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CORTISOL AND CORTISONE METABOLISM IN THE HUMAN FOETO-PLACENTAL UNIT AT MIDGESTATION

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SUMMARY

A mixture of cortisol-1,2-^{3}H and cortisone-4-^{14}C was injected in two cases at laparotomy into the intact foeto-placental circulation 15 min prior to the interruption of gestation. The metabolites present in the placentas and the various foetal tissues were isolated and identified.

Only 3–6 per cent of the administered radioactive material was recovered from the urine of the mothers. The cortisol, tetrahydrocortisol (3α, 11β, 17α, 21-tetrahydroxy-5β-pregnan-20-one) and 20β-hexahydrocortisol (5β-pregnane-3α, 11β, 17α, 20β, 21-pentol) isolated from all foetal tissues studied (liver, intestines, lungs, adrenals and residual foetal tissues) contained exclusively the ^{3}H label. In contrast, the cortisol isolated from the placentas contained both ^{3}H and ^{14}C labels.

The cortisone, tetrahydrocortisone (3α,17β,21-trihydroxy-5β-pregnane-11,20-dione) and 20β-hexahydrocortisone (3α,17α,20β,21-tetrahydroxy-5β-pregnan-11-one) isolated from all tissues studied contained both ^{3}H and ^{14}C labelled material. The cortisol obtained after solvolysis of the ester sulphate fraction of the adrenals and livers contained only ^{3}H, whereas, the cortisone liberated from the same fraction contained both ^{3}H and ^{14}C. It is concluded that the human foetus at midgestation converts cortisol extensively to cortisone, but it not able to convert the biologically inactive cortisone to the biologically active cortisol. On the other hand, the human placenta is able to effect the interconversion of these two steroids.

A scheme is presented describing the metabolism of cortisol and cortisone in the human foeto-placental unit at midgestation.

INTRODUCTION

IT IS ESTABLISHED that the adrenals of perfused midgestation human foetuses are capable of synthesizing substantial amounts of cortisol. This hormone was isolated from the adrenals following the perfusion of foetuses with progesterone [1], 17α,21-dihydroxy-pregnenolone [2, 3], 17α-hydroxy-pregnenolone and 17α-hydroxy-progesterone [4]. The foeto-placental metabolism of the cortisol formed is incompletely understood. However, it is known that term placental tissue is capable of oxidizing cortisol to cortisone [5] and that the amount of cortisone greatly exceeds that of cortisol in extracts of term placentas [6] or in cord blood [7].

In order to gain more information concerning the metabolism of cortisol in the foeto-placental unit, a mixture of ^{3}H-labelled cortisol and ^{14}C-labelled cortisone was injected into the intact umbilical circulation at laparotomy, and the metabolites formed in the various tissues were analyzed.

EXPERIMENTAL

Clinical material

Two healthy volunteers who were admitted to hospital for termination of pregnancy for social and medical reasons (in one of them surgical sterilization was also carried out) participated in this study.

Permission for interruption of gestation was granted upon request of the patients by the Swedish National Board of Health and Welfare in accordance with the statute of 1938 amended in 1946 and 1963. The periods of gestation of the two patients were 18 and 20 weeks, respectively.

Injection of labelled material

Two experiments were carried out. In the first case a mixture of 31·8 μCi of cortisol-^{3}H (specific activity: 3 μCi/μg*) and 6·4 μCi of cortisone-^{14}C (specific activity: 0·066 μCi/μg‡) was injected. In the second case the doses were 12 μCi of cortisol-^{3}H and 2 μCi of cortisone-^{14}C. The radioactive material was dissolved in 1 ml of saline solution and injected into the umbilical vein according to the method of Mikhail *et al.*[8]. The foeto-placental circulation was maintained for 15 min after which the products of conception were removed and the radioactive material present in the placenta and different foetal tissues was analyzed.

Abbreviations and trivial names

Tetrahydrocortisol (3α,11β,17α,21-tetrahydroxy-5β-pregnan-20-one); *tetrahydrocortisone* (3α,17α,21-trihydroxy-5β-pregnane-11,20-dione); *20β-dihydrocortisol* (11β,17α,20β,21-tetrahydroxy-4-pregnen-3-one); *20β-dihydro-cortisone* (17α,20β,21-trihydroxy-4-pregnene-3,11-dione); *20β-hexahydrocortisol* (5β-pregnane-3α,11β,17α,20β,21-pentol); *20β-hexahydrocortisone* (3α,17α,20β,21-tetrahydroxy-5β-pregnan-11-one); *adrenosterone* (4-androstene-3,11,17-trione); *11β-hydroxy-androstenedione* (11β-hydroxy-4-androstene-3,17-dione); *11β-hydroxy-etiocholanolone* (3α,11β-dihydroxy-5β-androstan-17-one); *11-keto-etiocholanolone* (3α-hydroxy-5β-androstene-11,17-dione); *cortisol 21-sulphate* (11β-17α-dihydroxy-4-pregnen-3,20-dion-21-yl-sulphate); *cortisone 21-sulphate* (17α-hydroxy-4-pregnene-3,11,20-trion-21-yl-sulphate).

C: Crystals; *dpm*: disintegrations per minute; *M.L.*: mother liquors; *PPC*: Paper partition chromatography.

Extraction and isolation of metabolites

The extraction of radioactive material, the hydrolysis of the conjugates, the chemical transformations of the individual steroids (reduction, oxidation and acetylation) and the measurement of the radioactivity were the same as previously described[9]. The method of identification of the individual steroids is indicated in Fig. 1.

Chromatographic systems

The following solvent systems were used for chromatography:

(1) Chloroform/formamide,
(2) Chloroform, benzene, methanol, water (1 : 1 : 1 : 1),
(3) Ethyl-acetate, toluene, methanol, water (1 : 9 : 5 : 5),
(4) Benzene, methanol, water (2 : 1 : 1),
(5) Isooctane, t-butanol, water (10 : 5 : 9),
(6) Benzene/formamide,
(7) Toluene/propanediol,

*Purchased from C.E.A. (Mol-Donk, Belgium).
†Purchased from New England Nuclear Corp. (Chicago, U.S.A.).

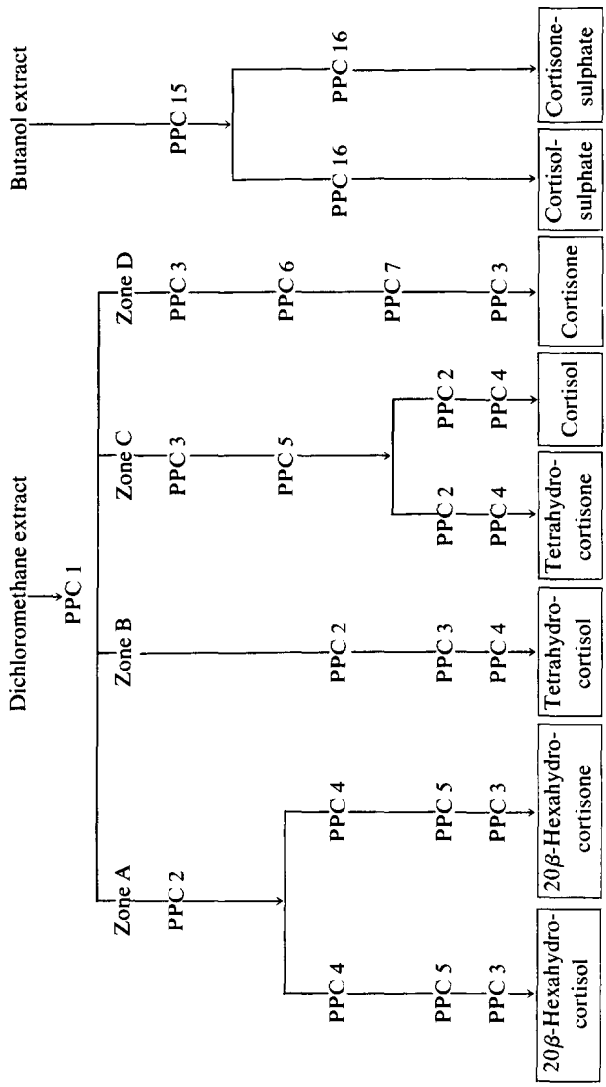

Fig. 1. Scheme of the method used for the isolation of the different cortisol and cortisone metabolites.

(8) Isooctane, toluene, methanol, water (7 : 1 : 4 : 4),
(9) Isooctane, toluene, methanol, water (3 : 1 : 2 : 2),
(10) Isooctane, toluene, methanol, water (1 : 1 : 1 : 1),
(11) Isooctane, toluene, methanol, water (8 : 2 : 5 : 5),
(12) Ligroin, toluene (1 : 1)/propanediol,
(13) Petroleum ether, benzene, methanol, water (33 : 17 : 40 : 10),
(14) Ligroin/propanediol,
(15) Butyl acetate, toluene, n-butanol, 4n NH_4OH, methanol (6 : 3 : 1 : 5 : 5),
(16) Butyl acetate, toluene, n-butanol, acetic acid, water, methanol (10 : 2 : 8 : 1 : 9 : 10).

RESULTS

Distribution of radioactive material

The distribution of the radioactive material recovered from the various sources is presented in Table 1.

Table 1. Distribution of radioactive material in the different foetal tissues, placenta and urine specimens after the simultaneous administration of cortisol-^{3}H and cortisone-^{14}C into the umbilical vein. (Values are expressed as percentage of the administered dose)

Tissues	Case I		Case II	
	^{3}H	^{14}C	^{3}H	^{14}C
Liver	24·3	27·1	27·0	25·2
Intestines	3·0	3·0	2·6	2·0
Adrenals	0·9	1·0	0·5	0·3
Kidneys	1·0	1·0	0·6	0·3
Lungs	1·2	1·5	0·8	0·5
Brain	8·3	9·2	—	—
Residual foetal tissues	25·5	26·4	25·4	26·0
Umbilical cord	0·5	0·9	1·0	0·9
Placenta	12·0	11·7	9·1	7·0
Total (tissues)	76·7	81·8	67·0	62·2
Urine				
1st day	5·0	3·2	3·0	2·6
2nd day	0·5	0·4	1·8	0·5
3rd day	0·1	0·1	0·5	traces
Total (urine)	5·6	3·7	5·3	3·1
Grand total	82·3	85·5	72·3	65·3

Most of the radioactive material was recovered from the liver and from the residual foetal tissues. The brain was analyzed separately only in one of the two cases: it contained a fairly high amount of radioactive material. The urinary excretion of the injected material, measured in urine collected over a period of 3 days, amounted to 3–6% of the administered dose.

The amount of conjugated radioactive material present in each tissue is shown in Table 2.

Table 2. Distribution of the conjugated radioactive material in the different foetal tissues and placenta after simultaneous perfusion of cortisol-^{3}H + cortisone-^{14}C. (Values are expressed as percentage of the total radioactivity recovered from each source)

Tissues	Case I		Case II	
	^{3}H	^{14}C	^{3}H	^{14}C
Liver	5	12	12	28
Intestines	7	18	15	13
Adrenals	15	25	18	29
Kidneys	3	2	1	2
Lungs	3	7	1	5
Brain	2	2	–	–
Residual foetal tissues	4	3	3	2
Placenta	2	4	9	8

The highest amounts of conjugated material in relation to the total radioactive material recovered were found in the adrenals, liver and intestines.

Identification of individual steroids

Cortisol. Part of the radioactive material eluted from PPC system No. 4 was acetylated. The product of acetylation had the same mobility as cortisone acetate when chromatographed in PPC systems Nos. 6, 7 and 9.

Another aliquot was oxidized with chromic acid; the product had the same mobility as authentic adrenosterone when chromatographed in systems 10 and 11. A third aliquot was mixed with cortisol and crystallized to constant S.A. The result is indicated in Table 3.

Tetrahydrocortisol. Part of the radioactive material eluted from PPC system No. 4 was acetylated. The product of acetylation exhibited the same mobility as tetrahydrocortisol-3,21-diacetate when subjected to PPC in systems Nos. 6, 7 and 14. Another aliquot was oxidized with sodium bismuthate. The product of oxidation had the same mobility as 11β-hydroxy-etiocholanolone when submitted to PPC in systems Nos. 12 and 14. A third aliquot was mixed with tetrahydrocortisol and crystallized; the result is indicated in Table 3.

Cortisone. Part of the fraction eluted from PPC No. 3 was acetylated. The product of acetylation had the same mobility as cortisone-21-acetate when subjected to PPC in systems Nos. 6, 7 and 10. Another aliquot was oxidized with chromic acid; the product of oxidation had the same mobility as authentic adrenosterone in system No. 12. A third aliquot was mixed with cortisone and crystallized (Table 3).

Tetrahydrocortisone. Part of the fraction eluted from PPC system No. 4 was acetylated. The product of acetylation had the same mobility as tetrahydrocortisone-3,21-diacetate when chromatographed in systems Nos. 8, 11, 12 and 13. Another aliquot was oxidized with chromic acid; the product of oxidation exhibited the same mobility as 11-keto-etiocholanolone in system N. 12. Another aliquot was crystallized with tetrahydrocortisone (Table 3).

20β-Hexahydrocortisol. Part of the fraction eluted from PPC system No. 3 was acetylated. The product of acetylation had the same mobility as 20β-hexahydrocortisol-3,20,21-triacetate when chromatographed in systems Nos. 12 and 14. Another aliquot was oxidized with sodium bismuthate. The product of oxidation had the same mobility as 11β-hydroxy-etiocholanolone when submitted to PPC in systems Nos. 11 and 12. Another aliquot was crystallized with authentic 20β-hexahydrocortisol (Table 3).

Table 3. Crystallization to constant specific activity (dpm/mg) of the various metabolites isolated

Steroids		C		M.L.	
		^3H	^{14}C	^3H	^{14}C
Cortisol					
(Liver)	a	3950	0	5500	0
	b	3780	0	3800	0
	c	3750	0	3600	0
(Intestines)	a	3300	0	4600	0
	b	3150	0	——	–†
	c	3250	0	3160	0
(Lung)	a	1900	0	3300	130
	b	1740	0	2100	0
	c	1600	0	1650	0
(Residual foetal tissues)	a	4750	0	6300	0
	b	4700	0	4850	0
Cortisone					
(Liver)	a	4810	1800	10100	5100
	b	5100	1760	4950	1800
(Residual foetal tissues)	a	1650	930	2600	1600
	b	1680	950	1690	940
Tetrahydrocortisol					
(Liver)	a	1800	0	2400	0
	b	1790	0	1700	0
Tetrahydrocortisone					
(Liver)	a	380	205	570	340
	b	410	195	405	200
20β-Hexahydrocortisol					
(Liver)	b	850	120	1250	600
	d	870	20	200	120
	a	885	0	890	40
20β-Hexahydrocortisone					
(Liver)	d	570	600	980	1350
	a	510	550	600	700
	b	505	540	520	570

a: Methanol; b: ethanol; c: ethyl acetate; d: methanol/ethyl acetate.
†Lost sample.

20β-Hexahydrocortisone. Part of the fraction eluted from PPC system No. 3 was acetylated. The product of acetylation showed the same mobility as 20β-hexahydrocortisone-3,20,21-triacetate when chromatographed in systems Nos. 12 and 14. Another part was oxidized with sodium bismuthate; the product of oxidation had the same mobility as 11-keto-etiocholanolone in systems Nos. 12 and 13. The result of crystallization with authentic 20β-hexahydrocortisone is indicated in Table 3.

Cortisol-sulphate- and cortisone-sulphate-like material. The conjugated radioactive material with the mobility of authentic cortisol- and cortisone-21-sulphates from system No. 16 was submitted to solvolysis and the liberated radioactivity was identified as cortisol and cortisone respectively.

Distribution of the isolated steroids

The distribution of the isolated steroids is presented in Table 4.

The greatest amount of tetrahydrocortisol was found in the liver, whereas large amounts of tetrahydrocortisone were present in the liver, intestines and residual foetal tissues. No reduction products were detected in the extracts of adrenals and placentas.

Isotopic ratios of the steroids isolated

These are presented in Table 5.

The data of Table 5 indicate that with the exception of the cortisol isolated from the placentas, the cortisol, tetrahydrocortisol and hexahydrocortisol isolated from all foetal tissues did not carry any ^{14}C label. On the other hand, the cortisone and tetrahydrocortisone isolated from all sources contained large quantities of the ^{3}H label.

DISCUSSION

The data presented in this study indicate that only a small part of the cortisol and cortisone injected into the umbilical circulation was transferred to the mother. It is of interest to note that Migeon *et al.*[10] found significant amounts of radioactive material in the foetal tissues following the administration of labelled cortisol to the mother. Thus the relative importance of transplacental transfer of circulating corticosteroids from mother to foetus and vice versa remains to be clarified.

The extent of conjugation of radioactive material in the tissues studied was rather limited, and most of the radioactive material in the conjugated fraction of the liver and adrenals was identified after solvolysis as unchanged cortisol and cortisone respectively. This is in contrast to our earlier findings on the metabolism of corticosterone and deoxycorticosterone[11,12]. The amount of tetrahydrocortisone in all tissues exceeded that of tetrahydrocortisol. This seems to suggest that the reduction in ring A is facilitated by the presence of an 11-oxo function, in contrast to the presence of an 11β-hydroxyl group. Furthermore, no reduction products were detected in the adrenals or placentas. This is in agreement with the foeto-placental metabolism of other biologically active steroids, such as testosterone[13].

The isotopic ratios of the isolated steroids revealed a very definite trend in the foeto-placental metabolism of cortisol and cortisone. The cortisol, as well as all the reduction products formed from it which were isolated from the foetal tissues, did not contain any ^{14}C label, indicating that no conversion of cortisone

Table 4. Distribution of the different steroids isolated after simultaneous administration of cortisol-^{3}H + cortisone-^{14}C into the umbilical vein at mid-pregnancy
(Values are percentages of the total radioactive material recovered from each source)

Steroids	Liver				Intestines				Lungs				Adrenals				Residual foetal tissues				Placenta			
	^{3}H		^{14}C		^{3}H		^{14}C		^{3}H		^{14}G		^{3}H		^{14}C		^{3}H		^{14}C		^{3}H		^{14}C	
Cases	I	II	I	II	I	II	I	II	I	II	I	II	I	II	I	II	I	II	I	II	I	II	I	II
Cortisol	25·0	16·0	0	0	62	45	0	0	28	22	0	0	45	42	0	0	40	55·0	0	0	2·5	5	8	1·5
Tetrahydrocortisol	12	8	0	0	6	12	0	0	3	1	0	0	–	–	–	–	3	5·5	0	0	–	–	–	–
20β-Hexahydrocortisol	16	20	0	0	2	4	0	0	–	–	–	–	–	–	–	–	1	0·5	0	0	–	–	–	–
Cortisone	9	5	18	25	12	6	50	45	25	8	35	21	6	11	15	25	12	3·0	38	22	12·0	25	14	16·0
Tetrahydrocortisone	13	6	28	8	3	4	23	15	4	2	8	6	–	–	–	–	8	5·0	10	8	–	–	–	–
20β-Hexahydrocortisone	2	1	12	5	–	–	–	–	–	–	–	–	–	–	–	–	1	2·0	4	2	–	–	–	–
Cortisol-sulfate	2	4	0	0	–	–	–	–	–	–	–	–	6	10	0	0	–	–	–	–	–	–	–	–
Cortisone-sulfate	1	1	5	6	–	–	–	–	–	–	–	–	1	3	12	10	–	–	–	–	–	–	–	–

– Not studied or insufficient material.

Table 5. Isotopic ratios ($^{14}C/^{3}H$) of the steroids isolated from the placenta and various foetal tissues. (Injected $^{14}C/^{3}H$: Case I: 0·20 – Case II: 0·16)

Source	Steroids											
	Cortisol		Tetrahydro-cortisol		20β-Hexahydro-cortisol		Cortisone		Tetrahydro-cortisone		20β-Hexahydro-cortisone	
Cases	I	II	I	II	I	II	I	II	I	II	I	II
Liver	0	0	0	0	0	0	0·4	0·8	0·4	0·2	1·2	0·8
Intestines	0	0	0	0	0	0	0·8	1·2	1·6	0·6	–	–
Lungs	0	0	0	0	–	–	0·3	0·4	0·4	0·5	–	–
Adrenals	0	0	–	–	–	–	0·5	0·4	–	–	–	–
Residual foetal tissues	0	0	0	0	0	0	0·6	1·1	0·3	0·25	0·8	0·16
Placenta	0·64	0·05	–	–	–	–	0·2	0·1	–	–	–	–

– Not studied or insufficient material.

to cortisol took place in the foetal organism. On the other hand, in all foetal tissues there was an extensive conversion of cortisol to cortisone. It appears therefore that the foetus converts major quantities of the biologically active cortisol to the biologically inactive cortisone but is not able to carry out the opposite reaction. On the other hand, the cortisol isolated from the placenta contained both ^{3}H and ^{14}C labels, suggesting that in this tissue there is a significant interconversion of cortisol and cortisone. However, since the cortisol isolated from all foetal tissues and its reduction products did not contain any ^{14}C label, the conclusion seems to be justified that little if any of the cortisol formed in the placenta from cortisone was secreted to the foetal compartment.

It remains to be established how much of the cortisol produced by the foetal adrenals is synthesized from placental precursors and how much is formed by *de novo* processes from acetate. Furthermore, in view of the likely role of foetal cortisol in the initiation of labour, it would seem important to estimate the amount of cortisol secreted by the foetus at various stages of gestation.

Thus the foetal metabolism of cortisol differs significantly from that taking place in adults, where there is an extensive interconversion of cortisol and cortisone [14]. It is relevant to note that the metabolism of cortisol and cortisone in guinea pig foetuses is very similar to that obtained in the present study [15].

The results of the present investigation can be integrated into a scheme describing the metabolism of cortisol and cortisone in the foeto-placental unit at midgestation. This scheme is presented in Fig. 2.

Placenta	Foetus			
Cortisol ⇌	Cortisol	→ Tetrahydrocortisol	→ 20β-Hexahydrocortisol	
⇵	↓	↓	↓	
Cortisone ⇌	Cortisone	→ Tetrahydrocortisone	→ 20β-Hexahydrocortisone	

→ Established pathway.
⇢ Suggested pathway.

Fig. 2. Metabolism of cortisol and cortisone in the foeto-placental unit at midgestation.

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