

Metabolism of Deoxycorticosterone and Deoxycorticosterone Sulfate in Men and Women

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ABSTRACT In this investigation we found that little of intravenously infused [^{14}C]deoxycorticosterone (DOC) was converted to [^{14}C]DOC-SO₄ that entered plasma. Moreover little of intravenously infused [^3H]DOC-SO₄ was metabolized by way of DOC except by intestinal bacterial enzymes. However evidence was obtained that plasma DOC is converted to DOC-SO₄ in liver, but little of the DOC-SO₄ formed in liver escapes into blood; rather the DOC-SO₄ enters bile and in the intestine is converted, in part, to progesterone (or metabolites thereof) by the action of bacterial enzymes. The estimated intrahepatic fractional conversion of DOC to DOC-SO₄ was significantly greater in premenopausal women (0.72 ± 0.118 , mean \pm SEM) than in men (0.28 ± 0.036 , $P < 0.005$).

INTRODUCTION

It now is known that plasma progesterone is converted to deoxycorticosterone (DOC)¹ in extraadrenal tissues (1, 2); indeed, this is the principal mechanism by which this mineralocorticosteroid is produced in women during the midluteal phase of the ovarian cycle (2, 3) and the mechanism that constitutes a major source of DOC during pregnancy (1). Interestingly, one tissue site of extraadrenal DOC formation may be the kidney because steroid 21-hydroxylase activity has been demonstrated in human adult (4) and fetal (5) kidney tissues. The levels of DOC in plasma of pregnant women (6–13) and in umbilical cord plasma of newborns (6,

10, 13–15) are extraordinarily high compared with those in men and nonpregnant women; but in addition, the levels of DOC-SO₄ in plasma of pregnant women and in umbilical cord plasma (13–16) are considerably greater than those of DOC.

Our study was undertaken to investigate the origin of DOC-SO₄ and to evaluate the metabolism of DOC and DOC-SO₄ in women and men. We sought primarily to ascertain whether DOC and DOC-SO₄ are interconverted, and if so, to determine the extent of such interconversion.

METHODS

Preparation and purification of radiolabeled steroids. [$1\alpha, 2\alpha(n)^3\text{H}$]DOC (44 Ci/mmol) was purchased from Amer-sham Corp. (Arlington Heights, IL) and [$4\text{-}^{14}\text{C}$]DOC (58.5 mCi/mmol) was purchased from New England Nuclear (Boston, MA). Radiolabeled DOC was purified by liquid-liquid partition chromatography on celite with the solvent system isooctane/*tert*-butanol/methanol/water (10:4:3:3, by vol). Nonradiolabeled DOC was purchased from Steraloids (Wilton, NH). [^3H]DOC-SO₄ and nonradiolabeled DOC-SO₄ were synthesized from purified [^3H]DOC and nonradiolabeled DOC by the method of Sobel et al. (17). DOC-SO₄ was purified by liquid-liquid partition chromatography on celite with the solvent system isooctane/*tert*-butanol/water/ammonium hydroxide (20:40:39:1, by vol) and by thin-layer chromatography (TLC) on silica gel 60 GF-254 (EM Reagents, Darmstadt, Germany) with the solvent systems chloroform/methanol/ammonium hydroxide (65:20:0.02, by vol) and ethyl acetate/ethanol/ammonium hydroxide (25:10:2, by vol). The purified [^3H]DOC-SO₄ was then mixed with [^{14}C]DOC. The DOC-SO₄ was hydrolyzed, and the radiolabeled DOC was extracted from the incubation mixture. The $^3\text{H}/^{14}\text{C}$ ratio of the recovered DOC was similar to that of that computed for the original mixture and remained so upon successive purification of the radiolabeled DOC according to procedures to be described below for urinary DOC(SO₄).

Subjects. The subjects of this study were healthy ambulatory volunteers who gave consent in writing to participate in these investigations. The consent form and protocol were approved by the Human Experimentation Review Committee of The University of Texas Health Science Center at Dallas. Six men, six premenopausal, and three postmenopausal women were studied. One subject (14) was in-

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¹*Abbreviations used in this paper:* DOC, deoxycorticosterone; DOC-SO₄, deoxycorticosterone sulfate; TLC, thin-layer chromatography; pregnanolone, 3 α -hydroxy-5 β -pregnan-20-one; pregnanediol, 5 β -pregnane-3 α ,20 α -diol; tetrahydro-DOC, 3 α ,21-dihydroxy-5 β -pregnan-20-one; [ρ]DOC-DOC-SO₄/BL, transfer constant of intrahepatic conversion of circulating DOC to DOC-SO₄.

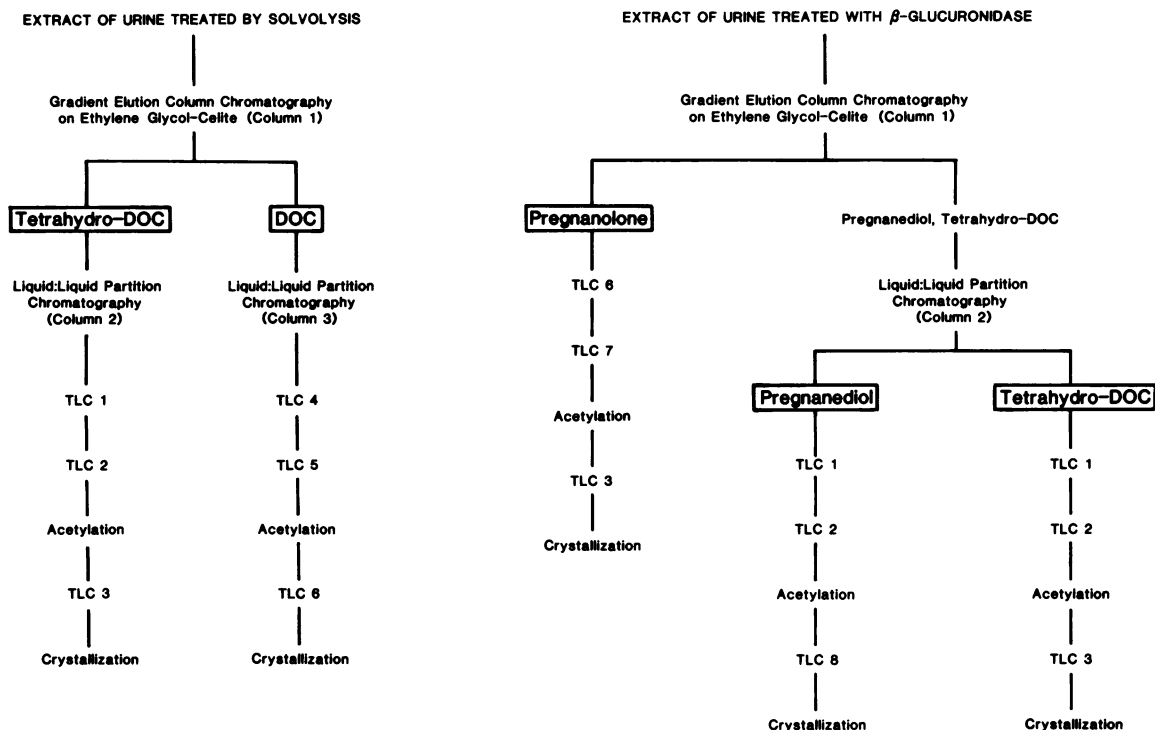


FIGURE 1 Procedure for purification of urinary metabolites of intravenously infused $[^3\text{H}]\text{DOC-SO}_4$ and $[^{14}\text{C}]\text{DOC}$. Column and TLC numbers correspond to the chromatographic systems given in Table I.

gesting an antibiotic, cephalexin (500 mg, four times daily), for treatment of a skin infection.

Tracer infusion and urine collection. 1 ml of ethanol containing $[^3\text{H}]\text{DOC-SO}_4$ (49–127 μCi) and $[^{14}\text{C}]\text{DOC}$ (0.9–2 μCi) was added to 9 ml of 0.15 M NaCl. This solution was infused intravenously during a 3–5-min period. Thereafter urine was collected for 5 d. In two subjects from whom urine was collected every 24 h, there was little carbon 14 or tritium in urine after day 3.

Purification of radiolabeled urinary metabolites. The 5-d urine collection was pooled and then divided into two equal parts. One portion was extracted with ethyl acetate (3 vol) to remove free steroids. The residual urine, after ethyl acetate extraction, was adjusted to pH 1 with concentrated HCl; sodium chloride was added to achieve a concentration of 20% (wt/vol), and the mixture was extracted with ethyl acetate (3 vol). Perchloric acid was added to the ethyl acetate extract to achieve a concentration of 0.1% (vol/vol); thereby solvolysis of the steroid sulfates was effected according to the procedure described by Burstein and Lieberman (18). The other portion of the 5-d urine collection was adjusted to pH 5.0 with glacial acetic acid. Sodium acetate buffer was added (20% vol/vol), and the mixture was incubated with β -glucuronidase (500,000 U) at room temperature for 3 d (or at 37°C for 1 d) to effect enzymatic hydrolysis of the steroid glucuronosides. The liberated steroids were extracted with ethyl acetate.

After addition of authentic nonradiolabeled steroids, several of the radiolabeled urinary metabolites of intravenously administered $[^3\text{H}]\text{DOC-SO}_4$ and $[^{14}\text{C}]\text{DOC}$ were purified by column chromatography, TLC, derivative (acetate) forma-

tion, TLC of the steroid acetate, and repeated crystallization of the steroid acetate together with 40 mg of the authentic nonradiolabeled acetate. The procedures used are given in diagrammatic form in Fig. 1. The solvent systems for column chromatography and TLC are given in Table I. Acetylation of steroids was conducted in a mixture of acetic anhydride and pyridine (1:1, vol/vol) at 37°C for 2 h. Crystallization of steroid acetates was accomplished by use of the solvent system diethyl ether and petroleum ether (bp $20\text{--}40^\circ\text{C}$) (with 1 drop of ethanol to effect solubilization of the DOC-acetate).

RESULTS

Establishment of radiochemical homogeneity of urinary metabolites. The $^3\text{H}/^{14}\text{C}$ ratios of the metabolites from the urine of subject 4 after each purification step are given in Table II. Generally the $^3\text{H}/^{14}\text{C}$ ratios of the urinary metabolites remained similar after the third chromatographic procedure.

Metabolism of intravenously infused $[^3\text{H}]\text{DOC-SO}_4$ and $[^{14}\text{C}]\text{DOC}$. In the men and women of this study there was little conversion of intravenously infused $[^{14}\text{C}]\text{DOC}$ to plasma DOC-SO_4 as evidenced by the very high $^3\text{H}/^{14}\text{C}$ ratio of urinary DOC-SO_4 compared with that of the injected tracers (Table III). Moreover, the fractional conversion of intravenously infused

TABLE I
Chromatographic Systems Used in the Purification of Radiolabeled Urinary Metabolites of [³H]DOC-SO₄ and [¹⁴C]DOC

Chromatograms*	Solvent system
Column 1—gradient elution on ethylene glycol-celite	isooctane/ethyl acetate (gradient)
Column 2—liquid:liquid partition on celite	isooctane/ <i>t</i> -butanol/methanol/water (25:10:9:6)
Column 3—liquid:liquid partition on celite	isooctane/ <i>t</i> -butanol/methanol/water (10:4:3:3)
TLC 1	ethyl acetate/isooctane (4:1)
TLC 2	benzene/ethanol (9:1)
TLC 3	ethyl acetate/isooctane (2:3)
TLC 4	ethyl acetate/isooctane (7:3)
TLC 5	dichloromethane/diethyl ether (4:1)
TLC 6	ethyl acetate/isooctane (1:1)
TLC 7	ethyl acetate/isooctane (3:2)
TLC 8	ethyl acetate/isooctane (3:7)

* The Arabic numbers of the chromatography systems correspond to those of Fig. 1.

[³H]DOC-SO₄ to [³H]DOC also was small as evidenced by the very low ³H/¹⁴C ratio of urinary tetrahydro-DOC (glucuronoside). In fact even the apparent small conversion of DOC-SO₄ to DOC may have been artifactual (except in subjects 3, 7, and 14) because of the possibility of spontaneous hydrolysis of small amounts of the [³H]DOC-SO₄ tracer before its infusion.

In all of the subjects of this study, except subject 14, urinary 3 α -hydroxy-5 β -pregnan-20-one (pregnanolone) and 5 β -pregnane-3 α ,20 α -diol (pregnanediol) glucuronosides were radiolabeled with both tritium and carbon 14. Indeed, the ³H/¹⁴C ratios of pregnanolone and pregnanediol isolated from the urine of some of the women of this study were similar to those of the

TABLE II
*Purification of Urinary Metabolites of Intravenously Infused [³H]DOC-SO₄ and [¹⁴C]DOC: Establishment of Radiochemical Homogeneity**

Steroids	³ H/ ¹⁴ C Ratio after each purification step									
	Chromatography†					Crystallization				Crystals
						Mother liquors				
	Column I	Column II	TLC I	TLC II	TLC III	1	2	3	4	Final
Glucuronosides										
Pregnanolone	120		144	192	195	198	184	190	186	185
Pregnanediol	70	156	170	180	184	188	188	189	184	187
Tetrahydro-DOC	1.4	0.4	0.3	0.3	0.3§					
Sulfates										
DOC	493	>1,500	>1,500	>1,500	>1,500§					
Tetrahydro-DOC	64	68	52	50	69	62	65	67		62

* Data presented are those obtained in study of subject 4; ³H/¹⁴C ratio of injected tracers = 59.3.

† ³H/¹⁴C ratios after successive chromatographic steps. The Roman numerals do not correspond necessarily to the Arabic numerals for chromatographic steps presented in Fig. 1 and Table I. For example, in each instance TLC-III is the thin-layer chromatography of the steroid after acetylation.

§ In some instances in which the ³H/¹⁴C ratio was <0.5 or >1,000, purification was not continued beyond chromatography, derivative formation, and chromatography of the steroid acetate.

TABLE III
³H/¹⁴C Ratios of Urinary Metabolites of [³H]DOC-SO₄ and [¹⁴C]DOC Infused Intravenously into Men and Women

Subject	Sex	Age (years)	Reproductive endocrine status	³ H/ ¹⁴ C Ratios*				
				Pregnanolone (Glucuronoside)	Pregnanediol (Glucuronoside)	Tetrahydro-DOC (Glucuronoside)	Tetrahydro-DOC (Sulfate)	DOC (Sulfate)
1	male	21	normal	258	255			
2	male	22	normal	226	223	0.8		
3	male	36	normal	156	199	2.4	74	>1,500
4	male	48	normal	215	217	>0.1	130	>1,500
5	male	50	normal	156	157	0.3	52	>1,500
6	male	52	normal	133	100	0.2	32	>1,500
7	female	54	postmenopausal (0.5)‡	63	61	7.1	9	813
8	female	55	postmenopausal (4)	120				
9	female	57	postmenopausal (7)	227	231	0.3	23	>1,500
10	female	25	premenopausal	69	65	0.3		
11	female	28	premenopausal	56	61	>0.1	23	>1,500
12	female	28	premenopausal	162				
13	female	30	premenopausal	73	73	0.2	103	>1,500
14	female	41	premenopausal§	ND¶	ND	10.5	27	636
15	female	43	premenopausal	44	54	>0.1	23	>1,500

* For ease of comparison, all ³H/¹⁴C ratios are corrected to a common ³H/¹⁴C ratio of the infused tracers, viz., 50. The actual ³H/¹⁴C ratios of the injected tracers varied from one subject to another, the low being 39 and the high being 75.1.

‡ Numbers in parentheses are the numbers of years that these women were postmenopausal at the time of study.

§ Subject 14 was ingesting antibiotics (Cephalexin, 500 mg, 4 times daily).

¶ ND = not detected.

infused tracers. On the other hand, the ³H/¹⁴C ratios of pregnanolone and pregnanediol from urine of the men of this study were considerably greater than that of the infused tracers. The identity of urinary pregnanolone and pregnanediol of subject 4 was rigorously established by a second method, viz., reverse isotope dilution. After column chromatography, TLC, derivatization, and TLC of the steroid acetates, the radiolabeled steroids were diluted with nonradiolabeled pregnanolone acetate or pregnanediol diacetate. The computed specific activities of the mixtures, the determined specific activities of the mixtures, and those of successive mother liquors and crystals after repeated crystallizations were almost identical.

The ³H/¹⁴C ratios of urinary tetrahydro-DOC(SO₄) were quite variable from subject to subject. For all subjects the ³H/¹⁴C ratio of urinary tetrahydro-DOC(SO₄) is greater than that of tetrahydro-DOC (glucuronoside) and less than that of DOC(SO₄), as presented in Table III.

DISCUSSION

The ³H/¹⁴C ratio of urinary DOC-SO₄ in all subjects was strikingly higher than that of the injected tracers, [³H]DOC-SO₄ and [¹⁴C]DOC, whereas the ³H/¹⁴C ratio of urinary tetrahydro-DOC (glucuronoside) was much lower than that of the tracers. These findings are in-

dicative that little of plasma DOC is converted to plasma DOC-SO₄, and little of plasma DOC-SO₄ is metabolized by hydrolysis to DOC (except, as will be discussed below, in intestine by action of bacterial enzymes).

The ³H/¹⁴C ratios of urinary tetrahydro-DOC-SO₄ were greater than those of urinary tetrahydro-DOC (glucuronoside) and less than those of urinary DOC-SO₄. We suggest that one interpretation of this finding is that urinary tetrahydro-DOC-SO₄ consists of a mixture of the 21-sulfate, the 3-sulfate, and possibly the 3,21-disulfate of this metabolite. Specifically, it is likely that [³H]DOC-SO₄ was converted directly to tetrahydro-DOC-21-SO₄, and [¹⁴C]DOC was converted to tetrahydro-DOC, and thence to tetrahydro-DOC-3-sulfate or to the 3,21-disulfate.

In each of the subjects except one, subject 14, there were large quantities of tritium and carbon 14 in urinary metabolites that were less polar than tetrahydro-DOC. In fact, one of these metabolites was strikingly less polar than the 21-hydroxylated metabolites of DOC and DOC-SO₄. These metabolites were identified as pregnanolone and pregnanediol.

In our investigation we found that in one regard the metabolism of intravenously administered [¹⁴C]DOC in men was different from that in most women, whereas the metabolism of [³H]DOC-SO₄ in men and women was similar.

In most of the premenopausal women of this study the $^3\text{H}/^{14}\text{C}$ ratios of urinary pregnanolone and pregnanediol were similar to or not much greater than that of the injected tracers. On the other hand, the $^3\text{H}/^{14}\text{C}$ ratios of urinary pregnanolone and pregnanediol in the men were considerably greater than that of the injected tracers. For DOC-SO_4 to be converted to progesterone and thence to pregnanolone and pregnanediol, hydrolysis of the sulfate ester and dehydroxylation of the 21-hydroxyl group must take place. We interpret these findings as follows. Large amounts of DOC-SO_4 , but not DOC, enter the bile. After reaching the intestine, bacterial enzymes act on DOC-SO_4 to effect hydrolysis and 21-dehydroxylation to yield progesterone. Progesterone is metabolized and reabsorbed, metabolized, and excreted in urine as metabolites of progesterone, viz., pregnanolone and pregnanediol glucuronosides. It is known that steroid 21-dehydroxylase activity is present in intestinal bacteria of the human but not in mammalian tissues (19, 20). In further support of this conclusion was the finding that large quantities of radiolabeled metabolites of progesterone were found in the urine of each person studied except subject 14. She was the only subject of this study who was ingesting an antibiotic. We conclude that the oral ingestion of this antibiotic led to a reduction in intestinal bacteria and in this manner the hydrolysis and dehydroxylation of DOC-SO_4 by bacterial enzymes was prevented.

As stated, it was found that after the intravenous infusion of $[^3\text{H}]\text{DOC-SO}_4$ and $[^{14}\text{C}]\text{DOC}$, the $^3\text{H}/^{14}\text{C}$ ratios of urinary pregnanolone and pregnanediol of many of the women were similar to that of the injected tracers. We interpret this to mean that most of the $[^{14}\text{C}]\text{DOC}$ in these women was sulfurylated in the liver, excreted in bile as $[^{14}\text{C}]\text{DOC-SO}_4$ and thence converted to progesterone in the intestine. This finding, together with the finding that there was little carbon-14 in urinary DOC-SO_4 , is indicative that little of the DOC-SO_4 formed in liver from $[^{14}\text{C}]\text{DOC}$ escaped the liver into peripheral blood and that the $[^3\text{H}]\text{DOC-SO}_4$ and $[^{14}\text{C}]\text{DOC}$ that reached the liver suffered a similar metabolic fate. Similarly, the finding that the $^3\text{H}/^{14}\text{C}$ ratios of urinary pregnanolone and pregnanediol in men were considerably greater than that of the injected tracers also is indicative that it is DOC-SO_4 and not DOC that enters bile before being metabolized further. However, more importantly, it has been demonstrated that after the intravenous infusion of radiolabeled DOC, there is no detectable unconjugated DOC in bile (21) and unlike other C-21 hydroxylated compounds, a considerable quantity of radiolabeled conjugated metabolites of DOC do appear in bile (21). It also is known from studies conducted in experi-

mental animals that there is considerably greater C-21 hydroxysteroid sulfotransferase activity in the liver of females than in males (22–25).

The preferential excretion of DOC-SO_4 into bile may be the consequence of the positioning of the sulfate moiety at carbon 21. The C-21-sulfate bears some analogy to the sulfurylated side chain of the bile acids. Recently, we have found (unpublished observations) that the metabolic clearance rate of plasma DOC-SO_4 is quite high (650–1,000 liters/24 h) compared with that of other steroid sulfates; e.g., the metabolic clearance rate of plasma dehydroisoandrosterone sulfate (26) and cholesteryl sulfate (27) in men and nonpregnant women is only 10 liters/24 h and those of estrone sulfate (28, 29), pregnenolone sulfate (30), and testosterone sulfate (31) are of the order of 150 liters/24 h. This may be due to preferential entry into bile of steroid-21-sulfates compared to steroid- 3β -sulfates or steroid- 17β -sulfates or else due to bacterial metabolism of DOC-SO_4 in a manner that precludes reabsorption of DOC-SO_4 and thus constitutes irreversible metabolism (i.e., conversion to progesterone and metabolites thereof). It has been shown that the rate of entry of dehydroisoandrosterone sulfate into bile is slow (32).

Therefore we suggest that progesterone is formed from DOC-SO_4 that enters bile and that little DOC enters bile except after conversion to the sulfate. This being the case, the $^3\text{H}/^{14}\text{C}$ ratio of urinary pregnanolone or pregnanediol after the infusion of $[^3\text{H}]\text{DOC-SO}_4$ and $[^{14}\text{C}]\text{DOC}$ can be used as a measure of C-21 hydroxysteroid sulfotransferase activity in liver. The intrahepatic fractional conversion of DOC to DOC-SO_4 can be computed from the relationship between the $^3\text{H}/^{14}\text{C}$ ratio of urinary pregnanolone and/or pregnanediol and that of the injected tracers provided several assumptions that must be made are valid. (a) It is DOC-SO_4 and not DOC that is secreted into bile. The evidence in favor of this proposition was cited above. (b) Progesterone is the product of intestinal enzymatic hydrolysis and dehydroxylation of DOC-SO_4 . The similarity of the $^3\text{H}/^{14}\text{C}$ ratios of urinary pregnanolone and pregnanediol are supportive of the validity of this assumption. (c) The metabolism of DOC-SO_4 is exclusively in liver. This assumption is not totally valid, but appears to be nearly so. After the intravenous infusion of $[^3\text{H}]\text{DOC-SO}_4$ there is some, but very little, tritium in urinary metabolites other than those of progesterone. For example in no instance could we recover >0.5% of the injected $[^3\text{H}]\text{DOC-SO}_4$ as $[^3\text{H}]\text{DOC-SO}_4$ in urine, and $[^3\text{H}]\text{DOC-SO}_4$ was the major tritium-labeled urinary metabolite other than pregnanolone and pregnanediol.

Based on these assumptions, we computed the transfer constant of intrahepatic conversion of circulating

DOC to DOC-SO₄ ($[\rho]\text{DOC-DOC-SO}_4/\text{BL}$). In the men of this study the $[\rho]\text{DOC-DOC-SO}_4/\text{BL}$ (0.28 ± 0.036 , mean \pm SEM) was significantly less than that of premenopausal women (0.72 ± 0.118 , $P < 0.005$). Interestingly, the $[\rho]\text{DOC-DOC-SO}_4/\text{BL}$ may decrease with time after the menopause. In the case of a woman studied 7 mo after the menopause (subject 7, Table III) the $[\rho]\text{DOC-DOC-SO}_4/\text{BL}$ was 0.89; in the woman who was 4 yr beyond menopause (subject 8) it was 0.42; and in the woman who was 7 yr postmenopausal (subject 9) it was 0.22.

We suggest that the difference in the metabolism of DOC between men and women is due to induction of C-21 hydroxysteroid sulfotransferase activity in women or else inhibition of such activity in men, or both. In experimental animals it has been shown that estrogen treatment causes increased steroid sulfotransferase activity in liver whereas androgen treatment causes the reverse (33).

In some regards the metabolism of DOC and DOC-SO₄ in the human is similar to that of corticosterone and corticosterone-sulfate. Namely, there is little or no interconversion of these compounds (34). On the other hand, there is one major difference in the metabolism of DOC-SO₄ and corticosterone sulfate; namely, large amounts of corticosterone sulfate and metabolites thereof are rapidly excreted in urine (34). This was not true of DOC-SO₄; namely, very little of DOC-SO₄ or metabolites thereof (other than those formed by intestinal bacterial enzymes) enter urine.

The origin and metabolism of DOC and DOC-SO₄ in pregnant women and their fetuses is of considerable interest and of potential significance in pregnancy physiology and pathophysiology. The levels of DOC and DOC-SO₄ in maternal and fetal blood are high compared with the levels of these compounds in men and nonpregnant women (6–16). Moreover, it is known that most of the DOC in the maternal and fetal compartments does not arise by maternal or fetal adrenal secretion because (a) DOC levels in near-term pregnant women do not fall during dexamethasone treatment (6, 8, 9, 11); (b) DOC levels in near-term pregnant women do not rise during ACTH treatment (8, 9, 11); (c) plasma progesterone is converted to DOC in extraadrenal tissues (1, 2); and (d) the levels of DOC and DOC-SO₄ in umbilical cord plasma of a newborn with adrenal aplasia were similar to those of normal newborns (16).

Thus, DOC in the maternal and fetal compartments arises primarily by the extraadrenal 21-hydroxylation of circulating progesterone. But what is the origin of DOC-SO₄ in plasma of pregnant women? In this study we found that little of circulating DOC is converted to DOC-SO₄ that enters plasma in men or nonpregnant

women. We suggest that during pregnancy, DOC-SO₄ is formed in extraadrenal, extrahepatic tissues in which progesterone is converted to DOC, e.g., kidney. We have demonstrated steroid 21-hydroxylase activity in fetal (5) and adult human (4) kidney tissue and have shown that estrogen treatment of women pregnant with a dead fetus causes an increase in extraadrenal 21-hydroxylation of plasma progesterone (35). It also is known that steroid 21-hydroxylase activity was high in the kidney of a pregnant woman (4). We (36) and others (37) have demonstrated that C-21 hydroxysteroid sulfotransferase activity is present in kidney of the human fetus. However, this enzyme activity could not be demonstrated in kidney of nonpregnant adults (38). The activity of this enzyme has not been measured in kidney tissue of pregnant women.

It has been a curiosity that estrogen is produced in such large amounts in normal pregnant women. However, it has been shown that certain estrogen-mediated responses are subject to further stimulation in the face of very high circulating levels of 17 β -estradiol. For example, in women pregnant with a dead fetus but with relatively high plasma levels of 17 β -estradiol, viz., 25 ng/ml, extraadrenal steroid 21-hydroxylase activity was stimulated further during diethylstilbestrol treatment (35). Therefore it is conceivable that estrogen in large amounts is required to effect optimal responses of certain estrogen responsive systems, e.g., extrahepatic C-21 hydroxysteroid sulfotransferase activity in tissue sites of conversion of circulating progesterone to DOC.

We speculate that failure of sulfurylation of DOC formed in the kidney of women in their first pregnancy may be instrumental in the pathogenesis of pregnancy-induced hypertension. If in the first pregnancy C-21 hydroxysteroid sulfotransferase activity in the kidney were not induced to optimal levels, the intracellular concentration of DOC formed in kidney may be elevated. DOC formed from plasma progesterone could act *in situ*, i.e., in kidney to effect the salt retention characteristic of preeclampsia. Interestingly, in primigravid women who were treated with diethylstilbestrol the incidence of preeclampsia was reduced strikingly (39).

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