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Research Article

During the third trimester of human pregnancy the concentrations of deoxycorticosterone (DOC) in maternal plasma are 4-50 times those in nonpregnant women and men. It has been suggested that the increased amount of DOC in maternal plasma originates in the fetal compartment. We considered an alternate explanation for the high levels of DOC in plasma or near-term pregnant women, viz., that DOC may be derived in part from 21-hydroxylation of maternal plama progesterone. To test this hyposthesis we measured the fractional conversion of plasma progesterone to DOC from the relationship between the 3H:14C ratio of the infused tracers, [3H]progesterone and [14C]-DOC, and the 3H:14C ratio or urinary 3 alpha,21-dihydroxy-5 beta-pregnan-20-one (tetrahydro-DOC). The fractional conversion of plasma progesterone to DOC ([rho](BU)P-DOC), measured in this manner, was 0.007 +/- 0.001 (mean +/- SEM, n = 26) in the subjects of this study. The values for [rho](BU)P-DOC varied widely among subjects (0.002-0.022) but the range of values for [rho](BU)P-DOC was similar among women pregnant with an anencephalic or dead fetus, nonpregnant and adrenalectomized women, and men. The transfer constant of conversion of progesterone to DOC in plasma, [rho](BB)P-DOC, remained constant in a nonpregnant woman during the infusion of nonradiolabeled progesterone at rates of 0-14 mg/h. Based on the results of these studied, we conclude that DOC is formed by extra-adrenal 21-hydroxylation of plasma […]

Conversion of Plasma Progesterone to Deoxycorticosterone in Men, Nonpregnant and Pregnant Women, and Adrenalectomized Subjects

EVIDENCE FOR STEROID 21-HYDROXYLASE ACTIVITY IN NONADRENAL TISSUES

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ABSTRACT During the third trimester of human pregnancy the concentrations of deoxycorticosterone (DOC) in maternal plasma are 4–50 times those in nonpregnant women and men. It has been suggested that the increased amount of DOC in maternal plasma originates in the fetal compartment. We considered an alternate explanation for the high levels of DOC in plasma of near-term pregnant women, viz., that DOC may be derived in part from 21-hydroxylation of maternal plasma progesterone. To test this hypothesis we measured the fractional conversion of plasma progesterone to DOC from the relationship between the ^{3}H:^{14}C ratio of the infused tracers, [^{3}H]progesterone and [^{14}C]-DOC, and the ^{3}H:^{14}C ratio of urinary 3α,21-dihydroxy-5β-pregnan-20-one (tetrahydro-DOC). The fractional conversion of plasma progesterone to DOC ($[\rho]_{BU}^{P-DOC}$), measured in this manner, was 0.007±0.001 (mean ±SEM, n = 26) in the subjects of this study. The values for $[\rho]_{BU}^{P-DOC}$ varied widely among subjects (0.002–0.022) but the range of values for $[\rho]_{BU}^{P-DOC}$ was similar among women pregnant with an anencephalic or dead fetus, nonpregnant and adrenalectomized women, and men. The transfer constant of conversion of progesterone to DOC in plasma, $[\rho]_{BB}^{P-DOC}$, remained constant in a nonpregnant woman during the infusion of nonradiolabeled progesterone at rates of 0–14 mg/h. Based on the results of these studies, we conclude that DOC is formed by extra-adrenal 21-hydroxylation of plasma progesterone and that the rate of formation of DOC by this pathway is proportional to the concentration of progesterone in plasma.

INTRODUCTION

In 1968, Sjövall and Sjövall (1) reported the finding of large quantities of 5α-pregnane-3α,20α,21-triol as the monosulfate in plasma obtained from pregnant women in the third trimester. Since then, several groups of investigators (2–7) including ourselves (8) have found that the levels of deoxycorticosterone (DOC)[1] in plasma of women late in pregnancy are 4–50 times those found in nonpregnant women and men. The mechanism(s) leading to the elevated DOC levels in the plasma of women late in pregnancy, however, is not established. The increased plasma levels of DOC appear to be due to increased production in that the production rate of DOC in one pregnant subject was 8.1 mg/24 h (2). Brown and Strott (9) found that the intravenous infusion of angiotensin II was not accompanied by an increase in plasma DOC concentrations; thus, it is unlikely that the pregnancy-induced increase in the levels of angiotensin II in plasma brings about an increase in adrenal secretion of DOC.

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[1] *Abbreviations used in this paper*: DOC, deoxycorticosterone; MCR-P_{DOC}, clearance of plasma of its progesterone content by DOC formation; $[\rho]_{BU}^{P-DOC}$, transfer constant of conversion of plasma progesterone to DOC; $[\rho]_{BB}^{P-DOC}$, transfer constant of conversion of progesterone to DOC in plasma; [^{3}H]P, N-[^{3}H]progesterone; tetrahydro-DOC, 3α,21-dihydroxy-5β-pregnan-20-one; TLC, thin-layer chromatography.

Most investigators have surmised that the high level of DOC in the maternal circulation is the result of the transfer to the mother of DOC produced in the fetus or fetal-placental unit. Their view was based on the findings that the levels of DOC and DOC-sulfate in umbilical cord plasma were higher than those in maternal plasma (2, 6, 10, 11) and that within 16–24 h after delivery, maternal plasma levels of DOC fell to levels found in nonpregnant women and men (2, 6). Importantly, it was also found that treatment of near-term pregnant women with dexamethasone or ACTH did not lead to significant changes in plasma DOC concentrations (2, 4, 5, 7, 12). However in pregnant women near term, urinary 3α,21-dihydroxy-5β-pregnan-20-one (tetrahydro-DOC) excretion was increased by ACTH-treatment (12). Nolten et al. (7) concluded that these results could be explained as follows: ACTH-treatment of pregnant women was associated with an increase in plasma cortisol concentration. They believed that DOC secretion was also increased after ACTH treatment but that the ACTH-induced increase in cortisol levels in plasma brought about a proportionate displacement of DOC from corticosteroid-binding globulin, resulting in an elevation in free DOC but little change in the total concentration of DOC in plasma.

We considered an alternate hypothesis to explain the finding of high levels of DOC in plasma of women late in pregnancy, viz., that DOC may be derived, in part, from the 21-hydroxylation of circulating progesterone. There are many precedents for the formation of biologically active steroid hormones from circulating precursors, e.g., the placental formation of 17β-estradiol from circulating maternal and fetal dehydroisoandrosterone sulfate (13–15), the extraglandular formation of testosterone (16) and estrone (17) from plasma androstenedione, as well as the formation of 17β-estradiol from plasma testosterone (18) and from plasma estrone (19).

During late human pregnancy, 250 mg or more of progesterone enter the maternal circulation each day (20, 21). Thus, even a small fractional conversion of maternal plasma progesterone to DOC could account for considerable DOC production compared to that normally secreted by the adrenal cortex. Moreover, the conversion of circulating progesterone to DOC would provide an explanation for the finding that neither dexamethasone nor ACTH treatment of pregnant women was followed by significant alterations in the levels of DOC in maternal plasma. To test the possibility that plasma progesterone is converted to DOC, we estimated the fractional conversion of plasma progesterone to DOC by the "urinary method." The transfer constant of conversion of plasma progesterone to DOC was computed from the relationship between the ^{3}H:^{14}C ratio of the infused tracers, [^{3}H]progesterone and [^{14}C]DOC, and the ^{3}H:^{14}C ratio of urinary tetrahydro-DOC in pregnant, nonpregnant, and adrenalectomized women, in men, and in a man with Cushing syndrome. The fractional conversion of plasma progesterone to DOC, measured in this manner, was similar among women pregnant with an anencephalic or dead fetus, nonpregnant women, nonpregnant adrenalectomized women, and men.

METHODS

Tracer purification and administration. The tracers, *N*-[7-^{3}H]progesterone ([^{3}H]P, 24 Ci/mmol) and [4-^{14}C]deoxycorticosterone ([^{14}C]DOC, 40 mCi/nmol) were purified by column chromatography on celite (22).

To evaluate the fractional conversion of progesterone to DOC in plasma, i.e., $[\rho]^{P-DOC}_{BB}$, two pregnant subjects (S-4A and S-5A, each of whom was pregnant with a dead fetus) were infused intravenously with ~173 μCi [^{3}H]P plus ~1.0 μCi [^{14}C]DOC at a constant rate for 4 h. 50-ml blood samples were obtained after 3 h 30 min, 3 h 45 min, and 4 h of infusion of radiolabeled steroids. Urine was collected for 72 h.

To ascertain whether wide fluctuations in plasma progesterone levels would result in variations in $[\rho]^{P-DOC}_{BB}$, the following study was conducted. 500 ml of blood were withdrawn from a 45-yr-old nonpregnant woman volunteer (subject S-14); the plasma (300 ml) was separated by plasmapheresis, and the erythrocytes were infused back into this woman before commencement of the study. Nonradiolabeled progesterone was dissolved in the plasma as described (23) to give a final concentration of 123 μg progesterone/ml plasma. The tracers, [^{3}H]P (~190 μCi) plus [^{14}C]DOC (~1.0 μCi), were infused intravenously into this subject at a constant rate for 6 h. After 3 h of radiolabeled steroid tracer infusion, a 50-ml sample of blood was withdrawn from the contralateral arm, and a second infusion consisting of her plasma containing nonradiolabeled progesterone was begun at a rate to deliver 2.5 mg progesterone/h; an amount similar to the estimated production rate of progesterone in early pregnancy. After 1 h of progesterone infusion, another 50-ml blood sample was obtained, and the rate of infusion of nonradiolabeled progesterone was increased to 10 mg/h; an amount similar to the estimated production rate of progesterone in the early third trimester of pregnancy. After 1 h, another 50-ml blood sample was obtained, and the rate of infusion of nonradiolabeled progesterone was increased to 14 mg/h; an amount similar to the estimated production rate of progesterone in pregnant women near term. This rate of progesterone infusion was continued for 1 h, a final 50-ml blood sample was obtained, and all infusions were discontinued. Urine was collected for 72 h. The experimental design employed in this study is illustrated in Fig. 1. The ^{3}H:^{14}C ratio of DOC in each of the

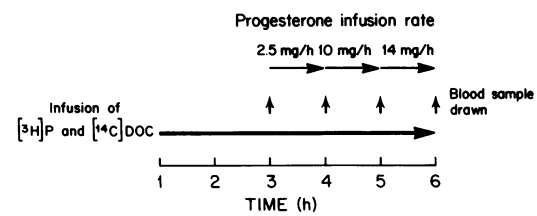

FIGURE 1 Experimental design employed to evaluate the effect of variations in plasma progesterone concentration on the relationship between $[\rho]^{P-DOC}_{BB}$ and $[\rho]^{P-DOC}_{BU}$.

plasma samples was determined, as was the ^{3}H:^{14}C ratio of urinary tetrahydro-DOC.

Purification of urinary tetrahydro-DOC. Urine samples were adjusted to pH 5.0, treated with β-glucuronidase (250,000 U/liter), and incubated for 3 d at room temperature. The liberated steroids were extracted with ethyl acetate. The solvent was evaporated and the residue was chromatographed by gradient elution column chromatography on ethylene glycol-celite (22). The fractions of this chromatogram containing tetrahydro-DOC were combined, the solvent was evaporated, and the residue was chromatographed by partition chromatography on celite employing the solvent system isooctane:*t*-butanol:methanol:water (25:10:8:7). The isolated tetrahydro-DOC was purified further by thin-layer chromatography (TLC) on silica gel G employing the solvent system methylene chloride:diethyl ether (7:3, vol/vol). The steroid(s) on the area of this chromatogram that comigrated with authentic tetrahydro-DOC was purified further. In some studies, an aliquot of the isolated tetrahydro-DOC was purified by high performance liquid chromatography. A liquid chromatograph (Waters Associates, Inc., Milford, Mass., model ALC-202/401) equipped with a refractive index and an ultraviolet detector was employed. A column (30-cm long by 4 mm Diam) containing μBondapak-C_{18} support (Waters Associates, Inc.) was employed for chromatographic separation. A mixture of methanol/water (7:3, vol/vol), at a flow rate of 2 ml/min, was used for elution in the isocratic mode. Tetrahydro-DOC was separated distinctly from other urinary C_{21}-steroid metabolites of progesterone (Table I).

An aliquot of the purified tetrahydro-DOC obtained after TLC or high performance liquid chromatography was treated with a mixture of pyridine and acetic anhydride (1:1, vol/vol) for 2 h at 37°C. The tetrahydro-DOC diacetate formed was purified by TLC using the solvent system isooctane:ethyl acetate (1:1, vol/vol). The tetrahydro-DOC diacetate was eluted from the chromatogram, mixed with authentic nonradioactive tetrahydro-DOC diacetate (15 mg) and crystallized to a constant ^{3}H:^{14}C ratio employing the solvent system pair diethyl ether-petroleum ether (20°–40°C).

Purification of plasma DOC. The blood samples were separated into plasma and erythrocyte fractions by centrifugation at 2,500 rpm for 10 min. 25 ml of plasma were transferred to culture tubes containing 1.0 mg DOC and 200 μg progesterone as recovery markers. The plasma samples were extracted first with 2 vol of petroleum ether and then with 2 vol of methylene chloride. The extracts were combined, washed once with water, and evaporated to dryness. The residue was chromatographed by TLC on silica gel G using the solvent system methylene chloride:diethyl ether (9:1, vol/vol). The region of the chromatogram containing DOC was localized by ultraviolet absorption. The isolated DOC was treated with a mixture of pyridine and acetic anhydride (1:1, vol/vol) for 2 h at 37°C. The DOC acetate formed was purified by TLC using the solvent system isooctane:ethyl acetate (1:1, vol/vol). The isolated DOC acetate was mixed with authentic nonradiolabeled DOC acetate (40 mg) and crystallized to constant ^{3}H:^{14}C ratios.

Measurement of progesterone and DOC in plasma. For the measurement of progesterone, plasma samples were mixed with 1,000 cpm of [^{3}H]P, 24 Ci/mmol, and extracted with 10 vol petroleum ether (30°–60°C). The petroleum ether phase was removed, evaporated, and the residue was chromatographed on celite-propylene glycol (1:1.25, wt/vol) columns; the fractions containing progesterone were collected and assayed by radioimmunoassay (24). For the measurement of DOC, plasma samples were mixed with 1,000 cpm of *N*-[1,2-^{3}H]DOC (46.8 Ci/mmol) and extracted with 10 vol diethyl ether. The organic phase was removed, evaporated, and the residue was chromatographed on celite-ethylene glycol (1:0.75, wt/vol) columns. The columns were treated sequentially with 5 ml each of isooctane and isooctane: ethyl acetate (92.5:7.5, vol/vol). The last 4 ml of the eluate, after applying the second solvent system, contained DOC which was quantified by radioimmunoassay. The antiserum (25) used in the DOC assay was generously supplied by Dr. C. E. Gomez-Sanchez, Department of Medicine, University of Texas Southwestern Medical School, Dallas, Texas. The antibody did not cross-react with the following steroids: cortisol, pregnenolone, 17α-hydroxyprogesterone, 11-dehydrocorticosterone, corticosterone, testosterone, 18-hydroxy-DOC, tetrahydro-DOC, or 17α-hydroxypregnenolone. The antiserum cross-reacted slightly with 17,21-dihydroxy-4-pregnene-3, 20-dione (3.3%) and 21-hydroxypregnenolone (3%). The only compound that cross-reacted significantly with the antiserum, other than DOC (100%), was progesterone (50%), which was separated from DOC during chromatographic isolation of DOC from the plasma extracts. The radioimmunoassay of DOC was conducted as follows. The column fraction containing DOC was evaporated and reconstituted in 0.1 M phosphate buffer–0.1% gelatin (assay buffer). DOC standards, in concentrations ranging from 3 to 400 pg/0.4 ml, were prepared similarly in assay buffer.

TABLE I
Retention Times of C_{21}-Steroids on High Performance Liquid Chromatography

Steroid	Retention time
	min
3α,21-Dihydroxy-5β-pregnan-20-one	5.04
5β-Pregnane-3,20-dione	7.64
5β-Pregnane-3α,20α-diol	9.84
5β-Pregnane-3β,20α-diol	7.60
3β-Hydroxy-5β-pregnan-20-one	8.40
3α-Hydroxy-5β-pregnan-20-one	9.08
20α-Hydroxy-5β-pregnan-3-one	8.48
3α,11β,21-Trihydroxy-5β-pregnan-20-one	2.80
5β-Pregnane-3α,17,20α-triol	7.56
3β,17-Dihydroxy-5β-pregnan-20-one	4.24
11β,17,21-Trihydroxy-5β-pregnane-3,20-dione	2.36
3α,11β,17,21-Tetrahydroxy-5β-pregnan-20-one	2.40
3α,17,21-triol-5β-pregnane-11,20-dione	2.48
5α-Pregnane-3,20-dione	8.04
5α-Pregnane-3β,20α-diol	7.72
5α-Pregnane-3α,20α-diol	11.70
20α-Hydroxy-5α-pregnan-3-one	7.88
20β-Hydroxy-5α-pregnan-3-one	10.00
3β-Hydroxy-5α-pregnan-20-one	9.00
3α-Hydroxy-5α-pregnan-20-one	9.80
3β,17-Dihydroxy-5α-pregnan-20-one	3.88
3α,11β,17,21-Tetrahydroxy-5α-pregnan-20-one	2.40
4-Pregnene-3,20-dione	4.96
21-Hydroxy-4-pregnene-3,20-dione	3.12
20α-Hydroxy-4-pregnen-3-one	6.60
20β-Hydroxy-4-pregnen-3-one	9.28
17,20α-Dihydroxy-4-pregnen-3-one	3.40
17,20β-Dihydroxy-4-pregnen-3-one	3.64
17,21-Dihydroxy-4-pregnen-3,20-dione	2.44

Standards and unknowns (the equivalent of 25% of the plasma extract) were incubated in duplicate with antiserum (diluted 1:5,000 in assay buffer) and 5,000 cpm [^{3}H]DOC. After an overnight incubation at 4°C, dextran-coated charcoal suspension (0.2 ml) was added. The tubes were agitated and incubated for 10 min at 4°C, and then centrifuged at 2,000 rpm for 10 min at 4°C. The supernatant fluid (containing antibody-bound steroid) was assayed for radioactivity in a liquid scintillation spectrometer. The concentration of DOC in unknown samples was computed and the values were corrected for procedural losses by the use of the internal recovery standard. The sensitivity of the method is 6 pg and the least amount of DOC detectable in the assay method, assuming 100% recovery of DOC during extraction and chromatography, is 24 pg DOC/ml plasma. To evaluate the accuracy of the method, known quantities of nonradiolabeled DOC (100, 200, 300, or 400 pg) were added to aliquots (in triplicate) of a plasma pool, containing 103 pg DOC/ml, which were then assayed for DOC as described above. The differences in DOC concentrations between the plasma pool with no DOC added and that in samples to which DOC was added were 99, 197, 295, and 393 pg, respectively. Within assay and between assay variations for the DOC radioimmunoassay procedure were 7 and 17%, respectively.

RESULTS

Transfer constant of conversion of plasma progesterone to DOC $[\rho]_{BU}^{P-DOC}$. Tetrahydro-DOC was isolated from the urine of each subject. The ^{3}H:^{14}C ratios of this metabolite, determined after each successive purification step (three subjects) after the initial gradient elution chromatogram, are presented in Table II. The ^{3}H:^{14}C ratio of tetrahydro-DOC changed little following the first three chromatographic procedures. Therefore, in subsequent studies urinary tetrahydro-DOC was purified as follows: gradient elution chromatography on ethylene glycol-celite, liquid-liquid partition chromatography on celite, TLC, acetylation, TLC of the diacetate, and crystallization. The amount of radiolabeled urinary tetrahydro-DOC recovered after chromatographic purification and the ^{3}H:^{14}C ratios after crystallization are presented in Table III.

The computed $[\rho]_{BU}^{P-DOC}$ in the subjects of this study, as well as the plasma levels of progesterone and DOC in the subjects pregnant with an anencephalic or dead fetus, are presented in Table IV. The transfer constant of conversion of plasma progesterone to DOC in these subjects was 0.007±0.001 (mean±SEM). The measurement of $[\rho]_{BU}^{P-DOC}$ was performed on two separate occasions in two subjects, S-4 and S-5. At the time of the first study (A) subject 4 was pregnant with a dead fetus at 20 wk gestation and subject 5 was pregnant with a dead fetus at 32 wk gestation. At the time of the second study (B) each woman was more than three months postpartum. In each subject, the values for $[\rho]_{BU}^{P-DOC}$ were similar in the two separate studies (Table V). The $[\rho]_{BU}^{P-DOC}$ values in three women pregnant with an anencephalic fetus (subjects S-1, S-6, and S-7) were similar to the mean $[\rho]_{BU}^{P-DOC}$ observed in all subjects. Therefore it is unlikely that a significant amount of maternal plasma progesterone is converted to DOC either in the fetus or placenta.

The values for $[\rho]_{BU}^{P-DOC}$ found in the two nonpregnant women who had sustained bilateral adrenalectomy were 0.003 and 0.007. The lowest value for $[\rho]_{BU}^{P-DOC}$ (0.002) was found in subject S-26, a 37-yr-old man with Cushing syndrome.

Transfer constant of conversion of progesterone to DOC in plasma $[\rho]_{BB}^{P-DOC}$. In two pregnant subjects (S-4A and S-5A) the $[\rho]_{BB}^{P-DOC}$ was evaluated by determining the ^{3}H:^{14}C ratio of DOC in plasma during the intravenous infusion of [^{3}H]P and [^{14}C]DOC. In these experiments the plasma samples obtained during the infusion were combined before the purification of radiolabeled DOC. The concentrations of radiolabeled DOC were computed and found to be, [^{3}H]DOC

TABLE II

Establishment of Radiochemical Homogeneity of Radiolabeled Tetrahydro-DOC Isolated from Urine. ^{3}H:^{14}C Ratios After Each Purification Procedure

	Chromatographic steps				Crystallizations					
Subject	Column chromatography on celite	TLC_1	HPLC	TLC_2	ML_1	ML_2	ML_3	ML_4	ML_5	Final crystals
S-13	3.60	3.24	2.71	3.15	3.17	2.91	2.86	2.65	2.97	2.79
S-15	1.14	0.96	0.93	0.93	0.94	0.93	1.03	0.90		0.85
S-16		2.74	2.57	2.55	2.54	2.44	2.26	2.67		2.07

Abbreviations used in this table: TLC_1, TLC of tetrahydro-DOC; TLC_2, TLC of tetrahydro-DOC diacetate; HPLC, high performance liquid chromatography; ML, mother liquor.

The ^{3}H:^{14}C ratios after gradient elution chromatography on celite, the first chromatographic step, are not shown. At this stage of purification, the tetrahydro-DOC was contaminated by [^{3}H]pregnanediol which was separated from tetrahydro-DOC during the second column chromatographic procedure.

TABLE III

Purification of Radiolabeled Tetrahydro-DOC From Urine after the Intravenous Administration of [^{3}H]P and [^{14}C]DOC

Subject	Radiolabeled tetrahydro-DOC recovered after purification by multiple chromatographic procedures			^{3}H:^{14}C Ratios after each crystallization						
	[^{3}H]	[^{14}C]	^{3}H:^{14}C ratio	ML_1	ML_2	ML_3	ML_4	ML_5	ML_6	Final crystals
	dpm									
1	96	40	2.40							
2	5,400	1,960	2.76	5.30	1.88	1.33	0.76	0.89	0.70	0.65
3	10,770	1,600	6.73	7.25	6.92	6.86				6.88
4A	34,630	22,200	1.56	1.74	1.64	1.54				1.56
4B	15,667	6,778	2.31	3.29	2.01	1.75	1.71			1.73
5A	23,760	14,400	1.65	2.14	1.37	0.70	0.63	1.11		
5B	6,000	4,676	1.28	1.33	1.12	1.11	1.41			1.09
6	2,842	880	3.23	2.88	2.39	2.28				2.24
7	13,740	16,360	0.84	0.86	0.80	0.79				0.78
8	6,550	460	14.24	15.16	15.34	15.47				14.25
9	4,960	3,220	1.54	1.71	1.55	1.53				1.49
10	1,480	440	3.36	2.92	2.72	2.87	1.80			1.77
11	14,230	1,560	9.12	8.09	7.64	7.79				7.72
12	4,130	620	6.66	2.81	1.36	1.35	1.68			1.13
13	2,110	670	3.15	3.17	2.91	2.86	2.65	2.97		2.79
14	8.760	17,520	0.50	0.49						0.50
15	1,190	1,200	0.99	0.94	0.93	1.03				0.85
16	1,910	750	2.55	2.04	2.44	2.26	2.67			2.07
17	52,030	5,420	9.60	33.93	7.27	4.34	3.26	3.26		3.07
18	24,000	7,500	3.20	4.99	3.73	3.53				3.55
19	7,110	1,100	6.46	6.45	7.70	7.19				6.41
20	3,913	2,248	1.74	1.40	0.92	0.93	1.02			0.99
21	6,800	4,148	1.64	0.94	0.69	0.84				0.82
22	11,600	5,630	2.06	0.97	0.82	1.01				0.82
23	5,817	3,472	1.67	1.24	1.01	1.00				0.99
24	9,330	4,300	2.17	2.38	2.14	2.21				2.12
25	9.210	3,760	2.45	2.36	2.26	2.24	2.27			2.19
26	4,730	6,660	0.71	0.71	0.69	0.68	0.66			0.65

Abbreviation used in this table: ML, mother liquor.

= 3.9×10^3 dpm/liter and [^{14}C]DOC = 6.4×10^3 dpm/liter for subject S-4A; and [^{3}H]DOC = 7.2×10^3 dpm/liter and [^{14}C]DOC = 7.7×10^3 dpm/liter for subject S-5A. In subject S-4A, the $[\rho]_{BB}^{P-DOC}$ was 0.004, whereas the $[\rho]_{BU}^{P-DOC}$ was 0.009. In subject S-5A, the ^{3}H:^{14}C ratio of DOC in plasma was somewhat greater than the ^{3}H:^{14}C ratio of urinary tetrahydro-DOC; therefore, the computed $[\rho]_{BU}^{P-DOC}$ in this subject, 0.004, was somewhat less than that computed for $[\rho]_{BB}^{P-DOC}$, viz., 0.006.

In subject S-14 [^{3}H]P and [^{14}C]DOC were infused intravenously at a constant rate for 6 h, while nonradiolabeled progesterone was infused at various rates (0–14 mg/h). The ^{3}H:^{14}C ratio of plasma DOC, and thus the $[\rho]_{BB}^{P-DOC}$, remained reasonably constant during the 3rd–6th h of tracer infusion, irrespective of the rate of infusion of nonradiolabeled progesterone (Table V). Additionally, in this subject $[\rho]_{BB}^{P-DOC}$ and $[\rho]_{BU}^{P-DOC}$ were similar (0.003). The levels of progesterone in the plasma of this subject during infusion of nonradiolabeled progesterone (Table V) were similar to those which should have been attained if the metabolic clearance rate of plasma progesterone in this woman were 2,500 liters/24 h. The plasma levels of DOC also rose during the infusion of progesterone; however, we cannot ascertain if steady-state conditions existed between the rate of progesterone infusion and the levels of progesterone and DOC in plasma since the rate of progesterone infusion was changed hourly.

DISCUSSION

The conversion of intravenously infused [^{3}H]P to urinary [^{3}H]tetrahydro-DOC was demonstrated in 26 subjects. The $[\rho]_{BU}^{P-DOC}$ computed from the ^{3}H:^{14}C ratio of urinary tetrahydro-DOC and that of the infused

TABLE IV

Transfer Constants of Conversion of Plasma Progesterone to DOC and Plasma Concentrations of DOC and Progesterone in the Subjects Pregnant with Anencephalic or Dead Fetus

Subject	Age	Sex	Race	Gestation (if pregnant)	Medical complications	Plasma concentrations		$[\rho]_{BU}^{P-DOC}$
						DOC	Progesterone	
	yr			*wk*		*ng/ml*		
1	28	Female	B	32	Anencephalic fetus	0.23	67	0.007
2	26	Female	B	6 postpartum				0.003
3	23	Female	W	6 postpartum				0.022
4A	22	Female	MA	20	Fetal demise	0.16	6	0.009
4B				Nonpregnant				0.009
5A	28	Female	B	32	Fetal demise	0.21	22	0.004
5B				Nonpregnant				0.005
6	23	Female	W	28	Anencephalic fetus	0.19	61	0.007
7	24	Female	MA	40	Anencephalic fetus	0.17	70	0.004
8	20	Female	B	Immediately postpartum	None			0.019
9	30	Female	MA	Immediately postpartum	None			0.003
10	21	Female	MA	Immediately postpartum	None			0.005
11	23	Female	MA	Immediately postpartum	None			0.016
12	35	Female	W	Nonpregmant	None			0.005
13	45	Female	W	Nonpregnant	None			0.010
14	45	Female	W	Nonpregnant	None			0.003
15	30	Female	W	Nonpregnant	Adrenalectomized			0.003
16	55	Female	W	Nonpregnant	Adrenalectomized			0.007
17	42	Female	W	Nonpregnant	None			0.008
18	25	Female	W	Nonpregnant	None			0.007
19	25	Female	W	Nonpregnant	None			0.015
20	28	Female	W	Nonpregnant	None			0.005
21	30	Female	W	Nonpregnant	None			0.003
22	26	Female	W	Nonpregnant	None			0.004
23	28	Female	W	Nonpregnant	None			0.004
24	40	Male	W	—	None			0.009
25	32	Male	W	—	None			0.009
26	32	Male	W	—	Cushing syndrome			0.002
								* Mean = 0.007
								SEM = 0.001
								SD = 0.005

Abbreviations used in this table: B, black; W, white; MA, Mexican-American; PIH, pregnancy-induced hypertension.

* In computing the mean, the averages of the values for subjects 4 and 5 were used.

tracers, [^{3}H]P and [^{14}C]DOC, in the subjects of this study was 0.007±0.001 (mean±SEM). Two subjects were studied on two occasions. The first study was conducted when they were pregnant with a dead fetus and the second study was conducted when they were more than 3 mo postpartum. The $[\rho]_{BU}^{P-DOC}$ was the same in each subject during and after pregnancy. These findings, together with the finding that the $[\rho]_{BU}^{P-DOC}$ in subjects pregnant with an anencephalic fetus were similar to values obtained in nonpregnant subjects, lead us to conclude that the $[\rho]_{BU}^{P-DOC}$ was not altered by pregnancy or by the levels of progesterone in the plasma of these subjects. The range for $[\rho]_{BU}^{P-DOC}$ among these subjects was great, viz., 0.002–0.022. However, while the $[\rho]_{BU}^{P-DOC}$ varied greatly among individuals, it was remarkably constant in the two persons studied on separate occasions. Therefore the rate of DOC formation from plasma progesterone was directly proportional to the plasma progesterone level.

In two adrenalectomized women the $[\rho]_{BU}^{P-DOC}$ was 0.003 and 0.007, values which are within the range for subjects with normal adrenal function. Based on these results and those obtained in a man with Cushing syndrome, in whom the $[\rho]_{BU}^{P-DOC}$ was only 0.002, it seems likely that little or none of the plasma progesterone was converted to DOC in the adrenal cortex. This conclusion can be reached through another line of deduction. If one assumes that plasma flow to the adrenal cortex were 1 ml·min^{-1}·g^{-1} tissue (27) and that the weight of the two adrenal glands were 10 g, the

TABLE V

Plasma Concentrations of DOC and Progesterone and Transfer Constants of Conversion of Plasma Progesterone to DOC in a Woman during the Infusion of Progesterone at Various Rates

Sample time	Plasma concentrations				Transfer constants of conversion of plasma progesterone to deoxycorticosterone		
	DOC	Progesterone	DOC*				
			Tritium	^{14}C	$[\rho]_{BB}^{P-DOC}$ ‡	$[\rho]_{BU}^{P-DOC}$ §	MCR-DOC
h	*ng/ml*		*dpm × 10⁻³/liter*				
0	0.036	0.5					
3	0.066	0.5	3.6	6.0	0.003		
4	0.086	25.7	4.0	6.2	0.004	[0.003]	[1,522]‖
5	0.100	106.5	3.1	6.6	0.003		
6	0.140	143.7	3.0	5.9	0.003		

* Rate of tracer infusion = [^{3}H]P 1.69 × 10^9 dpm/24 h, [^{14}C]DOC 9.4 × 10^6 dpm/24 h.

‡ $[\rho]_{BB}^{P-DOC}$ = fractional conversion of progesterone to DOC in plasma computed from the ^3H:^{14}C ratios of plasma DOC.

§ $[\rho]_{BU}^{P-DOC}$ = fractional conversion of plasma progesterone to DOC computed from the ^3H:^{14}C ratio of urinary tetrahydro-DOC.

‖ MCR-DOC computed from the mean concentration of [^{14}C]DOC in plasma.

plasma flow to the adrenal cortices would be ~15 liters/24 h. Assuming further a metabolic clearance rate (MCR) for plasma progesterone (P) of 2,500 liters/24 h (21), the clearance of plasma of its progesterone content by DOC formation (MCR-P_{DOC}) can be computed as follows: MCR-P_{DOC} = MCR-P × $[\rho]_{BU}^{P-DOC}$. Using the mean value for $[\rho]_{BU}^{P-DOC}$ of this study, 0.007, the MCR-P_{DOC} = 17.5 liters/24 h, a value greater than adrenal plasma flow. Using the value for $[\rho]_{BU}^{P-DOC}$ that was found in subject S-3 (0.022), the MCR-P_{DOC} becomes 55.0 liters/24 h, a value almost four times that of the computed adrenal plasma flow. Moreover, it is difficult to envision complete extraction of circulating progesterone by adrenal cells and quantitative 21-hydroxylation without further metabolism in the adrenal cortex, assumptions which must be made even if one were to postulate that 15 liters of plasma were cleared of progesterone by DOC formation in the adrenal cortex. Indeed, if the progesterone that enters the adrenal cells from the circulation were metabolized in a manner similar to that of progesterone that is synthesized within these cells, it can be computed that no more than 1% of progesterone entering the adrenal would be secreted as DOC. This obtains since most progesterone within the adrenal cells is converted to cortisol (15–20 mg/24 h) (28), corticosterone (2–4 mg/24 h) (28) and other products secreted by the adrenal whereas the secretion rate of DOC is less than 0.25 mg/24 h (29, 30). Thus, it can be deduced that no more than 150 ml plasma/24 h could be cleared of progesterone through DOC formation in the adrenal.

Based on the results of the present study, therefore, extra-adrenal 21-hydroxylation of circulating progesterone can be added to the growing list of examples of the use of plasma prehormones in the formation of biologically active steroids. The importance of extraglandular steroid hormone production from circulating prehormones has been demonstrated in the case of 17β-estradiol and estrone formation from plasma C_{19}-steroids, testosterone formation from circulating andostenedione as well as 5α-dihydrotestosterone production from plasma testosterone (13–19). To our knowledge this is the first demonstration of the formation of the potent mineralocorticosteroid, DOC, from circulating precursors in adults. Bird et al. (31) found radiolabeled DOC-sulfate in adrenal tissue after infusing radiolabeled progesterone to previable human abortuses and concluded that the DOC-sulfate was formed from circulating progesterone in the fetal adrenal gland. Other investigators have demonstrated 21-hydroxylase activity in incubations of fetal testicular (32) tissue and human ovarian (33) tissue that was believed to be abnormal. However, it seems unlikely that the conversion of plasma progesterone to DOC observed in the present study occurred in the gonads of these subjects. As in the case of the adrenal cortex, near complete extraction and 21-hydroxylation of circulating progesterone by the gonads would have been required to account for the estimated MCR-P_{DOC}.

The finding of wide variations in $[\rho]_{BU}^{P-DOC}$ among the subjects of this study, while the $[\rho]_{BU}^{P-DOC}$ in the same subject remained fixed, is of considerable interest. Ordinarily the fractional conversion of a steroid prehormone to a product hormone is similar among normal persons. For example, the fractional conversion of plasma androstenedione to estrone (17, 19) and to plasma testosterone (16, 19) varies little among normal young adults. Similarly the transfer constant of con-

version of plasma testosterone to 17β-estradiol is similar among normal men (18, 19) and among nonpregnant women (18, 19). This was not the case for the fractional conversion of plasma progesterone to DOC. Indeed, a 10-fold variation in the $[\rho]_{BU}^{P\text{-}DOC}$ was found among normal persons. If the DOC derived from plasma progesterone were physiologically important, it follows that a much greater impact of such conversion could exist in persons with greater capacity to convert plasma progesterone to DOC. This might be especially important when high levels of plasma progesterone are present, e.g., during the luteal phase of the ovarian cycle and during pregnancy. In women late in pregnancy, in whom 250 mg progesterone are produced each day, the amount of DOC derived from plasma progesterone could vary from 0.5 to 5.5 mg/d depending on whether their $[\rho]_{BU}^{P\text{-}DOC}$ was 0.002 or 0.022, whereas adrenal secretion of DOC has been estimated to be 0.05–0.25 mg/24 h (29, 30).

However, based on these findings alone, we could not ascertain whether the DOC derived from plasma progesterone entered the circulation as DOC. Consider the following. As illustrated diagrammatically in Fig. 2, progesterone in blood enters the cellular site(s) of conversion of progesterone to DOC. The DOC formed may then undergo one of several metabolic fates in its cellular site of formation. DOC could enter the circulation in part, or totally, as DOC. On the other hand, the DOC derived from circulating progesterone could be metabolized in its cellular site of origin to tetrahydro-DOC (or tetrahydro-DOC glucuronoside) and then enter the blood as tetrahydro-DOC and thence be excreted in the urine. To measure the fractional conversion of progesterone to DOC in plasma, i.e., $[\rho]_{BB}^{P\text{-}DOC}$, it is necessary to measure the ^3H:^{14}C ratio of DOC in plasma during steady state conditions while infusing radiolabeled progesterone and radiolabeled DOC. Because of the amount of radioactivity required for such studies, we have been able to measure $[\rho]_{BB}^{P\text{-}DOC}$ in only two pregnant women, in each of whom the fetus was dead. In subject S-4A, whose plasma progesterone level was 6 ng/ml, the $[\rho]_{BB}^{P\text{-}DOC}$ (0.004) was less than that computed for $[\rho]_{BU}^{P\text{-}DOC}$ (0.009). In subject S-5A, however, whose plasma progesterone concentration was 22 ng/ml, the $[\rho]_{BB}^{P\text{-}DOC}$ (0.006), and $[\rho]_{BU}^{P\text{-}DOC}$ [0.004 and 0.005 (postpartum)], were more similar.

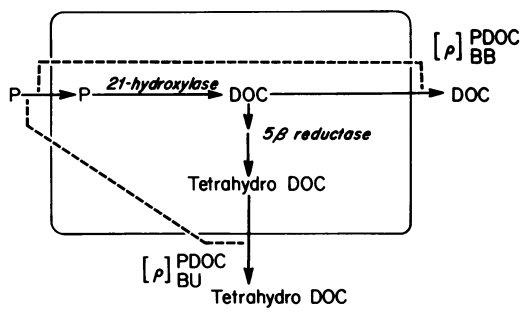

FIGURE 2 Possible pathways of metabolism of progesterone and DOC in the cellular site(s) of conversion of plasma progesterone to DOC.

Based on these observations, we speculated that the relationship between the conversion of progesterone to DOC in plasma and the fractional conversion computed from the ^3H:^{14}C ratio of urinary tetrahydro-DOC might be influenced by the intracellular concentration of progesterone. This seemed to be an attractive hypothesis since the $[\rho]_{BU}^{P\text{-}DOC}$ was not altered by changes in plasma progesterone concentration and therefore, the amount of 21-hydroxylase activity in the cellular site(s) of DOC formation was not saturated even during pregnancy. Rather, it was postulated that the amount of DOC formed from plasma progesterone that entered blood could be limited by the *in situ* conversion of DOC to tetrahydro-DOC. In turn, the rate of tetrahydro-DOC formation in the cellular site(s) of conversion of progesterone to DOC could be influenced by competition of intracellular progesterone and DOC for the 5β-reductase enzyme. We tested this hypothesis in a study of subject S-14. During a 6 h intravenous infusion of [^{3}H]P and [^{14}C]DOC at a constant rate, nonradiolabeled progesterone was infused intravenously at various rates from 0 to 14 mg/h. During the infusion of tracers alone the $[\rho]_{BB}^{P\text{-}DOC}$ was 0.004 and the $[\rho]_{BB}^{P\text{-}DOC}$ found at all rates of infusion of nonradiolabeled progesterone was 0.003 or 0.004, values which were similar to the $[\rho]_{BU}^{P\text{-}DOC}$ measured in this subject, 0.003. Moreover, the levels of DOC in plasma rose as progesterone was infused into this woman. The failure to find similar values for $[\rho]_{BB}^{P\text{-}DOC}$ and $[\rho]_{BU}^{P\text{-}DOC}$ in subject S-4A (who was pregnant with a dead fetus) remains unexplained. This result may have been due to experimental error. On the other hand it may be that the fraction of DOC derived from plasma progesterone that enters the circulation differs among individuals.

Other investigators have concluded that the high levels of DOC in the plasma of women late in pregnancy are due to transfer to the mother of DOC formed in the fetus. The basis for this conclusion was the finding of higher levels of DOC in the umbilical cord plasma of the newborn than in the mother together with the observation that the levels of DOC in maternal plasma were not altered by ACTH or dexamethasone treatment.

However, Brown and co-workers (2) found that the mean plasma level of DOC in umbilical arterial plasma was 1.92 ng/ml, whereas that of umbilical venous plasma was 1.71 ng/ml. Therefore the arteriovenous difference was small, 0.21 ng/ml, and could not account for the striking increase in concentration of

DOC in maternal plasma. A similar analysis of the potential contribution of DOC-sulfate in the fetus to DOC in maternal plasma cannot be made at this time because there are no data available concerning the arteriovenous difference in DOC-sulfate concentrations in umbilical cord blood. The levels of DOC-sulfate in cord blood are considerably greater than those of DOC. Thus a portion of the DOC in the maternal circulation may have arisen by transfer of DOC-sulfate from the fetus. It this were the case, it is likely that the DOC-sulfate is hydrolyzed by the action of steroid sulfatase in the trophoblast.

An alternate explanation for one source of DOC in plasma of women during the last few weeks of pregnancy is provided by the results of the present study. The conversion of circulating progesterone to DOC could give rise to a large amount of DOC in women late in pregnancy. If we assume that the progesterone production rates at term are 250 mg/24 h, the contribution of plasma progesterone to DOC can be computed using the values for $[\rho]_{BU}^{P-DOC}$ measured in this study. By doing so we estimate that the amount of DOC produced from plasma progesterone in near-term pregnant women could vary from 0.75 to 5.5 mg/24 h. If the production rate of progesterone were 600 mg/24 h (34), as in some gravidas, the extra-adrenal formation of DOC by 21-hydroxylation of progesterone could exceed 12 mg/24 h.

It is unlikely that a significant quantity of maternal plasma progesterone was converted to DOC in the fetus. This obtains for several reasons: (*a*) the transfer of maternal plasma progesterone to the fetus is small (35, 36); (*b*) the $[\rho]_{BU}^{P-DOC}$ in pregnant women was similar to that found in nonpregnant women; and (*c*) the $[\rho]_{BU}^{P-DOC}$ in a given woman was the same during and after pregnancy.

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