3}H]-estrone to [^{3}H]-estradiol in the fetal kidney was also studied in *"in vitro"* experiments by incubating [^{3}H]-estrone with cell suspensions or subcellular fractions. There was a considerable conversion of [^{3}H]-estrone to [^{3}H]-estradiol by cell suspensions or the cytosol fraction and some conversion after incubation with purified nuclei (Table 7).

DISCUSSION

Comparative studies concerned with the formation of macromolecule complexes of [^{3}H]-estradiol, [^{3}H]-aldosterone or [^{3}H]-progesterone in the fetal guinea pig kidney show both *in vivo* and *in vitro* significant differences: [^{3}H]-estradiol and [^{3}H]-aldosterone form macromolecule complexes in both the cytosol and nucleus but no binding for progesterone was found in the different subcellular fractions. The analytical study of the total radioactive material of the different subcellular fractions in the progesterone experiments showed that most of this material consisted of non-metabolized progesterone. Similar analysis of the radioactive material in the macromolecule complexes from the [^{3}H]-estradiol and [^{3}H]-aldosterone experiments also showed that most of this is made up of non-metabolized [^{3}H]-estradiol or [^{3}H]-aldosterone, respectively. A possible explanation for the absence of [^{3}H]-progesterone complexes could be the high concentration of endogenous progesterone circulating in the fetal plasma (4·8–5·3 μg/100 ml of plasma[6]).

As previously shown[13], estradiol had no effect on the formation of [^{3}H]-aldosterone complexes in the fetal kidney. Furthermore, the present work revealed

Table 7. "In vitro" conversion of [^{3}H]-estrone to [^{3}H]-estradiol by the fetal kidney of guinea pig

Subcellular fraction incubated	Fraction studied	[^{3}H]-Estradiol %*	[^{3}H]-Estrone %*
Exp. I Total Cell	Total fraction	32	35
Exp. II Cytosol	Total fraction	13	32
	Bound fraction	43	42
Exp. III Purified nuclei			
1. 0·3 M NaCl Extract	Bound fraction	1·5	95
2. 1 M NaCl Extract	Bound fraction	4·6	90

In *Exp. I*, a cell suspension of 1 g of fetal kidney was incubated with 10 μCi of [^{3}H]-estrone (4.75×10^{-8} M) in 4 ml of Krebs–Henseleit buffer for 30 min at 37°C. In *Exp. II*, 6 ml of the cytosol fraction were incubated with 5 μCi of [^{3}H]-estrone for 15 min at 37°C. In Exp. III, the purified nuclei from 1 gr of fetal kidney were incubated with 10 μCi of [^{3}H]-estrone in 2 ml of Krebs buffer for 15 min at 37°C. The data represent the average of 2 experiments.
* The percentages of [^{3}H]-estrone and [^{3}H]-estradiol correspond to the percentage of the total radioactivity in the dichloromethane extract which contains the unconjugated material.

that aldosterone did not compete in the formation of [^{3}H]-estradiol complexes in the fetal kidney, neither *in vivo* nor *in vitro*: so it appears that at this period of development the sites of binding of aldosterone and estradiol or the macromolecules that bind aldosterone and estradiol in the fetal kidney are different since there is no competition between the two for binding. The studies with [^{3}H]-estradiol indicated that the estradiol binding sites appeared to have some affinity for two other estrogens, estrone and estriol, but neither cortisol nor testosterone competed in the formation of [^{3}H]-estradiol macromolecule complexes.

The application of the Scatchard method established that in the cytosol fraction of the fetal kidney, [^{3}H]-estradiol appears to bind to two different sets of binding sites. The dissociation constant of the first group was found to be $K_{d1}(4°C) = 2{\cdot}5 \times 10^{-10}$ M with n (number of sites) $= 4{\cdot}5 \times 10^{-14}$ mol/mg protein and of the second group, $K_{d2}(4°C) = 7{\cdot}7 \times 10^{-9}$ M with $n = 1{\cdot}4 \times 10^{-13}$ mol/mg protein. It is interesting to note that the K_d of the first group is of the same order as that of the binding of estradiol with the specific receptor of the cytosol fraction in the uterus of immature rats[23, 24].

The sucrose density gradients of the two nuclear extracts (0·3 M NaCl and 1 M NaCl) showed a 3·7 S component on which estradiol had a significant competitive effect but cortisol had no effect. The possible connection of this component with the macromolecule complexes of the cytosol fraction is to be explored. Furthermore, the sedimentation coefficient value of this nuclear component is slightly different from that found in the nucleus of the uterus of rats [23, 24].

In previous work, it was demonstrated that the formation of [^{3}H]-aldosterone macromolecule complexes in the nuclei of the fetal kidney is temperature dependent[13]. Similar data were found in the studies carried out on the nuclear [^{3}H]-estradiol complexes obtained from the same fetal tissue (unpublished data). In connection with this temperature dependency of the nuclear complexes, a series of experiments was carried out. Purified nuclei were incubated at 4°C and at 37°C with [^{3}H]-estradiol cytosol complex or with [^{3}H]-estradiol cytosol complex previously activated at 30°C. Although the quantity of radioactivity of the cytosol complex transferred into the nuclei was too small to permit further determination of whether this radioactive material was bound in any of the nuclear extracts, it was, nevertheless, sufficient to indicate that the penetration of the [^{3}H]-estradiol cytosol complex into the nucleus is also temperature dependent (Table 4). Furthermore, the penetration of cytosol complex into the nucleus was greater when the [^{3}H]-estradiol cytosol complex was pre-incubated at

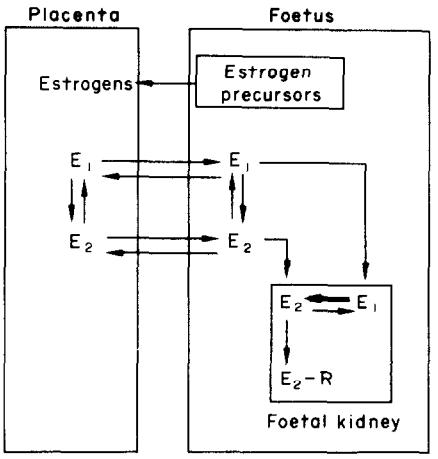

Figure 6
Hypothetical sequence of the formation of estradiol macromolecule complexes in the fetal kidney of guinea pig. E_2: Estradiol; E_1: Estrone; E_2-R: Estradiol-receptor.

30°C before subsequent incubation with the purified nuclei, showing that temperature is necessary to activate the cytosol complex prior to its penetration into the nucleus. It is interesting to note that the nuclear complexes of different steroid hormones are also temperature dependent in other target tissues of adult animals, i.e. estradiol in the uterus of the immature female rat[25], 5α-dihydrotestosterone in the rat ventral prostate[26], etc.

Since the competition studies had revealed that estrone competes in the formation of the [^{3}H]-estradiol complexes, it was important to know whether this effect was due to estrone or to a conversion of estrone to estradiol. In this respect, the results showed that there was indeed a large conversion of estrone to estradiol *in vivo* in the fetal kidney which was substantially higher than the conversion in fetal liver, intestine, brain, lungs and in the placenta. *In vitro* incubation of kidney cell suspensions or subcellular fractions (Table 7) revealed a significant conversion of estrone to estradiol in all fractions. Estradiol thus formed from estrone could then bind to specific estradiol binding sites. A suggested sequence of the formation of estradiol macromolecule complexes in the fetal kidney of the guinea pig is indicated in Fig. 6.

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REFERENCES

1. Bloch Tissenbaum B. and Benirschke K.: *Biochim biophys. Acta* **60** (1962) 182–184.

2. Villee D. B., Engel L. L., Loring J. M. and Villee C. A.: *Endocrinology* **69** (1961) 354–372.
3. Diczfalusy E.: *Excerpta Med. Found. Intern. Congr. Ser.* **183** (1968) 65–109.
4. Pasqualini, J. R.: *Excerpta Med. Intl. Congr. Ser.* **219** (1971) 487–495.
5. Pasqualini J. R., D'Amore B. and Constant B. (unpublished data).
6. Castellet R. and Pasqualini J. R.: *C.r. hebd. Séanc. Acad. Sci. (Paris)* Serie D **276** (1973) 1205–1208.
7. Unpublished data.
8. Jensen E. V. and Jacobson H. I.: *Recent Progr. Hormone Res.* **18** (1962) 387–414.
9. Munck A. and Brinck-Johnsen T.: *J. biol. Chem.* **243** (1968) 5556–5562.
10. Fang S., Anderson K. M. and Liao S.: *J. biol. Chem.* **244** (1969) 6584–6595.
11. O'Malley B. W., McGuire W. L., Kohler P. O. and Korenman S. G.: *Recent Progr. Horm. Res.* **25** (1969) 105–160.
12. Herman T. S., Fimognari G. M. and Edelman I. S.: *J. biol. Chem.* **243** (1968) 3849–3856.
13. Pasqualini J. R., Sumida C. and Gelly C.: *J. steroid Biochem.* **3** (1972) 543–556.
14. Pasqualini J. R., Sumida C. and Gelly C.: *Acta Endocr. (Kbh)* **77** (1974) 356–367.
15. Pasqualini J. R., Sumida C., Gelly C. and Nguyen B. L.: *C.r. hebd. Séanc. Acad. Sci. (Paris)* Series D **276** (1973) 3359–3362.
16. Pasqualini J. R. and Palmada M.: *C.r. hebd. Séanc. Acad. Sci. (Paris)* Serie D **274** (1972) 1218–1221.
17. Pasqualini J. R. and Perassi R.: unpublished data.
18. Krebs H. A. and Henseleit K.: *Hoppe Seyler's Z. Physiol. Chem.* **210** (1932) 33–66.
19. Chauveau J., Moule Y. and Rouiller C.: *Expl. Cell Res.* **11** (1965) 317–321.
20. Scatchard G.: *Ann N.Y. Acad. Sci.* **51** (1945) 550–572.
21. Lowry O. H., Rosebrough N. J., Farr A. L. and Randell R. J.: *J. biol. Chem.* **193** (1951) 265–275.
22. Rosenthal H. E.: *Anal. Biochem.* **20** (1967) 525–532.
23. Gorski J., Toft D., Shyamala G., Smith D., and Notides A.: *Recent Prog. Hormone Res.* **24** (1968) 45–80.
24. Desombre E. R., Puca G. A. and Jensen E. V.: *Proc. Natn. Acad. Sci. USA*, **64** (1969) 148–154.
25. Jensen E. V., Molha S., Gorell T., Tanaka S. and Desombre E. R.: *J. steroid Biochem.* **3** (1972) 445–458.
26. Fang S. and Liao S.: *J. Biol. Chem.* **246** (1971) 16–24.

DISCUSSION

Villee:

I was very interested in your finding of these receptors in the guinea pig kidney. We have been studying estradiol receptors in the hamster kidney for some years because of the role they play in the generation of renal carcinomas. These receptors have properties which are not significantly different from the properties of the receptors in the hamster uterus or the hamster hypothalamus. The association constants, sedimentation coefficients, etc. are very similar. The new finding I wanted to report to you is that recently in collaboration with Prof. Pantic at the University of Belgrade we've carried out some autoradiographic studies and we found, somewhat to our surprise, that the estrogen (estradiol) is bound almost entirely in the primary convoluted tubules and not in any other part of the kidney. I wonder if you have carried out any autoradiographic studies with the guinea pig kidney and whether in your system it's the primary convoluted tubules that do the binding.

Pasqualini:

Thank you for your interesting comment. Concerning your question on the autoradiographic studies, at the moment this project is in realization but we don't have any data yet.

Crabbé:

I'd like to know whether there is a chronological evolution in terms of appearance and development of these receptors during the fetal life in guinea pigs. Is there a progressive increase as gestation goes on and is there some kind of dissociation between the time one can detect one type of receptor versus the other one?

Pasqualini:

Our studies were carried out using fetuses between 35 and 55 days of gestation in which we have not yet done a systematic study on the evolution of the receptors during fetal development. At the present time, studies are in progress to compare the specific binding of estradiol in kidneys of fetuses with those of newborn, immature and adult guinea pigs, both females and males.

Kolpakov:

I would like to make a comment. I think that the very high levels of estradiol in the guinea pig mother determines the receptor in the fetus during its development. This also determines receptors in nuclear sites for corticosteroids since this is the situation you have with the aldosterone concentration which is higher at this period of life. This is a possible cause of the formation of aldosterone complexes.

Your experiments and ours are rather similar because in your experiments you have hyper-aldosteronism from the maternal compartment. In our experiments we have hyper-aldosteronism from sodium deficiency. Maybe this is the cause of the formation of aldosterone receptors or binding sites of target organs.

Pasqualini:

Your comment on the relationship between the concentration of hormones circulating in the plasma and receptors in the cells is interesting. One explanation for the significant quantity of estradiol and aldosterone receptors in the nucleus of the fetal guinea pig kidney could be that due to the high circulating concentrations of these two hormones in the fetal plasma, the cytosol receptors are already present in the nucleus. Nevertheless, another possibility is that during fetal development, in this tissue, the hormone reaches the nuclear receptor directly without an intermediate cytosol complex. Recent data give some support to this last hypothesis since after incubating the chromatin fraction (1 M NaCl) with [^{3}H] -estradiol, specific binding of estradiol was found with a K_D of 3·4 $\times$ 10^{-10} M (Sumida C., Gelly C. and Pasqualini J. R. (*C.r. hebd. Séanc. Acad. Sci. (Paris)* **279** (1974), 1793–1796).