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IN VITRO TRANSFORMATION OF [^{3}H]-DEOXYCORTICOSTERONE INTO [^{3}H]-3β,5α-TETRAHYDRO-DEOXYCORTICOSTERONE AND INTO [^{3}H]-3α,5β-TETRAHYDRO-DEOXYCORTICOSTERONE IN THE FETAL, NEW BORN AND IMMATURE GUINEA PIG LIVERS

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SUMMARY

In the present paper the transformation of [^{3}H]-deoxycorticosterone into its tetrahydro derivatives, the two equatorial isomers [^{3}H]-3β,5α-tetrahydro-deoxycorticosterone and the [^{3}H]-3α,5β-tetrahydro-deoxycorticosterone was studied in the fetal, newborn and immature guinea pig livers. A systematic study of the transformation of [^{3}H]-deoxycorticosterone into [^{3}H]-3β,5α-tetrahydro-deoxycorticosterone during fetal development showed that fetal liver at least from 35 days of gestation could convert 29–45% of the total radioactivity into [^{3}H]-3β,5α-tetrahydro-deoxycorticosterone and this increased to 62–73% at the end of gestation (59 days). This conversion diminished significantly after birth and in immature animals and there were only trace quantities at 28 days old. One the other hand the other equatorial isomer, [^{3}H]-3α,5β-tetrahydro-deoxycorticosterone, was not detectable in the fetal liver but this conversion is present after birth and increases significantly in immature animals. A study of the influence of sex on these reductions of [^{3}H]-deoxycorticosterone showed no significant differences during fetal evolution and at least until 28 days after birth.

INTRODUCTION

3β,5α-Tetrahydro-deoxycorticosterone (3β,21-dihydroxy-5α-pregnan-20-one) was isolated and identified from the urine of pregnant women but not in non-pregnant women nor in men [1]. Also it was demonstrated that its excretion rate increases significantly during pregnancy so that the maximal values are found at the third trimester (300–1080 μg/24 h) [2].

In previous studies it was demonstrated that fetal liver of guinea pig (45–55 days of gestation) can convert [^{3}H]-deoxycorticosterone to [^{3}H]-3β,5α-tetrahydro-deoxycorticosterone with a high yield [3]. Consequently it was interesting to study the quantitative evolution of this transformation during fetal development in this animal species and the transformation of [^{3}H]-deoxycorticosterone to the other tetrahydro equatorial isomer of deoxycorticosterone, 3α,21-dihydroxy-5β-pregnan-20-one. A comparative study of the transformation of [^{3}H]-deoxycorticosterone in male and female fetal livers is also presented.

EXPERIMENTAL

Biological material. The fetal livers of male and female Hartley Albino guinea pigs were used. The males and females were mated for 24 h, consequently the period of gestation could be determined with an error of $\pm$24 h. The livers of newborns (16 h) and immature animals (9 days and 28 days) were also used.

Chemicals. [1,2-^{3}H]-deoxycorticosterone (S.A. 46.8 Ci/mmol) was purchased from NEN Chemicals, Frankfurt, West Germany. Its purity was controlled by paper chromatography in the systems: toluene–propanediol and isooctane–methanol–water (5:3:2 by vol.) and after acetylation in the system isooctane–propanediol. Synthetic deoxycorticosterone was purchased from Steraloids Inc., Wilton, NH, U.S.A. Synthetic 3β,5α-tetrahydro-deoxycorticosterone and 3α,5β-tetrahydro-deoxycorticosterone were purchased from Synsteroids, Molravia, CA, U.S.A.

Incubation. Slices (0.1 mm) from 1 gm of fetal, new born and immature livers were incubated with 10 μCi of [^{3}H]-deoxycorticosterone in 5 ml of Krebs–Henseleit buffer [4] at 37° for 3 h with shaking. The reaction was stopped by the addition of ethanol. After incubation the tissues were disintegrated with sand and the proteins were precipitated with 80 percent v/v ethanol and left for 48 h at $-10°$. The ethanol extract was evaporated to dryness and dissolved again in ethanol 90 percent (v/v), left for 48 h at $-10°$, centrifuged, evaporated to dryness, dissolved in water and extracted three times with 1 vol. of dichloromethane and twice with 1 vol. of *n*-butanol.

The following paper chromatographic systems were used: S-1: toluene–propanediol; S-2: isooctane–

toluene–methanol–water (4:1:3:2 by vol.); S-3: isooctane–methanol–water (5:3:2 by vol.); S-4: isooctane. (In this system the 2 cm band at the origin was sprayed with a mixture of methanol/propanediol (v/v) before depositing the steroids) [5]. Acetylation was carried out overnight at room temperature with a mixture of pyridine–acetic anhydride. Radioactivity was measured in a liquid scintillation spectrometer (Packard Model 3330 TRICARB). The unconjugated radioactivity was measured in a POPOP-PPO–toluene scintillation solution. The conjugated radioactive material was measured in the same solution after the addition of 0.5 ml methanol.

RESULTS

(I) *Identification of* [^3H]-3β,5α-*tetrahydro-deoxycorticosterone*

The dichloromethane extracts were chromatographed in the system S-1 (3 h). The zone with the same chromatographic migration as that of synthetic 3β,5α-tetrahydro-deoxycorticosterone was eluted and chromatographed successively in the systems S-1 (5 h), S-2 (4 h) and S-3 (6 h). In all of these chromatographic systems the migration of the radioactive material had the same R_f as synthetic 3β,5α-tetrahydro-deoxycorticosterone. An aliquot of the eluted radioactive material from the last system was acetylated and the acetate derivative had the same R_f as synthetic 3β,5α-tetrahydro-deoxycorticosterone 3,21-diacetate in the system S-4.

Another aliquot was mixed with 15 mg of authentic 3β,5α-tetrahydro-deoxycorticosterone and crystallized successively in different solvents. The data of the S.A. of the crytals are indicated in Table 1.

(II) *Identification of* [^3H]-3α,5β-*tetrahydro-deoxycorticosterone*

The dichloromethane extracts were chromatographed in the system S-1 (3 h). The zone having the same chromatographic migration as that of synthetic 3α,5β-tetrahydro-deoxycorticosterone was eluted and chromatographed successively in the systems S-1 (5 h), S-2 (4 h) and S-3 (6 h). In all of these chromatographic systems the migration of the radioactive material had the same R_f as synthetic 3α,5β-tetrahydro-deoxycorticosterone. An aliquot of the eluted radioactive material from the last system was acetylated and the acetate derivative had the same R_f as synthetic 3α,5β-tetrahydro-deoxycorticosterone 3,21-diacetate in system S-4. Another aliquot was mixed with 15 mg of authentic 3α,5β-tetrahydro-deoxycorticosterone and crystallized in different solvents. The data of the S.A. of the crystals are shown in Table 1.

Table 1. Crystallization to constant specific activity (d.p.m./mg) of isolated radioactive steroids

Steroid	Source	Crystals (d.p.m./mg)	Solvent
[^{3}H]-3β,5α-tetrahydro-deoxycorticosterone	Fetal liver	4615	(a)
		4540	(b)
		4570	(c)
[^{3}H]-3α,5β-tetrahydro-deoxycorticosterone	Immature liver	5635	(b)
		5565	(d)
		5430	(d)

Solvents: (a) methanol, (b) benzene, (c) ethyl acetate (d) ethyl acetate-isooctane.

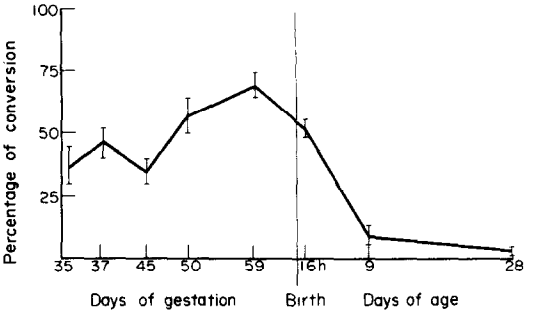

Fig. 1. *In vitro* conversion of [^{3}H]-deoxycorticosterone to [^{3}H]-3β,5α-tetrahydro-deoxycorticosterone by the fetal, newborn and immature guinea pig liver. 1 g of tissue was incubated with 10 μCi of [^{3}H]-deoxycorticosterone in 5 ml of Krebs–Henseleit buffer at 37° for 3 h. 1 g of liver tissue was obtained from 5–10 fetuses or from 2 newborn or immature animals. The values represent the averages of 4 experiments.

(III) *Conversion of* [^3H]-*deoxycorticosterone to* [^3H]-3β,5α-*tetrahydro-deoxycorticosterone and to* [^3H]-3α,5β-*tetrahydro-deoxycorticosterone by the fetal, new born and immature guinea pig livers*

Figure 1 shows the percentage of the transformation of [^{3}H]-deoxycorticosterone into [^{3}H]-3β,5α-tetrahydro-deoxycorticosterone by fetal liver during the fetal evolution of guinea pig as well as in 16 h and 9 days old.

As can be seen in this figure, during the period studied from 35 days to the end of gestation, there exists a great conversion of [^{3}H]-deoxycorticosterone into [^{3}H]-3β,5α-tetrahydro-deoxycorticosterone. The maximal values (an average of 67% of conversion) are obtained at 59 days of gestation. After birth, there is a significant decrease in this conversion and only 5.5% (average) is converted at 9 days and traces if any are detected at 28 days.

In a comparative study to establish the conversion of [^{3}H]-deoxycorticosterone into [^{3}H]-3β,5α-tetrahydro-deoxycorticosterone and into [^{3}H]-3α,5β-tetrahydro-deoxycorticosterone, it was observed that [^{3}H]-3α,5β-tetrahydro-deoxycorticosterone was not detected during fetal development (Table 2). However, this conversion became significant at 9 days and 28 days after birth.

(IV) *Transformation of* [^3H]-*deoxycorticosterone in male and female fetal, new born and immature guinea pig livers*

Table 3 indicates the distribution of radioactivity in the dichloromethane, *n*-butanol and aqueous phases after incubation of [^{3}H]-deoxycorticosterone

Table 2. Percentage of conversion of [^{3}H]-deoxycorticosterone to [^{3}H]-3β,5α-tetrahydro-deoxycorticosterone and to [^{3}H]-3α,5β-tetrahydro-deoxycorticosterone by the fetal, new born and immature guinea pig liver

Liver	Age	[^{3}H]-3β,5α-tetrahydro-deoxycorticosterone %	[^{3}H]-3α,5β-tetrahydro-deoxycorticosterone %
Fetal	35 days	37.2	N.D.
Fetal	59 days	67.2	N.D.
New born	16 h	51.7	N.D.
Immature	9 days	5.5	42.2
	28 days	Traces	43.1

The experimental conditions are the same as indicated in Fig. 1. The values correspond to the percentage of the total radioactivity incubated and represent the average of 4 experiments. N.D. = not detectable.

with liver of fetuses of 35, 37, 45, 50, and 59 days of gestation and of guinea pigs of 16 h, 9 days and 28 days after birth. It is observed that in both males and females the major portion of the radioactivity was found in the dichloromethane phase but there is a significant increase in the *n*-butanol phase at 28 days after birth.

The conversion of [^{3}H]-deoxycorticosterone to [^{3}H]-3β,5α-tetrahydro-deoxycorticosterone in males and females is indicated in Table 4. As is observed there is no significant sex difference in the reduction of [^{3}H]-deoxycorticosterone into the 3β,5α,tetrahydro derivative.

DISCUSSION

The data obtained in the present study confirm previous observations in this laboratory that the fetal liver of guinea pig converts tritiated deoxycorticosterone into tritiated 3β,5α-tetrahydro-deoxycorticosterone to a very great extent [3]. Furthermore, as is indicated in the present paper a high percentage of

Table 3. Distribution of the radioactivity in the dichloromethane, *n*-butanol and aqueous phases after *in vitro* incubation of [^{3}H]-deoxycorticosterone with fetal, new born and immature guinea pig livers

Age of fetus in days	Male liver			Female liver		
	Dichloromethane %*	*n*-butanol %*	Aqueous Phase %*	Dichloromethane %*	*n*-butanol %*	Aqueous Phase %*
35	86.75	12.75	0.50	83.85	15.70	0.45
37	89.95	9.60	0.45	83.60	15.95	0.45
45	85.50	14.00	0.50	85.50	13.80	0.70
50	86.35	12.55	1.10	87.55	11.80	0.65
59	93.50	6.30	0.20	95.80	3.90	0.30
New born 16 h	87.90	11.30	0.80	84.70	14.60	0.70
Immature 9 days	86.00	13.70	0.30	72.10	25.50	2.40
28 days	66.50	30.20	3.30	57.70	37.80	4.50

Experimental conditions are the same as in Fig. 1. * Percentage of the total radioactivity. The extractions were carried out first with dichloromethane, followed by *n*-butanol. The values in the aqueous phases represent the radioactivity remaining after extraction. The data represent the average of 2 experiments.

Table 4. Percentage of transformation of [^{3}H]-deoxycorticosterone to [^{3}H]-3β,5α-tetrahydro-deoxycorticosterone in the male and female guinea pig liver during life evolution

Sex	Fetuses (age in Days) 35 %*	37 %*	45 %*	50 %*	59 %*	New born 16 h %*	Immature (age in days) 9 %*	28 %*
Female	37.5	41.1	30.9	59.8	69.7	54.5	5.8	Traces
Male	36.9	45.8	33	54	64.7	49	5.1	Traces

N.D. = not detectable. * Percentage of the total radioactivity incubated. Experimental conditions are the same as indicated in Fig. 1. The data represent the average of 2 experiments.

conversion is obtained at least from 35 days until the end of gestation. The most interesting aspect is the significant decrease after birth in the percentage of conversion of deoxycorticosterone to 3β,5α-tetrahydro-deoxycorticosterone with a concomitant increase in the conversion of deoxycorticosterone into the other tetrahydro derivative equatorial isomer, the 3α,5β-tetrahydro-deoxycorticosterone (Table 2). This conversion into the 3α,5β-tetrahydro derivative increases with postnatal age and the transformation of deoxycorticosterone into the 3β,5α-tetrahydro derivative diminishes after birth and only traces, if any, are detectable at 28 days.

Comparative data concerning the transformation of deoxycorticosterone in the human fetus have shown that [^{3}H]-deoxycorticosterone is converted into [^{3}H]-3β,5α-tetrahydro-deoxycorticosterone only in the fetal liver (fetuses of 18–22 weeks) but the conversion of [^{3}H]-deoxycorticosterone into the [^{3}H]-3α,5β-tetrahydro isomer is many times greater [6]. It is interesting to note that in adult humans (men and non-pregnant women) the only tetrahydro metabolites of deoxycorticosterone found are the 3α, 5β equatorial isomer [7] and the 3α, 5α axial isomer (3α 21-dihydroxy-5α-pregnan-20-one) [8].

In relation to the influence of sex during fetal development on the reduction of deoxycorticosterone it is observed in the present paper that there are no significant differences during the fetal period studied nor in the immature animals at least until 28 days old.

In connection with this it is interesting to note that in the rat sex differences in the reduction of cortisol were found only from 30 days after birth [9], at which time it was observed that in female rats cortisol is transformed mainly into 5α-tetrahydro-cortisol (3α,11β,17,21-tetrahydroxy-5α-pregnan-20-one) and into 5α-dehydro-cortisol (11β,17,21-trihydroxy-5α-pregnane-3,20-dione) to a greater extent than in male rats. These results are in agreement with the data presented previously by Forchielli *et al.*[10] who found that the incubation of 11-deoxycortisol with a microsomal preparation of female rat liver gives principally 5α derivatives and with male rat liver principally 5β derivatives. At present studies are being carried out to explore the possible influence of sex on the transformation of other corticosteroids in guinea pigs.

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