Compound W, a 3,3'-Diiodothyronine Sulfate Cross-Reactive Substance in Serum from Pregnant Women—A Potential Marker for Fetal Thyroid Function

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ABSTRACT: Compound W, a 3,3'-diiodothyronine sulfate (T_2S) cross-reactive material in maternal serum, was found to be useful as a marker for fetal hypothyroidism. In the present report, we explored its biochemical properties and studied its concentrations in cord and in maternal serum obtained from various gestational periods and at term from different continents. Mean W concentrations, expressed as nmol/L T_2S -equivalent, in maternal serum during gestation showed a moderate increase at 20–26 wk (1.57 nmol/L) and an accelerated increase to 34–40 wk (3.59 nmol/L). The mean serum level was relatively low in nonpregnant women (0.17 nmol/L). Compound W levels in cord and maternal serum at term were not significantly different among samples obtained from Taiwan compared with samples from the United States. The mean cord serum “corrected” (by hot acid digestion) concentrations of W were significantly higher than maternal serum concentrations at birth and were also higher in venous than in paired arterial samples, suggesting that the placenta may play a role in its production. We compared a total of 45 iodothyronine analogs by antibody, gel filtration, and HPLC chromatographic studies and found only one compound, N,N-dimethyl- T_2S , that has close similarities to Compound W. Further studies are needed. (*Pediatr Res* 61: 307–312, 2007)

Employing a sensitive RIA for T_2S , we found high concentrations in fetal and pregnant serum in humans (1). We further identified that this T_2S -cross-reactive material is not authentic T_2S and does not co-chromatograph with synthetic T_2S on HPLC. Thus, the name Compound W was coined to represent this material in fetal and maternal serum (1). Levels increased with the progression of pregnancy and rapidly peaked before parturition. At delivery, a 20-fold increase in “ T_2S ” was found compared with nonpregnant women and returned to baseline within 7–10 d. Serial measurements of serum W in pregnant women have been found to be useful as a noninvasive technique for the diagnosis of fetal hypothyroidism (2,3).

The present study was undertaken to determine the normal distribution of elevated Compound W levels in different stages of pregnancy as well as from various geographic areas and correlate between paired maternal and cord blood samples, and to explore its origin and chemical structure.

MATERIALS AND METHODS

T_2S RIA. 3,3'- T_2S and [^{125}I] T_2S were prepared by the method of Eelkman-Rooda and co-workers (4,5). T_2S was further purified and quantitatively recovered by reverse-phase HPLC with a preparative column, as described previously (1,2,4).

The T_2S RIA procedure was a modification of the RIA described previously (1,2). Serum samples (0.2–1.0 mL) were extracted with 2 vol 95% ethanol (final concentration, 63%) before assay. Preliminary experiments showed that the extraction efficiency of T_2S in serum exceeded 96%. Final T_2S concentrations were not corrected for recovery. The lower limit of detection of the assay was 3.3 fmol (2 pg), or 33.1 pmol/L in a 300 μ L ethanol extract of serum. Intra-assay variations were 1.9–9.1% and interassay variations were 6.0–19.5%, depending on the measured concentrations (6).

With respect to dose-responsiveness, we found close parallelism to the T_2S standard curve in the range between 10 and 250 pg/tube. The mean percentage deviation (± 1 SE) from the expected values was $11.1 \pm 1.6\%$ in the 10–100 pg/tube range and $6.1 \pm 2.3\%$ in the 100–250 pg/tube range. Higher deviations ($22.6 \pm 3.2\%$) were found in measurements exceeding 250 pg/tube. In the measurement of Compound W, 100 μ L of ethanol extract was used, which usually contains 5–100 pg per tube, corresponding to a T_2S equivalence of 15–300 ng/dL (or 0.248–4.96 nmol/L) in the serum sample.

rT_3S , T_3S , and T_4S RIA. [^{125}I] rT_3S , [^{125}I] T_3S , and [^{125}I] T_4S were prepared using a modification of the technique of Eelkman-Rooda *et al.* (4) using [^{125}I] rT_3 , [^{125}I] T_3 , and [^{125}I] T_4 and chlorosulfonic acid as reagents, as we previously reported (7,8). [^{125}I] rT_3S , [^{125}I] T_3S , and [^{125}I] T_4S were separated from the reaction products using anion gel chromatography. In addition, rT_3S , T_3S , and T_4S were prepared in a similar fashion using rT_3 , T_3 , and T_4 as substrates for sulfation. The reaction products were purified and quantitatively recovered by reverse-phase HPLC and were used as standards.

The rT_3S , T_3S , and T_4S RIA were performed using our previously described methods (7,8). For the T_4S RIA, the lower limit of detection averaged 2 ng/dL (23.3 pmol/L); interassay variation was 12.7% and intra-assay variation was 7.4%. For the T_3S RIA, the lower limit of detection averaged 2 ng/dL (27.4 pmol/L), and the inter- and intra-assay coefficients of variation were 11% and 6.9%, respectively. The lower limit for detection in the rT_3S assay was 1 ng/dL (13.7 pmol/L), with an interassay variation of 15.8% and intra-assay variation averaging 4.6%.

Abbreviations: D1, type 1 deiodinase; D3, type 3 deiodinase; rT_3 , 3,3',5'-triiodothyronine; rT_3S , 3,3',5'- T_3S 3,3',5'-triiodothyronine sulfate; T_2 , 3,3'-diiodothyronine; T_2S , 3,3'- T_2S 3,3'-diiodothyronine sulfate; T_3 , 3,3',5'-triiodothyronine; T_4 , thyroxine; T_4S , thyroxine sulfate; TH, thyroid hormone; 3'- T_1 , 3'-iodothyronine

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RESULTS

Identification and characterization of Compound W. T₂S immunoreactivity was studied in maternal or cord serum extracts by acid hydrolysis, HPLC, and RIA. Five to 10 mL cord or maternal serum were extracted with 2 vol 95% ethanol and subsequently lyophilized. The dried extracts were dissolved in H₂O and purified with an LH-20 column, as previously described (1,6). Radioactive or nonradioactive T₂S-supplemented serum extracts from nonpregnant women were used to identify the T₂S peak on HPLC. To study hot-acid stability, the serum samples were digested in 0.5 N HCl at 80°C for 1 h to hydrolyze the authentic T₂S and then assayed as described by Mol and Visser (1,5).

Synthesis of N,N-dimethyl-T₂S (DMT₂S). A synthesized novel thyroid hormone derivative has been found to co-chromatograph with W on HPLC and cross-react to T₂S antibody. Equimolar T₂S and iodomethane were allowed to react in dimethylformamide at room temperature for 30 s and dried in N₂. Dried samples were dissolved in 0.02 N NaOH and purified by HPLC. The purified product was analyzed by T₂S RIA and high-resolution mass spectrometry (MS) (9,10). RIA analysis revealed a T₂S-reactive peak, the elution time of which coincided with W peak on HPLC. The purified compound was found nonreactive to ninhydrin reagent, suggesting the loss of the amino group in the alanine side-chain. LC/MS/MS analysis, using a custom-built capillary RPC18 column (150 μ $\times$ 5 cm) HPLC system coupled to a trap mass spectrum, demonstrated a spectrum consistent with N,N-dimethyl-T₂S (DMT₂S) (Beckman Research Institute, City of Hope, Los Angeles, CA).

Sources of samples and materials. Serum samples were obtained from 195 pregnant women (17–40 y of age) with estimated gestational ages ranging from 3 to 40 wk and 33 venous cord blood at delivery from Taiwan. All blood-sampling protocols were approved by the Tri-Service General Hospital clinical research committee, and informed consent was obtained from all patients. In addition, 473 paired and 258 nonpaired maternal-cord, male euthyroid, and hyperthyroid patients' banked serum samples from Thailand, Colombia, Taiwan, and the United States were obtained.

3-Iodothyronine (3-T₁), 3'-iodothyronine (3'-T₁), 3,3'-diiodothyronine (3,3'-T₂), 3',5'-diiodothyronine (3',5'-T₂), 3,3',5'-triiodothyronine (3,3',5'-T₃), 3,3',5'-triiodothyronine (3,3',5'-T₃), thyroxine (T₄), 3,5-diiodothyronamine (3,5-diam), 3,3',5'-triiodothyronamine (3,3',5'-triam), and 3,3',5,5'-tetraiodothyronamine (tetram) were purchased from Henning-Berlin Co. (Berlin, Germany). Ninhydrin reagent, 3,3',5'-triiodothyroacetic acid (3,3',5'-triac), 3,3',5,5'-tetraiodothyroacetic acid (tetrac), 3,5-diiodothyropropionic acid (3,5-diprop), and 3,3',5'-triiodothyropropionic acid (3,3',5'-triprop) were purchased from Sigma Chemical Co. (St. Louis, MO). Sulfoconjugated analogs, including T₂ sulfamates, were synthesized by the methods of Mol and Visser (5). Glucuronidated analogs were produced by the methods of Hays and Hsu (11). 3,3'-Diiodothyroacetic acid (3,3'-diac) and 3,3'-diiodothyropropionic acid (3,3'-diprop) were synthesized by the iodination of 3-monoiodothyroacetic acid (3-monac) and 3-monoiodothyropropionic acid (3-monoprop). 3-Monac, 3-monoprop, and O-methyl-T₂ were obtained through the kind assistance of Dr. Hans Cohmann.

[¹²⁵I]3,3'-T₂ was prepared by radioiodination using the method described previously (12). [¹²⁵I]rT₃, [¹²⁵I]T₃, and [¹²⁵I]T₄ were purchased from NEN-PerkinElmer (Boston, MA). BSA, 1-ethyl-3-(3-dimethyl-aminopropyl) carbodiimide, iodomethane, and dimethylformamide were purchased from Sigma Chemical Co. (St. Louis, MO). Chlorosulfonic acid (99%) was purchased from Aldrich Chemical Co. (Milwaukee, WI). The Biochrom 1010 ODS preparative column was obtained from Regis (Morton Grove, IL).

Statistical analysis. Unpaired *t* test was used to assess between-group differences. ANOVA was used to test multigroup comparisons. If significant differences were present, Dunnett's multicomparison test was used to compare the control or baseline mean and the mean values of other groups (13). Significance was defined as *p* < 0.05. Results are reported as the mean $\pm$ 1 SD (or 1 SE in Fig. 3). In addition, simple linear regression analysis of the serum Compound W concentrations was used to test the correlation between at-term pregnant women samples and the paired cord samples (14).

Normal values of serum T₂S-equivalent levels in pregnant women.

A total of 195 serum samples from pregnant women from Taiwan at gestational ages ranging from 3 to 40 wk were examined. Serum mean concentrations of T₂S-cross-reactive material (expressed as T₂S equivalent) were elevated at 3–7 wk (*n* = 37, 0.98 nmol/L) compared with nonpregnant women (*n* = 17, 0.17 nmol/L; *p* < 0.01). Mean levels were found not to increase significantly at 8–13 wk (*n* = 32, 0.83 nmol/L; *p* = 0.17 versus 3–7 wk) or at 14–19 wk (*n* = 37, 1.17 nmol/L; *p* = 0.12 versus 3–7 wk). Moderate increases were noted at 20–26 wk (*n* = 17, 1.57 nmol/L; *p* < 0.01 versus 3–7 wk) and at 27–33 wk (*n* = 50, 2.38 nmol/L; *p* < 0.01 versus 3–7 wk). An accelerated increase was noted at 34–40 wk (*n* = 22, 3.59 nmol/L; *p* < 0.01 versus 3–7 wk) (Table 1, Fig. 1). The 20–40 wk increase correlates with the timing and progressive increase in fetal thyroid hormone secretion *in utero*. T₂S-equivalent values are similar in nonpregnant females and males (*n* = 30, 0.23 nmol/L) and are minimally increased in hyperthyroid patients (*n* = 22, equal number of males and females, 0.31 nmol/L; *p* = 0.01 versus nonpregnant women).

Correlation between fetal and maternal serum T₂S-equivalent and Compound W Concentrations. Figure 2 shows the concentrations of T₂S equivalent in 436 pairs of maternal serum and cord blood samples. The correlation coefficient of the linear regression was 0.686 (*p* < 0.01). In Figure 3, the mean ($\pm$ 1 SE) of the maternal serum concentrations of T₂S-reactive material (3.81 $\pm$ 0.07 nmol/L or 1.36 nmol/L for 1 SD) was significantly lower than that of the paired cord serum concentrations (4.91 $\pm$ 0.06 nmol/L or 1.31 nmol/L for 1 SD; *p* < 0.01 versus maternal levels). In our previous study, the authentic T₂S was hydrolyzed by hot-acid digestion (1). Using this procedure, the recovery of T₂S-cross-reactive material (mean $\pm$ 1 SE) was 77.1 $\pm$ 2.2% in cord serum (*n* = 6) and 87.4 $\pm$ 3.2% in maternal serum (*n* = 12; Fig. 3). The maternal and paired cord serum concentrations of T₂S-reactive material in Figure 2 were adjusted by these percentages to obtain the hot-acid-resistant T₂S-equivalent activity, or the “corrected” Compound W level. The “corrected” W mean level ($\pm$ 1 SE) was still significantly higher in cord serum (3.79 $\pm$ 0.05 nmol/L) than in maternal serum (3.33 $\pm$ 0.06 nmol/L; *p* < 0.01).

T₂S-equivalent values in cord and maternal serum at term from different geographic regions. Cord and at-term maternal serum samples from Asia (Taiwan) and the United States from normal pregnancy were studied. The serum T₂S-equivalent concentrations in cord and at-term maternal samples were

Table 1. Normal values of Compound W in pregnant women, nonpregnant women, and newborns

	Weeks of pregnancy						Cord	NP*
	3–7	8–13	14–19	20–26*	27–33*	34–40*		
<i>N</i>	37	32	37	17	50	22	33	17
Serum concentration (nmol/L)	0.98 $\pm$ 0.50	0.83 $\pm$ 0.35	1.17 $\pm$ 0.59	1.57 $\pm$ 0.86	2.38 $\pm$ 0.80	3.59 $\pm$ 1.86	4.34 $\pm$ 1.10	0.17 $\pm$ 0.05
Range (nmol/L)	0.48–1.48	0.48–1.18	0.58–1.76	0.81–2.41	1.58–3.18	1.73–5.43	3.24–5.43	0.12–0.22

Concentrations and ranges in mean $\pm$ SD T₂S-equivalent. For ng/dL, multiply by 60.5. NP, nonpregnant women.

* *p* < 0.05 compared with 3–7 weeks pregnancy

Figure 1. Normal values of T_2S -cross-reactive material in serum from pregnant women, nonpregnant women, and newborns. Vertical bars are mean $\pm$ 1 SD. The numbers of samples in each gestational period are shown in Table 1. * $p < 0.05$ compared with 3–7 wk pregnancy.

Figure 2. Levels of T_2S -cross-reactive material in paired maternal and cord serum at term. The solid line is the trend-line from linear regression analysis for the correlation ($n = 436$, $R = 0.686$). Dashed lines indicate mean $\pm$ 1 SD for maternal and cord serum levels.

measured and found to not differ significantly from one another (Table 2). However, cord and maternal serum samples from Thailand and Colombia were found to have slightly but significantly higher values (Table 2).

Possible origin of Compound W. Figure 4 shows the serum concentrations of sulfated iodothyronines from cord arterial and venous samples ($n = 8-9$). There were no significant differences between the mean T_3S , T_4S , or rT_3S concentrations of arterial and venous serum samples. However, the venous T_2S -equivalent concentration was higher than arterial in seven of the paired samples and lower in two. The mean “corrected” W concentration in nine pairs of cord serum was found to be significantly higher in venous samples ($2.67 \pm$

Figure 3. Recovery of T_2S -cross-reactive material following hot-acid treatment. (Top) Percentage of T_2S -cross-reactive fraction that is hot-acid stable in cord ($n = 6$) and maternal ($n = 12$) serum samples. (Bottom) Total and corrected W values (shaded bars) of 436 paired cord and maternal serum samples at term. Vertical bars indicate 1 SE. * $p < 0.05$ vs. maternal samples.

0.94 nmol/L) than in arterial samples (2.27 ± 0.91 nmol/L; $p < 0.04$).

Compound W: characterization and comparison with other analogs. A total of 45 known synthetic thyroid hormone analogs, synthesized, obtained commercially, or obtained as gifts from other laboratories, were subjected to HPLC and T_2S RIA, and their characteristics were compared with those of Compound W (Table 3).

The cross-reactivities of various other thyroid hormone analogs were studied, and only T_4S , TriacS, rT_3S , and T_3S cross-reacted significantly (3.2%, 1.7%, 1.4%, and 0.02%, respectively) (1). Other nonsulfated iodothyronines and sulfated steroids cross-reacted $<0.0001\%$ (1). Only N,N-dimethyl- T_2S was found to be in close similarity to Compound W in its retention time on HPLC and reaction to T_2S -specific antibody.

DISCUSSION

In developing mammals (including humans), a deficiency or excess of thyroid hormone (TH) in the developing brain during the fetal and neonatal periods can lead to morphologic and functional abnormalities (15–18). The most severe form of TH deficiency in human fetus and neonate is the syndrome of cretinism. A milder form of fetal TH deficiency is observed in children of women with subclinical hypothyroidism during pregnancy as assessed by IQ and other neuropsychological tests (19–21).

In sheep, a precocial species similar to humans, it has been shown that a significant amount of T_3 formed in the fetal

Table 2. Fetal and maternal blood Compound W levels among different countries

Country City	Thailand		Colombia		Taiwan Taipei	USA Los Angeles
	Bangkok	Nan	La Cumbre	Other		
Cord(T ₂ S eq.) (N)	(244)	(176)	(49)	(31)	(33)	(80)
nmol/L	4.92 ± 1.31	5.09 ± 1.47	5.13 ± 1.00	5.13 ± 1.17	4.34 ± 1.10	4.36 ± 1.21
Maternal(T ₂ S eq.) (N)	(252)	(175)	—	—	(43)	(73)
nmol/L	3.86 ± 1.36	4.17 ± 1.59	—	—	3.66 ± 1.29	3.43 ± 1.20

For ng/dL, multiply by 60.5.

circulation is converted to T₂S and transferred to the maternal compartment for deiodination/excretion (22,23). This process may serve to maintain low circulating T₃ levels in the fetus. Since T₂S appears to be quantitatively derived from circulating T₃ in the fetus, a significant increase or decrease in T₂S in the maternal circulation would suggest hyper- or hypothyroidism in the fetus (22). Based on previous studies in sheep, our working hypothesis is that placental tissue, with its iodothyronine sulfotransferase(s) and type 3 deiodinase (D3), may be the site of sulfation that facilitates the unidirectional transfer of T₂S from the fetal to the maternal circulation in pregnant ewes (in humans T₂S is proposed to be further metabolized to Compound W; Fig. 5). The postulated immediate precursor for W is 3,3'-T₂S. T₂S could be derived from T₃S by the activities of type 1 deiodinase (D1), which generally is low in fetuses. A more likely route would be from the inner-ring deiodination of T₄ to rT₃ by D3, and then to T₂ which is an excellent substrate for sulfotransferases hSULT1A1 and hSULT1E1 (3). T₃ is a poor substrate to both sulfotransferases and could be readily converted to T₂ by D3. The higher level of W in venous cord blood than that in arterial samples suggests that the placenta may play a role in its production and preferential transfer to the maternal compartment (Fig. 4, Fig. 5). By removing fetal circulating iodothyronines, *e.g.* T₃, it

may critically regulate the homeostasis of thyroid hormones in the fetus, especially in the later stage of development when D1 begins to mature in fetal tissues (8,24,25).

Prior studies identified that a T₂S-cross-reactive material, Compound W, is not authentic T₂S, and does not co-chromatograph with synthetic T₂S on HPLC in fetal and maternal serum (1). Maternal levels increased with the progression of pregnancy and rapidly peaked before parturition. Serial measurements of serum W in pregnant women have been found to be useful as a noninvasive technique for the diagnosis of fetal hypothyroidism in pregnancies associated with fetal goiter (2,3). In the present report, we established the “normal” levels, expressed as T₂S-equivalent, in different gestation periods (Table 1) and also showed the majority of the T₂S-cross-reactive material is W in maternal serum at term (Fig. 3). The increased levels of serum W in early pregnancy (Fig. 1), before the beginning of fetal thyroid hormone production, are in agreement with the increase in maternal T₄ needs and the apparent maternal to fetal transfer of T₄ (Fig. 5). The presence of TH in the fetus (26,27) and the access to the placenta appear to be critical for the production of Compound W. We also showed that there are similar cord and maternal levels at term in samples from the United States compared with those from Taiwan, where all the “normal” samples in Table 1 were obtained. Samples from other regions, including Thailand and Colombia, were found to have a slight but significant increase in cord and maternal serum at term (Table 2). Since the exact iodine intakes in these regions are unknown, the cause(s) of this difference is not clear and deserves further study.

Authentic T₂S is hydrolyzed by hot-acid digestion and constitutes a minor fraction in total T₂S-cross-reactivity in serum from mother at term or from cord (Fig. 3). The adjustment of Compound W values for the hot-acid-resistant T₂S-equivalent activity revealed that fetal “corrected” Compound W levels are significantly higher than those in maternal sera (Fig. 3). On the other hand, significantly more authentic T₂S in cord samples than in maternal samples is consistent with higher circulating sulfated iodothyronines in developing mammals, likely a result of lower D1 activity compared with adults (3).

The higher level of W in venous cord blood than that in arterial samples is consistent with our postulation that the placenta may play an essential role in its production and preferential transfer to the maternal compartment (Fig. 4). Interestingly, other iodothyronine sulfates, including T₃S, T₄S, and rT₃S, are found at essentially the same levels in

Figure 4. Sulfated iodothyronine (or the equivalence in T₂S) levels in arterial (A) and venous (V) cord serum. Horizontal bars indicate the mean. *For conversion to nmol/L T₂S, multiply by 0.0165; nmol/L T₃S and rT₃S, multiply by 0.0156; nmol/L T₄S, multiply by 0.0148.

Table 3. HPLC retention times of various T_4 analogs

T_4 analog	HPLC retention (min)*		Cross-reactivity (T_2S), %
	22:78	35:65	
3-Iodothyronine (3- T_1)	6	—	<0.0001
3- T_1S	3	—	
3'- T_1	10	—	<0.0001
3'- T_1S	6	—	0.008
3'- T_1G	2.5	—	
3,3'-Diiodothyronine (3,3'- T_2)	—	3.5	
3,3'- T_2S	7.5	—	
3,3'- T_2G	4	—	
3',5'- T_2	24	—	<0.0001
3',5'- T_2G	4	—	
3,5- T_2	13	—	<0.0001
3,5- T_2S	6	—	<0.0001
3,3',5-Triiodothyronine (3,3',5- T_3)	—	5.5	<0.0001
T_3S	14.5	—	0.02
T_3G	7.5	—	
3,3',5'-Triiodothyronine (rT_3)	—	7	<0.0001
rT_3S	11.5	—	1.4
rT_3G	5	—	
Thyroxine (T_4)	—	10	<0.0001
T_4S	22	—	3.2
T_4G	8.5	—	
3-Iodothyroacetic acid (3-Monac)	—	7	
3,3'-Diiodothyroacetic acid (3,3'-Diac)	—	23	
3,3'-DiacS	42.5**	—	
3,3'-DiacG	21.5	—	
3,3',5-Triiodothyroacetic acid (Triac)	—	31.5	<0.0001
TriacS	88.5**	—	1.7
TriacG	—	4.5	
3,3',5,5'-Tetraiodothyroacetic acid sulfate (TetracS)	143.5**	—	
3-Monoiodothyropropionic acid (3-Monoprop)	—	13	
3,3'-Diiodothyropropionic acid (3,3'-Diprop)	—	39	
3,3'-DipropS	—	4	
3,5-Diprop	—	4.5	
3,5-DipropS	19.1	—	
3,3',5-Triiodothyropropionic acid (3,3',5-Triprip)	—	68	
3,3',5-TripripS	—	7	
T_2 Sulfamate	42	—	
T_2S Sulfamate	12.5	—	
O-Methyl- T_2	26	—	
O-Methyl- T_2S	12	—	
3,5-Diiodothyronamine (3,5-Diam)	35.6	—	
3,5-DiamS	17.3	—	
3,3',5-Triiodothyronamine (3,3',5-Triam)	—	8.5	
3,3',5,5'-Tetraiodothyronamine (Tetram)	—	20.5	
N,N-Dimethyl- T_2S	10	—	? 100
Compound W	10	—	? 100

* Standard run was 25 min with acetonitrile and ammonium acetate buffer at ratio of 22:78, followed by 35:65 for another 25–80 min as needed.

** Elution continued at 22:78 ratio without changing to 35:65.

Figure 5. Postulated routes of fetal to maternal transfer of iodothyronine sulfates in sheep (and humans). A postulated mechanism to remove fetal circulating T_3 in late gestation in sheep (>120 d of gestation, term = 150 d). D_1 , D_2 , and D_3 : type 1, type 2, and type 3 deiodinases; ST : iodothyronine sulfotransferases. Heavy solid lines indicate pathways that are more active in fetuses than in adults; thin solid lines, pathways that are less active in fetuses. Dashed lines indicate postulated fetal iodothyronine substrates as precursors for T_2S transferred into maternal circulation; T_3 and 3,3'- T_2 could be converted to T_2S in the uteroplacental unit. In humans, the T_2S could be further metabolized to Compound W. The light dotted line depicts T_4 of maternal origin moving to the fetal compartment in the first trimester, before the fetal thyroid begins functioning, to act as the substrate to the Compound W that is measured in the first trimester.

venous and arterial serum. Recently, Williams *et al.* (28) have demonstrated that serum T_4S levels exhibit a postnatal transient increase in preterm infants. It is not known whether there is a similar increase in T_2S . It would be interesting to study the serum content of preterm infants; an increase in T_2S without a significant corresponding increase in Compound W, *i.e.*, a significant decrease in percentage of W compared with cord (Fig. 3), would suggest nonfetal origin of Compound W.

Serum levels of T_2S -immuno cross-reactive material have been recently measured by Chopra (29). In this UCLA study, compared with normal subjects (mean T_2S -equivalent value, 0.86 nmol/L) there is a significant rise in T_2S -immuno cross-reactive material in pregnant women with different gestational ages (2.7 nmol/L) and in newborn cord serum samples (18 nmol/L). It is not clear why the normal serum level was 5-fold higher than that in our nonpregnant women (0.17 nmol/L). Chopra's data are somewhat difficult to interpret, since HPLC and hot-acid digestion were not used to separate T_2S from Compound W in the study (29). Nevertheless, Chopra's data independently demonstrate that there is an increase in T_2S -cross-reactive material in pregnant and fetal serum. In the Chinese mainland, Chen *et al.* (33) found a mean serum concentration of 0.23 nmol/L in nonpregnant women and a progressive rise with gestational age to a mean of 2.79 nmol/L in pregnant women in the third trimester, similar to our values in normal pregnancy (Table 1).

The chemical structure of Compound W remains to be elucidated. In the present report we evaluated 45 thyroid hormone analogs and found that only one, N,N-dimethyl- T_2S , is biochemically and immunologically similar to Compound W. However, this novel compound has not yet been identified in human biologic fluids, nor has its presence been determined

in pregnant women or fetuses. Furthermore its stability and binding to serum proteins *in vivo* have yet to be studied. It does appear that transferred maternal T₂S of fetal origin is further metabolized to Compound W in human pregnancy, which may prolong biologic half-life and facilitate measurement in the maternal circulation during pregnancy (1). Thus far, Compound W has not been identified in sera from other Mammalia, including rats, mice, pigs, horses, cattle, sheep or guinea pigs (Wu SY, Huang WS, and Fisher DA, unpublished observations). Our studies in humans, however, indeed suggest that Compound W is derived from thyroid hormones of fetal origin and can be a practical noninvasive marker for fetal thyroid function (1,2,34–36).

Even though the biochemical structure of Compound W is yet to be established, its clinical evaluation as a useful potential marker for fetal hypothyroidism should be continued using the available methodology. In evaluation of PTU-treated mothers with Graves' disease, Van Middlesworth found that subnormal W levels imply excessive antithyroid medication (Van Middlesworth L. *et al.* 2005 A potential monitor of antithyroid therapy during pregnancy. Presented in International Thyroid Congress, Buenos Aires, Argentina, Oct. 30–Nov. 4, 2005); application of W measurements in pregnancies associated with fetal goiter, in association with serial ultrasonographic measurement, may allow reduced reliance on cordocentesis for fetal therapy management in these cases (34,37).

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