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17β-HYDROXYSTEROID OXIDOREDUCTASE ACTIVITY IN HUMAN MATERNAL AND UMBILICAL CORD SERA

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Summary—The specific activity of 17β-hydroxysteroid oxidoreductase (17β-HSOR) in human umbilical cord arterial serum has been reported to be similar to that of maternal serum and 5- to 15-times higher than that of cord venous serum. Based on these findings, it was proposed that 17β-HSOR in cord arterial serum arises from fetal tissue sources other than placenta. In the course of studies of the role of 17β-HSOR in the modulation of bioactive estrogen levels in the human fetus, we determined that: (i) the specific activity of 17β-HSOR in maternal serum is 2.1- to 55-times higher than that in either umbilical cord venous serum or cord arterial serum; (ii) the specific activity of 17β-HSOR in umbilical cord venous and cord arterial sera are similar; (iii) anti-human placental cytosolic 17β-HSOR antibody inactivates the 17β-HSOR in maternal, umbilical cord arterial, and cord venous sera but not in maternal or fetal erythrocytes; (iv) the specific activity of 17β-HSOR in maternal serum (expressed per mg protein) is higher than that in umbilical cord serum and maternal and fetal erythrocytes, and is approximately 700-times lower than that of the placental microsomal enzyme; (v) the preferred cofactor for maternal serum 17β-HSOR is $NADP^+$; (vi) 17β-HSOR is associated with the high speed supernatant fraction of maternal serum rather than with the particulate fraction; and, (vii) the patterns of binding of [^{3}H]estradiol-17β to proteins in maternal and umbilical cord arterial sera and those of 17β-HSOR activity, determined in corresponding fractions obtained after sucrose density gradient centrifugation, are approximately coincidental at $S_{20,\omega}$ 4.6–5. The findings of higher 17β-HSOR levels in maternal serum compared with umbilical cord arterial serum and the inactivation of the cord arterial serum enzyme by an antibody that recognizes human placental cytosolic 17β-HSOR is suggestive that 17β-HSOR in cord arterial serum is of placental origin.

INTRODUCTION

17β-Hydroxysteroid oxidoreductase (17β-HSOR, E.C. 1.1.1.62) in serum of pregnant women and fetuses catalyzes the interconversion of estradiol-17β (E2) and estrone (E1) *in vitro* when appropriate cofactors are added to the incubation mixtures [1–8]. 17β-HSOR in maternal serum originates in the placenta, and the specific activity of the serum enzyme increases with advancing gestation [1]. From findings that the specific activity of 17β-HSOR in umbilical cord arterial serum was similar to that in maternal serum and 5- to 15-fold higher than that in umbilical cord venous serum, Plotti *et al.* [5] suggested that 17β-HSOR in cord arterial serum orginates in fetal tissues and not in the placenta.

In studies conducted by western blot analysis by use of an anti-human placental cytosolic 17β-HSOR antibody, we found that 17β-HSORs in homogenates and cytosolic fractions of various human fetal tissues had similar molecular weights as that of the denatured placental cytosolic enzyme (M_r ~34,000) [9], however, 17β-HSORs in washed microsomes, prepared from the same tissues, were not recognized by the antibody except for the placental and fetal brain enzyme(s) [9]. Thus we considered the possibility that placental cytosolic 17β-HSOR was present as a contaminant in homogenates and cytosolic fractions of fetal tissues as a consequence of nonspecific uptake of the enzyme from fetal plasma. To study this possibility, we determined the specific activity of 17β-HSOR in maternal, umbilical cord arterial, and cord venous sera in a manner similar to that of Plotti *et al.*[5] in an attempt to discern whether the source of cord arterial serum enzyme was the placenta or the fetus.

17β-HSOR activity in human maternal and umbilical cord sera can be determined with either E1 or E2, radiolabeled at stable nuclear positions, as the substrate. Initially, we determined 17β-HSOR in human maternal and cord sera by use of [6,7-^{3}H]E2

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with either $NADP^+$ or NAD^+ as cofactor. The product, [6,7-^{3}H]E1, was purified by TLC before and after acetylation and, thereafter, quantified as described [10]. Because of the large number of samples to be analyzed in this study and to simplify the quantification process, we developed an abbreviated assay to determine 17β-HSOR activity *in vitro* by use of [17α-^{3}H]E2 as the substrate with either $NADP^+$ or NAD^+ as cofactor. The assay is based on the obligatory transfer of tritide from the C-17α-position of the substrate to the oxidized form of the cofactor with resulting formation of nonradiolabeled E1 and either $NADP^3H$ or NAD^3H. After selective removal of unmetabolized radiolabeled steroidal substrate, the radioactivity that remains associated with the reduced cofactor is used for quantification of enzymatic activity.

In the determination of 17β-HSOR activity, the substitution of deuterium for hydrogen at the C-17α-position of E2 results in a primary kinetic isotope effect of approximately 2.6–2.8 [11], therefore, we determined the isotope effect introduced by substitution of tritium for hydrogen at the C-17α-position of E2. For this purpose, we used serum of a pregnant woman as the source of the enzyme and compared the specific activity of 17β-HSOR determined with both [6,7-^{3}H]E2 and [17α-^{3}H]E2 as substrates and $NADP^+$ as cofactor. The isotope effect was taken into consideration for quantification of 17β-HSOR activity. By use of this technique we determined: (i) 17β-HSOR levels in maternal, umbilical cord arterial, and cord venous sera: (ii) the effect of an anti-human placental cytosolic 17β-HSOR antibody on 17β-HSOR activity in maternal, umbilical cord arterial and cord venous sera and maternal and fetal erythrocytes; (iii) the preferred cofactor for serum 17β-HSOR; (iv) the effect of glycerol on 17β-HSOR activity of maternal and umbilical cord vein sera upon freezing and thawing; (v) whether 17β-HSOR was associated with particulate or supernatant fractions of maternal serum; and (vi) the patterns of binding of radiolabeled E2 to proteins in maternal and umbilical cord arterial sera and those of 17β-HSOR activity to establish whether the patterns of E2 binding and enzymatic activity were coincidental.

EXPERIMENTAL

Steroids

[6,7-(N)-^{3}H]E2 (sp. act, 59 Ci/mmol) and [4-^{14}C]-E1 (sp. act 59 mCi/mmol) were purchased from New England Nuclear (Boston, MA) and were purified by partition chromatography on celite columns [isooctane-*tert*-butanol-methanol-water (50:20:16:14)] prior to use. Nonradiolabeled steroids were purchased from Sigma Chemical Co. (St Louis, Mo.). [17α-^{3}H]E2 was synthesized [12]: in brief, estrone acetate was reduced with sodium borotritide, first in isopropanol for 72 h at 24°C and thereafter by addition of methanol (15 h at 24°C), to give [17α-^{3}H]E2 3-acetate. This steroid and the recovered E1 acetate were hydrolyzed by treatment with potassium bicarbonate in methanol-water (1:1, v/v) for 24 h at 24°C. The mixture was acidified with acetic acid and the products, [17α-^{3}H]E2 and E1, were extracted with ethyl acetate and separated by gradient chromatography on a column of celite-ethylene glycol to yield the purified [17α-^{3}H]E2.

Chemicals

β-Nicotinamide adenine dinucleotide phosphate, sodium salt ($NADP^+$), β-nicotinamide adenine dinucleotide, sodium salt (NAD^+), and hemoglobin were purchased from Sigma Chemical Co. (St Louis, Mo.). Dichloromethane, ethyl acetate, and methanol of nanograde quality, and chloroform of analytical reagent grade were purchased from Mallinkrodt Chemical Works (St Louis, Mo.). Precoated silica gel thin-layer plastic sheets (Polygram Sil G-HY; 20 × 20 cm; 0.25 mm thick) were purchased from Brinkmann Instruments (Houston, Tex.).

Blood collection and processing

Umbilical cord bloods were obtained at the time of spontaneous, term delivery. The umbilical cords were double clamped approximately 8–10 cm apart using hemostats, and arterial and venous bloods were collected separately from the severed cord sections through hypodermic needles into syringes. Also, at delivery, peripheral maternal venous blood (5–10 ml) was collected. The 3 blood specimens were processed simultaneously with immediate separation of serum via centrifugation at 400 ***g*** for 10 min. The determination of enzymatic activity was initiated within 30 min of delivery.

Quantification of 17β-HSOR activity in maternal and cord serum

A technique to remove the radiolabeled steroid substrate and the nonradiolabeled steroid product, E1, selectively in the presence of NADPH, was developed. The following protocol was used to ascertain the efficacy of the method. Aliquots (1 ml) in quintuplicate of a solution that mimicked the conditions used for determination of 17β-HSOR, made of potassium phosphate buffer (0.1 M, pH 7.4), nonradiolabeled E2 (30 μM), and either NADPH (1 mM) or $NADP^+$ (1 mM), were transferred to 25 × 150 mm teflon-capped tubes. Control samples (1 ml, in duplicate) consisted of a mixture of buffer and E2, without the cofactors. Chloroform (5 ml) was added to each sample and the contents were mixed thoroughly, the chloroform and water layers were separated by centrifugation at 200 ***g*** for 20 min, and the organic solvent layers were transferred to clean tubes. The water layers were extracted 3 more times with chloroform (5 ml), the pooled chloroform layers ($\sim$20 ml) were treated with anhydrous sodium sulfate to remove water and, thereafter, filtered through

Whatman No. 1 qualitative filter paper into clean glass tubes. The chloroform was evaporated with a stream of nitrogen and the residues were dissolved in potassium phosphate buffer (0.1 M, pH 7.4; 1 ml) to determine absorbances. The chloroform-extracted aqueous layers were transferred to clean tubes, and the residual chloroform in these samples was evaporated by bubbling nitrogen into the solutions. In addition, controls made of chloroform alone (20 ml, in duplicate) were evaporated and the dry residues were redissolved in potassium phosphate buffer (0.1 M, pH 7.4; 1 ml). Absorbances in all the samples prepared as described were determined both at 340 nm and 260 nm either in the undiluted samples or in 1:10 diluted samples, depending on the concentration of cofactor, to determine whether chloroform extraction removed $NADP^+$ and NADPH from the aqueous media.

17β-Hydroxysteroid oxidoreductase activity was determined by use of freshly obtained serum (1 ml) that was mixed with potassium phosphate buffer (pH 7.4; 0.2 M; 1 ml) containing [17α-^{3}H]E2 (0.56 μCi), glycerol (40%, v/v), E2 (60 μM), and $NADP^+$ (2 mg) to give serum diluted 1:1 (2 ml) that contained phosphate buffer (0.1 M), glycerol (20%, v/v), E2 (30 μM), and $NADP^+$ (1 mg/ml). When the volume of serum available was less than 1 ml, the largest aliquot possible was taken, mixed with double distilled water to give a volume of 1 ml, and processed as described. Control incubations were as described but substituting double distilled water for serum. Incubations were conducted at 37°C for 1 h in capped tubes (teflon caps) and the reactions were terminated by chilling in ice-water and addition of chloroform (6 ml). The contents were mixed thoroughly and the mixtures were centrifuged for 20 min at 200 *g* to separate the aqueous layers from the chloroform layers. The chloroform layers containing the steroid were removed by use of a pasteur pipette and the remaining aqueous layers were extracted with chloroform three more times, as described. Aliquots (0.5 ml) of the aqueous layers that contained the product, $NADP^3H$, were assayed for radioactivity in a Packard Tri-Carb liquid scintillation spectrometer model 3330 (Downers Grove, Ill.). The scintillation fluid used was ACS-II (Amersham, Arlington Heights, Ill.). The tritium radioactivity in the aliquots was corrected for volume dilution and used to compute the specific activity of 17β-HSOR by taking into consideration the specific activity of the substrate, the volume of serum used for incubation, the time of incubation, and the primary kinetic isotope effect.

The kinetic isotope effect was determined by assaying aliquots of maternal serum both by use of [17α-^{3}H]E2, as described above, and by use of [6,7-^{3}H]E2 as the substrate and $NADP^+$, as cofactor, as described [10]. Full details of the determination of the isotope effect are given in the legend to Table 2.

Anti-human placental cytosolic 17β-HSOR antibody

An antibody elicited against purified human placental cytosolic 17β-HSOR was raised in a rabbit [9]. The antibody was used in studies designed to evaluate its neutralizing activity on 17β-HSOR in maternal serum, umbilical cord serum, erythrocytes, and placental microsomes.

E2-binding to serum proteins determined by sucrose density gradient centrifugation

Maternal and umbilical cord arterial sera (0.5 ml each) were mixed with [6,7-^{3}H]E2 (1.75×10^6 dpm) and incubated at 4°C for 1 h. Thereafter, the samples were mixed with acetylated [^{14}C]albumin (6600 dpm, internal marker) and layered on sucrose density gradients (SDG; 5%–20%; 12.1 ml) contained in preparative centrifuge tubes in a SW40 rotor. In parallel, using the same sera samples, aliquots (5×0.5 ml) were incubated at 4°C for 1 h in the absence of radiolabeled E2 and, thereafter, layered on SDGs as described. The samples were centrifuged at 33,000 rpm for 26 h, and aliquots (39×0.3 ml) were collected through the top of each centrifuge tube. The fractions obtained from the serum samples that were incubated with [6,7-^{3}H]E2 were assayed for radioactivity to determine the binding patterns. Corresponding fractions of the remaining serum samples, centrifuged in the absence of radiolabeled E2, were pooled together to give 39 fractions: aliquots (1 ml) were mixed with phosphate buffered saline solution, pH 7.4 (0.1 ml) containing [17α-^{3}H]E2 (0.56 μCi; 20 μM) and $NADP^+$ (1 mM) and incubated at 37°C for 1 h. Thereafter, the samples were processed as described to determine 17β-HSOR activity. The sedimentation coefficients ($S_{\omega,20}$) were determined as described [13].

Protein determination

The method of Lowry *et al.* [14], with bovine serum albumin as standard, was used to determine protein concentration in serum, and the cyanmethemoglobin method [15], with human hemoglobin as the standard, was used to assess the protein content in erythrocytes.

Statistics

Where appropriate, the data were treated by analysis of variance and by Newman–Keuls multiple comparison analysis.

RESULTS

17β-HSOR assay: comparison of methods to remove the steroid substrate selectively from the incubation mixture without removal of the cofactor

(a) Activated charcoal extraction. The classical use of activated charcoal to remove the steroid from the incubation medium also resulted in the removal of

Table 1. Validation of the chloroform extraction technique for the determination of NADPH in the aqueous layer[a]

Sample	Absorption[b] 340 nm Water	340 nm Chloroform	260 nm Water	260 nm Chloroform
Chloroform	—	0.021*	—	0.078*
Buffer + E2	0.026*	0.022*	—	0.115*
Buffer + E2 + $NADP^+$	0.023 ± 0.0030	0.024 ± 0.0021	17.83 ± 0.75	0.120 ± 0.016
Buffer + E2 + NADPH	4.95 ± 0.072	0.039 ± 0.0054	14.05 ± 0.10	0.111 ± 0.015

[a] The samples were prepared and processed as described in Expermental.
[b] The absorbances are expressed as the mean ± SD of quintuplicate samples except when indicated by an asterisk, where average absorptions of duplicate samples are presented.

most of the NADPH (>80%) (data not shown). Thus, the activated charcoal technique is not appropriate for selective removal of [17α-^{3}H]E2 in the presence of $NADP^3H$.

(b) Validation of the chloroform extraction technique for determination of $NADP^3H$. As demonstrated in this study, the chloroform extraction technique is appropriate to remove E2 selectively without removal of reduced or oxidized cofactor from the aqueous incubation medium. As illustrated in Table 1, the strong absorbance at 340 nm, which is the characteristic absorption maximum for NADPH, was observed only with the aqueous samples containing NADPH and not in the solubilized residues of the chloroform extracts, obtained after drying (chloroform extracts were obtained by extraction of aqueous solutions of NADPH, of samples containing $NADP^+$, or control samples without cofactors). A strong absorption at 260 nm was observed with the aqueous solutions containing either $NADP^+$ or NADPH, but not with the solubilized residues of the chloroform extracts: the cofactors remained associated exclusively with the water layer after repeat chloroform extraction. Thus, the chloroform extraction technique is appropriate for determination of NADPH and 17β-HSOR in serum when using [17α-3H]E2 as the substrate and $NADP^+$ as the cofactor.

17β-HSOR assay: kinetic isotope effect with [17α-^{3}H]E2 as the substrate

The primary kinetic isotope effect introduced by substitution of tritium for hydrogen at the C-17α-position of [17α-^{3}H]E2 was determined in parallel incubations by use of both [17α-^{3}H]E2 and [6,7-^{3}H]E2 as substrates in concentrations of 30 μM, with $NADP^+$ as the cofactor. Serum of a pregnant woman near term was used as the source of 17β-HSOR. The product of [6,7-^{3}H]E2 metabolism, viz., [6,7-^{3}H]E1, was quantified as described previously [10], and that

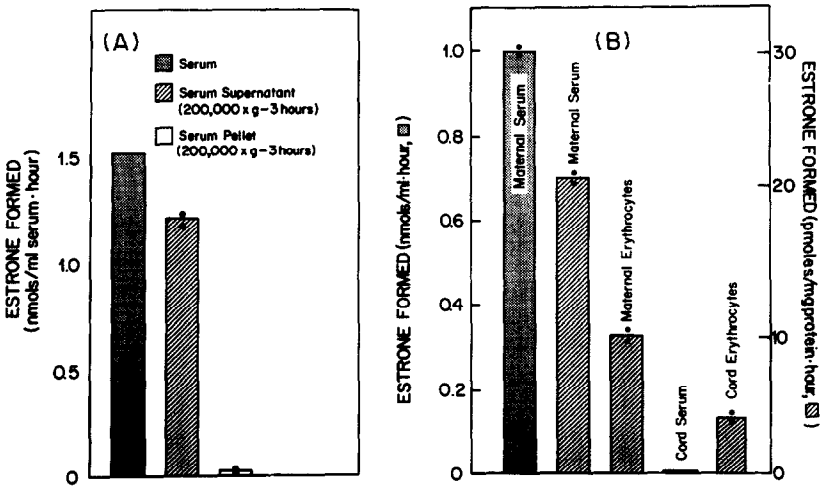

Fig. 1. (A) The specific activity of 17β-HSOR was determined in freshly obtained maternal serum that was kept at 4°C for 3 h, and in a pellet and supernatant fraction of the same serum that were obtained after centrifugation at 4°C (200,000 *g* for 3 h); [17α-^{3}H]E2 (0.56 μCi; 30 μM) was used as the substrate and $NADP^+$ (1 mM) as the cofactor, as described in the text. 17β-HSOR activity (nmols/ml serum·h) was calculated taking into consideration the amounts of pelletable and supernatant fractions corresponding to 1 ml of unfractionated serum. (B) The specific activity of 17β-HSOR in maternal serum and pooled cord serum (venous plus arterial) and in erythrocytes (lysates) obtained from maternal blood and umbilical cord blood (venous plus arterial) were determined as described in the text by use of [17α-^{3}H]E2 (0.56 μCi; 30 μM) as the substrate and $NADP^+$ (1 mM) as the cofactor. Erythrocyte lysates were prepared by sonication by use of a Sonicator Cell Disruptor Model W185F (Plainview, N.Y.) (15 pulses at setting No. 3, with cooling in ice-water).

of [17α-^{3}H]E2 metabolism, viz., NADP^{3}H, was quantified as described in the Experimental section. The ratio of specific activities obtained by use of the C-17α-protonated and C-17α-tritiated substrates represent the primary kinetic isotope effect, which was found to be 2.6 (Table 2).

17β-HSOR assay: preferred cofactor for determination of serum 17β-HSOR activity and effect of glycerol on serum freezing and thawing

$NADP^+$ and NAD^+ were evaluated as cofactors for 17β-HSOR in maternal, cord arterial, and cord venous sera. $NADP^+$ appeared to be more efficient as a cofactor than NAD^+ in maternal serum, and there were no apparent differences in enzyme activity in cord venous and cord arterial sera with either $NADP^+$ or NAD^+ (Table 3). $NADP^+$, therefore, was used to assay 17β-HSOR in serum.

Maternal and cord vein sera were mixed with glycerol (8:2, v/v) and kept at −20°C for 24 h; thereafter, 17β-HSOR activity was determined and compared with the enzymatic activity determined previously in corresponding fresh sera. We found, as have others with maternal serum [6, 7], that in frozen-thawed preparations of maternal and cord vein sera containing glycerol the specific activity of 17β-HSOR was similar to that obtained with freshly prepared sera. 17β-HSOR activities reported in this study, however, were determined by use of freshly prepared serum samples.

Distribution of 17β-HSOR activity in particulate and supernatant fractions of maternal serum and comparison of specific activity of the enzyme in serum and erythrocytes

Freshly prepared maternal serum was centrifuged at 200,000 *g* for 3 h: a particulate fraction and a supernatant fraction were obtained and 17β-HSOR activity in these fractions was determined: the enzyme was present almost exclusively in the supernatant fraction (Fig. 1A).

For comparison purposes, 17β-HSOR activity was determined in maternal, pooled umbilical cord (arterial plus venous), and cord arterial sera and in lysates of corresponding erythrocytes as well as in term placental microsomes. In these studies (Fig. 1B and Table 4, Experiment No. 2), the specific activity of 17β-HSOR, expressed per mg protein, was higher in maternal serum than in cord sera and erythrocytes, but the specific activity of placental microsomal 17β-HSOR was approximately 700-times higher than that of maternal serum enzyme (Table 4)

Effect of an anti-human placental cytosolic 17β-HSOR antibody on 17β-HSORs of maternal and umbilical cord sera, maternal and fetal erythrocytes, and placental microsomes

Preincubation of maternal, umbilical cord arterial, and umbilical cord venous sera at 24°C for 2 h with an antiplacental 17β-HSOR antibody [9] resulted in a

Table 2. *Kinetic isotope effect introduced by use of [17α-^{3}H]estradiol-17β as the substrate in the determination of 17β-HSOR activity in human maternal serum*[a]

Substrate	*N*	Maternal serum	Product: [6,7-^{3}H]E1 ^{3}H:^{14}C ratio (cpm:cpm) (Mean ± SD)				Tritium in the aqueous layer (cpm) (mean ± SD)	17β-HSOR spec. act. pmols E1 formed		Primary kinetic isotope effect
			TLC 1	TLC 2 (after acetylation)	Last mother liquor	Final crystals		mg protein-h (mean ± SD)		
[6,7-^{3}H]E2	4	+	9.57 ± 2.18	9.93 ± 2.33	9.74 ± 1.86	10.1 ± 1.87		2017 ± 394		
	1	−		0.12	0.06	0.02				
[17α-^{3}H]E2	5	+	0.17				14,633 ± 1154	786 ± 62		2.6
	5	−					227 ± 17			

[a]Freshly obtained human maternal serum (7 ml) was mixed with potasssium phosphate buffer (7 ml; 0.2 M; pH 7.4) that contained glycerol (40% v/v) and E2 (60 μM). Aliquots (1 ml) were incubated with either [6,7-^{3}H]E2 (1.46 μCi) or [17α-^{3}H]E2 (1.89 μCi) and $NADP^+$ (1 mM) at 37°C for 1 h. At the end of the incubation with [6,7-^{3}H]E2,[4-^{14}C]E1 (12,100 dpm) was added as the internal recovery standard. Control incubations were conducted in the absence of maternal serum. Estrone formation was quantified by [6,7-^{3}H]E1 isolation [10] or by determination of radioactivity in NADP^{3}H, depending on the substrate used for incubation, as described in the text. The ^{3}H to ^{14}C ratios of the final crystals and the mother liquors, obtained in the fifth crystallization procedure, are presented. *N* = Number of incubations.

significant decrease in 17β-HSOR activity in these fluids (~60–84%). Placental microsomal 17β-HSOR was inhibited approximately 55%, but there was no inhibition of 17β-HSOR activity in lysates of maternal or fetal erythrocytes (Table 4).

Patterns of binding of radiolabeled E2 to proteins in maternal and umbilical cord arterial sera and of 17β-HSOR activity

The patterns of binding activity of [6,7-^{3}H]E2 to proteins in maternal and umbilical cord arterial sera and of specific activity of 17β-HSOR were obtained by use of fractions collected after sucrose density gradient centrifugation, as presented in Fig. 2. The patterns of [^{3}H]E2-binding activity and 17β-HSOR activity (with a suggestion of two peaks) appeared to be approximately coincidental at ~$S_{\omega,20}$ 4.6–5 and were similar in both maternal and cord arterial sera. A peak of 17β-HSOR activity also was detected at ~$S_{\omega,20}$ 18 by use of maternal serum, however, a corresponding peak of binding activity was not present (Fig. 2).

17β-HSOR activity in maternal, cord venous, and cord arterial sera

The specific activity of 17β-HSOR in serum samples obtained from maternal blood and corresponding umbilical cord venous and arterial bloods obtained at the time of spontaneous vaginal delivery or cesarean section are presented in Table 5. The weight and sex of the fetuses as well as maternal weight also are given in Table 5. The ratios of specific activities of 17β-HSOR in the maternal serum samples and corresponding umbilical cord arterial serum ranged from 2.3 to 55, and those of maternal serum and cord venous serum ranged for 2.1 to 26.5. The mean ± SD for the specific activities of 17β-HSOR in maternal serum, cord arterial serum, and cord venous serum were 1.89 ± 1.38, 0.23 ± 0.12, and 0.16 ± 0.10 nmols estrone formed/ml serum · h, respectively; the differences in specific activity of the enzyme in maternal serum and those in umbilical cord arterial and cord venous sera were highly significant ($P < 0.01$), while the specific activities of the

Table 3. Evaluation of NAD^+ and $NADP^+$ as cofactors for 17β-HSOR in maternal and cord sera[a]

Patient	Serum	Cofactor NAD^+	Cofactor $NADP^+$	17β-HSOR nmol/ml/serum·h
L.N.[b]	Maternal	+	–	0.20[d]
		–	+	2.31[d]
	Pooled cord	+	–	0.09[d]
		–	+	0.08[d]
L.H.[c]	Maternal	+	–	0.29[d]
		–	+	1.38[d]
	Cord vein	–	+	0.06[e]
	Cord artery	–	+	0.06[e]
M.C.[b]	Maternal	+	–	0.24[d]
		–	+	2.56[d]
	Cord Vein	–	+	0.04[e]
S.R.[b]	Maternal	+	–	0.21[d]
		–	+	2.55[d]
	Pooled cord	–	+	0.05[e]
D.J.S.[b]	Maternal	+	–	0.16[d]
		–	+	0.75[d]
	Pooled cord	+	–	0.12[e]
		–	+	0.09[e]

[a]The specific activity of 17β-HSOR in freshly obtained maternal, umbilical cord arterial, cord venous, and pooled cord (venous plus arterial) sera was determined by use of [17α-^{3}H]E2 (0.56 μCi; 30 μM) as the substrate and either $NADP^+$ or NAD^+ (1 mM) as the cofactor in the presence of glycerol (20%) in either single or duplicate incubations conducted as described in the text. [b]Vaginal delviery; [c]cesarean section; [d]average of duplicate determinations; [e]single determination.

Table 4. Effect of an anti-human placental cytosolic 17β-HSOR antibody on 17β-HSOR activity of maternal, cord arterial, and cord venous sera, placental microsomes, and lysates of maternal and fetal erythrocytes[a]

Experiment No.	Tissue	Antibody	17β-HSOR pmols mg protein-h	17β-HSOR nmols[c] ml-h	P	Inhibition %
1.[b]	Maternal serum	–		1.00 ± 0.04[d]	<0.001	75
		+		0.25 ± 0.03[d]		
	Cord arterial serum	–		0.08[e]	—	81
		+		0.015[e]		
	Cord venous serum	–		0.02[f]	—	60
		+		0.008[f]		
2.[b]	Maternal serum	–	26 ± 4[d]	1.700 ± 0.13[d]	<0.001	84
		+	3.9 ± 0.3[d]	0.26 ± 0.12[d]		
	Cord arterial serum	–	5.3 ± 0.4[d]	0.27 ± 0.08[d]	<0.01	69
		+	1.6 ± 0.3[d]	0.08 ± 0.03[d]		
	Maternal erythrocytes	–	4.5 ± 0.3[d]	1.38 ± 0.45[d]	NS	—
		+	4.8 ± 0.5[d]	1.46 ± 0.23[d]		
	Cord arterial erythrocytes	–	8.2 ± 0.6[d]	2.66 ± 0.86[d]	NS	—
		+	10.7 ± 0.5[d]	3.46 ± 0.06[d]		
	Placental microsomes	–	18,100 ± 630[d]	—	<0.01	55
		+	8,200 ± 1900[d]	—		

[a]Maternal, cord arterial, and cord venous sera, placenta microsomes, and lysates of maternal and fetal erythrocytes, mixed with glycerol (20%) and phosphate buffer as described in the text, were incubated at 24°C for 2 h with and without anti-human placental cytosolic 17β-HSOR antibody (0.2 ml). Thereafter, the specific activity of 17β-HSOR was determined by incubation of these mixtures with [17α-^{3}H]E2 (0.56 μCi; 30 μM) and $NADP^+$ (1 mM) at 37°C for 1 h, as described in Experimental. [b]Protein concentrations were not determined in the sera used in Experiment No. 1. The protein concentrations of sera and erythrocytes used in Experiment No. 2 were as follows: maternal serum, 66.5 mg/ml; cord arterial serum, 50.9 mg/ml; packed maternal erythrocytes, 304 mg/ml; packed cord arterial erythrocytes, 321 mg/ml. [c]The specific activity of erythrocytes is expressed as nmol/ml packed erythrocytes · h. [d]Mean ± SEM of triplicate determinations. [e]Duplicate determinations. Single determination. NS, not significant.

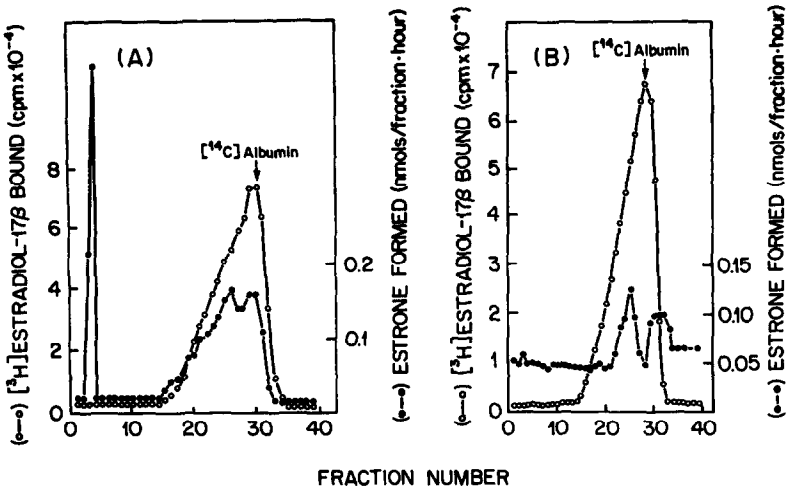

Fig. 2. Estradiol-17β binding (○—○) to proteins in maternal and cord arterial sera was determined by sucrose density gradient centrifugation, as described in the text. The specific activity of 17β-HSOR (●—●) in the fractions collected by centrifugation also was determined, as described. (A) Maternal serum. (B) cord arterial serum.

enzyme in cord arterial and cord venous sera were similar. There was no correlation between the mode of delivery, weight of the neonate, sex of the neonate or maternal weight with 17β-HSOR activity in maternal serum, cord venous serum or cord arterial serum (Table 5).

DISCUSSION

The 17β-HSOR-catalyzed reaction requires the participation of either reduced or oxidized cofactors, viz., NAD(P)H or $NAD(P)^+$, depending on the steroidal substrate, e.g. E1 or E2, respectively. The mechanism of oxidation of E2 to E1, catalyzed by

Table 5. Neonate and maternal weight, neonatal sex, mode of delivery, and 17β-hydroxysteroid oxidoreductase activity in maternal venous serum and umbilical cord arterial and venous serum

Neonate No.	Neonate weight (kg)	Sex	Maternal weight (kg)	Mode of delivery	17β-hydroxysteroid oxidoreductase activity (nmols product formed/ml serum·h) Maternal serum	Cord arterial serum	Cord venous serum
1	3.350	male	79.9	Repeat C/S, NIL, 40 wk	1.14 ± 0.30	0.54 ± 0.03	0.03 ± 0.006
2	4.870	female	73.1	Repeat C/S, NIL, term	1.99 ± 0.01	0.13 ± 0.01	0.13 ± 0.01
3	3.830	female	92.6	C/S, term, elective	1.58 ± 0.01	0.18 ± 0.02	0.13 ± 0.01
4	3.580	female	79.5	Breech, term, in labor	1.85 ± 0.01	—[b]	0.10 ± 0.01
5	3.480	male	74.9	C/S, chorio, mec.	1.33 ± 0.02	0.13 ± 0.02	—[b]
6	3.970	male	54.5	C/S, term, thin mec., fetal distress	7.15 ± 0.11	0.27 ± 0.02	0.13 ± 0.02
7	3.130	male	65.8	Stat C/S, abrup, 42 wk	2.80 ± 0.29	0.25 ± 0.02	0.23 ± 0.02
8	3.100	male	72.6	SVD, term	3.13 ± 0.33	0.18 ± 0.01	0.12 ± 0.01
9	3.770	female	78.1	SVD, term	2.90 ± 0.17	0.21 ± 0.02	0.12 ± 0.04
10	3.370	male	52.2	SVD, term	2.56 ± 0.16	—[b]	0.14 ± 0.03
11	0.525	male	69.0	Repeat C/S, NIL, ROM	1.23 ± 0.11	0.26 ± 0.04	0.53 ± 0.07
12	3.790	male	62.2	Repeat C/S, NIL	1.04 ± 0.08	0.15 ± 0.02	0.11 ± 0.04
13	1.700	male	76.3	SVD, PROM, 31 wk	1.50 ± 0.17	0.24 ± 0.03	0.09 ± 0.04
14	3.000	female	77.2	SVD, term	2.65 ± 0.10	0.24 ± 0.02	0.10 ± 0.02
15	3.440	female	89.0	Repeat C/S, NIL, term	0.97	0.15	0.23
16	3.250	male	68.1	Repeat C/S, NIL, term	0.86	0.09	0.28
17	2.840	male	84.0	Repeat C/S, NIL, term	1.57	0.56	0.19
18	3.015	male	97.6	Stat C/S, thick mec., term	1.35	0.19	0.23
19	3.070	male	49.0	Repeat C/S, NIL, term	1.07	0.18	0.10
20	3.650	female	69.0	Repeat C/S, NIL, term	1.04	0.29	0.14
21	3.475	male	99.9	C/S, CPD-chorio	0.84	0.07	0.11
22	2.950	male	56.8	Repeat C/S, NIL, term	1.10	0.19	0.09

Abbreviations used are: C/S, cesarean section; NIL, not in labor; mec., meconium; SVD, spontaneous vaginal delivery; Stat C/S, emergency cesarean section; ROM, rupture of membranes; PROM, preterm rupture of membranes: CPD, cephalopelvic disproportion. 17β-HSOR activities in samples No. 1–14 were determined in triplicate: the mean values and standard deviations are reported. 17β-HSOR activities in samples No. 15–21 were determined in single incubations.

The ratios of specific activities of 17β-HSOR in maternal and umbilical cord sera ranged from 2.3 to 55, and those of maternal and cord venous sera ranged from 2.1 to 26.5. The mean and standard deviations for the specific activities of 17β-HSOR in maternal, cord arterial, and cord venous sera were 1.89 ± 1.38, 0.23 ± 0.12, and 0.16 ± 0.10 nmols estrone formed/ml serum·h, respectively. The differences in specific activity of 17β-HSOR in maternal serum compared with those in cord arterial and cord venous sera were highly significant ($P < 0.01$).

17β-HSOR, involves the transfer of hydride (H:) from the C-17α-position of E2 to the C-4 position of nicotinamide of either NAD^+ or $NADP^+$ to give NADH or NADPH [16]. In a similar manner, tritide at the C-17α-position of [17α-^{3}H]E2 is transferred to $NADP^+$ to give $NADP^3H$ and nonradiolabeled E1 (Fig. 3). After incubation, the selective removal of unreacted radiolabeled E2 from the incubation medium permits the quantification of the $NADP^3H$ formed and, thus, 17β-HSOR activity. The primary kinetic isotope effect of 2.6 introduced by substitution of tritium for hydrogen at the C-17α position of E2 is taken into consideration in the computation of the specific activity of the enzyme. The isotope effect determined with [17α-^{3}H]E2 as the substrate was similar to that found by Adams *et al.* [11] by use of 17α-deuterated E2 as substrate.

Breuer *et al.* [17] reported that NAD^+ was slightly better than $NADP^+$ as a cofactor for maternal serum 17β-HSOR (0.86 nmols product formed/0.5 ml serum · 2 h vs. 0.72 nmols product formed/0.5 ml serum · 2 h), and Lubbert[6] reported that NAD^+ was twice as effective as $NADP^+$ as a cofactor for the maternal serum enzyme. In the present study we found that $NADP^+$ was more effective than NAD^+ as a cofactor for maternal serum 17β-HSOR, however, there were not apparent differences in fetal serum 17β-HSOR activity by use of either NAD^+ or $NADP^+$. The cause(s) for these discrepancies and the different relative activities of maternal and umbilical cord sera 17β-HSORs with NAD^+ and $NADP^+$ as cofactors is not readily evident. If maternal and umbilical cord sera 17β-HSOR(s) were of placental origin, we would expect that the response to added cofactors would be the same, and this is not the case.

It has been shown that 17β-HSOR activity in maternal serum decreases with freezing [6] and that the enzymatic activity can be preserved by addition of glycerol [6, 7]. In the present study we confirmed that glycerol preserved 17β-HSOR activity in maternal serum and also in cord vein serum for at least 24 h upon cooling at −20°C.

To establish whether 17β-HSOR activity in maternal serum was associated with light particles in serum, e.g. placental microvilli [18], we centrifuged serum at 200,000 *g* for 3 h to separate the pelletable fraction from the supernatant fraction; upon analysis, we found that 17β-HSOR activity was present almost exclusively in the supernatant fraction of serum, thus confirming the soluble nature of the enzyme.

The specific activities of 17β-HSORs in maternal, pooled cord (venous plus arterial), and cord arterial sera were compared with those of erythrocytes prepared from corresponding blood samples and with that of placental microsomes. The specific activity of 17β-HSOR in maternal serum (expressed per mg protein) was higher than that in maternal erythrocytes, pooled umbilical cord erythrocytes, cord arterial erythrocytes, pooled cord serum and cord arterial serum. The blood samples used in these studies were not hemolyzed, thus the presence of 17β-HSOR in serum was not the result of contamination by erythrocyte 17β-HSOR. These findings serve to emphasize the fact that the specific activity of 17β-HSOR in umbilical cord serum is low compared with that in maternal serum. The specific activity of maternal serum 17β-HSOR, however, was approximately 700 times lower than that of the enzyme in placental microsomes.

The patterns of binding of [6,7-^{3}H]E2 to proteins in maternal and umbilical cord arterial sera and those of 17β-HSOR activity were similar and approximately superimposable. These patterns were obtained by analysis of fractions collected after sucrose density gradient centrifugation. The binding of [^{3}H]E2 possibly takes place to albumin, sex steroid binding globulin, and/or 17β-HSOR [19]. In this study, 17β-HSOR activity in maternal serum also was detected at $\sim S_{\omega,20}$ 18, however, there was not a corresponding peak of [^{3}H]E2 binding activity. The enzymatic activity in this heavy fraction may be due to an aggregate of 17β-HSOR produced by the extended centrifugation at 4°C, since the activity was found close to the bottom of the centrifugation tube. The lack of [^{3}H]E2 binding may be due to the absence of the other binding proteins, mentioned above.

In the present study, we established that the levels of 17β-HSOR in maternal serum were 2.1- to 55-fold higher than those in corresponding umbilical cord arterial and venous sera. Moreover, 17β-HSOR levels in both umbilical cord arterial and venous sera were similar. While 17β-HSOR levels in maternal and cord venous sera were comparable to those reported by other investigators [1–8], 17β-HSOR levels in cord arterial serum were much lower than those reported by Plotti *et al.* [5]. We also demonstrated that an anti-human placental cytosolic

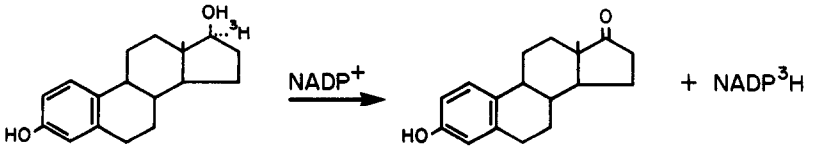

Fig. 3. The reaction involved in the assay of 17β-HSOR is illustrated. (1) [17α-^{3}H]Estradiol-17β; (2) Estrone; $NADP^3H$, [4-^{3}H]Nicotinamide adenine dinucleotide phosphate.

17β-HSOR antibody [9] inhibited maternal and umbilical cord sera 17β-HSOR(s) as well as the placental microsomal enzyme, but not the enzyme(s) present in maternal and fetal erythrocytes.

On the basis of findings of higher 17β-HSOR levels in maternal serum compared with those in fetal arterial serum, and on the inhibitory effect of the anti-human placental cytosolic 17β-HSOR antibody on maternal, cord arterial, and cord venous sera 17β-HSOR activity we suggest that umbilical cord arterial serum 17β-HSOR is of placental origin, as is the enzyme present in maternal [17] and umbilical cord venous sera [5]. It can be argued, however, that the cross reactivity of umbilical cord arterial enzyme with antiplacental-17β-HSOR antibody does not necessarily support the concept that the fetal serum enzyme is of placental origin since it is conceivable that some fetal tissue(s) may produce a soluble enzyme (immunologically similar to placental 17β-HSOR) that leaks into fetal serum and, thus, cross-reacts with the antibody. Additional studies, therefore, are necessary to ascertain unequivocally the origin of the umbilical cord arterial enzyme.

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