A Radioimmunoassay for Measurement of 3,3′-Diiodothyronine Sulfate: Studies in Thyroidal and Nonthyroidal Diseases, Pregnancy, and Fetal/Neonatal Life

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Data suggesting that (1) sulfation of the phenolic hydroxyl of iodothyronines plays an important role in thyroid hormone metabolism and (2) maternal serum 3,3′-diiodothyronone sulfate (3,3′-T_2S) may reflect on the status of fetal thyroid function stimulated us to develop a radioimmunoassay (RIA) for measurement of T_2S. Our T_2S RIA is highly sensitive, practical, and reproducible. T_4S, T_3S, and T_1S crossreacted 3.1%, 0.81%, and 5.3%, respectively; thyroxine (T_4), triiodothyronine (T_3), and reverse (r)T_3, 3,3′-T_2 and 3′-T_1 crossreacted <0.1%. Although rT_3 sulfate (rT_3S) crossreacted 55% in 3,3′-T_2S RIA, its serum levels are very low and have little influence on serum T_2S values reported here. T_2S was measured in ethanol extracts of serum, amniotic fluid, and urine. Recovery of nonradioactive T_2S added to serum was 96%. The dose-response curves of inhibition of binding of ^{125}I-T_2S to anti-T_2S by serial dilutions of ethanol extracts of serum or urine were essentially parallel to the standard curve. The detection threshold of the RIA varied between 0.17 and 0.50 nmol/L (or 10 and 30 ng/dL). The coefficient of variation (CV) averaged 9% within an assay and 13% between assays. The serum concentration of T_2S was [mean ± SE, nmol/L] 0.86 ± 0.59 in 36 normal subjects, 2.2 ± 0.06 in 10 hyperthyroid patients ($P < .05$), 0.73 ± 0.10 in 11 hypothyroid patients (not significant [NS]), 6.0 ± 1.5 in 16 patients with systemic nonthyroidal illness ($P < .001$), 18 ± 2.5 in 16 newborn cord blood sera ($P < .02$), 2.7 ± 0.32 in 25 pregnant women [15 to 40 weeks gestation, $P < .001$], 0.94 ± 0.10 in 10 hypothyroid women receiving T_4 replacement therapy (NS), and 2.0 ± 0.38 in 11 hypothyroid women treated with T_4 replacement and oral contraceptives ($P < .02$); serum T_2S levels in the third trimester of pregnancy were similar to those in the second trimester of pregnancy. T_2S concentration in amniotic fluid was 12.5 ± 2.7 nmol/L (n = 7) at 15 to 20 weeks gestation, and it decreased markedly to 3.3 ± 1.3 nmol/L (n = 3) at 35 to 38 weeks gestation. Urinary excretion of T_2S in random urine samples of 19 normal subjects was 10.9 ± 1.3 nmol/g creatinine. (1) T_2S is a normal component of human serum, urine, and amniotic fluid, and serum T_2S levels change substantially in several physiologic and pathologic conditions; (2) high serum T_2S in pregnancy may signify increased transfer of T_2S from fetal to maternal compartment, estrogen-induced increase in T_2S production, decreased clearance, or a combination of these factors. The data do not support the notion that fetal thyroid function is the only or the predominant factor responsible for high serum T_2S in pregnant women.

SEVERAL STUDIES HAVE shown that sulfation of the phenolic hydroxyl contributes importantly to iodothyronine metabolism in health and disease. Sulfoconjugates of several iodothyronines including thyroxine (T_4), 3,5,3′-triiodothyronine (T_3), reverse (r) T_3 (3,3′, 5′-T_3) have been identified in human biological fluids.[1-4] Additionally, maternal serum levels of 3,3′-diidothyronine sulfate (T_2S) or a related compound (compound W) may reflect on the status of fetal thyroid function.[5,6] To further evaluate this important consideration and to study serum T_2S concentration in health and disease, we have developed a sensitive, practical, and reproducible radioimmunoassay (RIA) of T_2S and applied it to measurement of T_2S in human serum, urine, and amniotic fluid. The RIA method and the initial data obtained with it form the subject of this report.

MATERIALS AND METHODS

3,3′-T_2S and ^{125}I-labeled 3,3′-T_2S were prepared in our laboratory using the method of Eelkman-Rooda et al[7] and Mol and Visser.[8] Preparation of T_2S required purified L-3,3′-T_2. For preparation of ^{125}I-labeled T_2S, L-3-T_1 was first iodinated with ^{125}I as described previously, and the products of radioiodination (3,3′-T_2, rT_3, and iodine) were separated chromatographically[9] and ^{125}I- 3,3′-T_2 was sulfated as previously described.[7,8]

The RIA used an anti-T_2S antibody obtained from 1 of 2 New Zealand white female rabbits (~4 kg in weight) immunized with an emulsion of 1-mL solution of L-3,3′-T_2S-bovine serum albumin (BSA) conjugate (containing ~2 mg BSA) and an equal volume of complete Freund's adjuvant subcutaneously in 4 to 6 sites on the back. Booster injections comprised 1 mL conjugate and an equal volume of incomplete Freund's adjuvant. They were given at 4- to 6-week intervals. Antiserum selected for our RIA was obtained at 3 weeks after the fifth immunization. It bound approximately 33% of a tracer amount (~10 pg) of ^{125}I-3,3′-T_2S at a final dilution of 1:100,000 in 1 mL 0.075 mol/L barbital buffer (pH 8.6) containing 0.25% normal rabbit serum (NRS). Ethanol does not affect the binding of ^{125}I-3,3′-T_2S to anti-T_2S in concentrations up to 25%. Therefore, we used ethanol extracts of sera and other body fluids containing approximately 63% ethanol for measurements of T_2S; the final ethanol concentration in the RIA tube was 19%.

RIA Procedure

The basic RIA procedure was a modification for the RIA described previously for T_3S.[3] The following reagents were added to 10 × 75 mm disposable glass tubes in 2 or more replicates: (1) 0.075 mol/L barbital buffer (pH 8.6) containing 1 g/L sodium azide and 0.25% NRS in a volume sufficient to adjust the final volume to 1 mL; (2) 300 μL ethanol (95% ethanol-water, 2:1, vol/vol) in standard curve tubes and an equal volume of an ethanol extract (ethanol-serum, 2:1, vol/vol) of test specimen, representing 100 μL original specimen; and (3) nonradioactive T_2S for the standard curve. One hundred microliters of various solutions of T_2S were added to place 10 pg (16 fmol) to 10 ng (16 pmol) T_3S in tubes for an 8- to 12-point standard curve. T_2S had

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been dissolved in 0.01 mol/L sodium hydroxide to a concentration of 10 mg/mL. Subsequent dilutions and those used in the RIA standard curve were made in barbital buffer; (4) 100 μL of 1:10,000 diluted T_2S-binding rabbit antiserum; and (5) approximately 10 to 15,000 cpm [^{125}I]T_2S (~5 pg T_2S) in 100 μL barbital buffer. The tubes were mixed after each addition and then incubated at 4°C for 20 to 24 hours. Selection of this period of incubation was based on preliminary experiments, which indicated that near-maximal binding had occurred by this time. A sufficient volume (~25 to 50 μL) of a previously tittered goat antirabbit gamma globulin (second antibody) and 0.6 mL 10% polyethylene glycol (Carbowax 6000; Sigma Chemical, St Louis, MO) were then added. The tubes were mixed, incubated at 4°C for 10 minutes, and centrifuged at 1,000 × *g* at 4 to 5°C for 30 minutes. Subsequent steps of separation of bound from free radioactivity, correction for nonspecific binding or trapping of [^{125}I]T_2S in the precipitate (~3%), and plotting of standard curve were undertaken as described previously for RIAs of other iodothyronines.[10-14] The T_2S content in 100-μL test specimens was read off the standard curve, and results are expressed as nanogram per deciliter or nmol/L.

Sources of Specimens

Serum samples were obtained from 36 normal subjects, 18 to 73 years of age (controls); 10 clinically hyperthyroid patients with Graves' disease, 20 to 51 years of age; 11 clinically hypothyroid patients, 30 to 78 years of age; and 16 patients with a variety of systemic nonthyroidal illnesses (NTIs), 26 to 67 years of age. Among NTIs, the major clinical disorders included liver disease (2 patients), renal failure (3 patients), heart disease (congestive heart failure, 6 patients), lung disease (cor pulmonale, 2 patients), and/or neoplastic disease (5 patients). The diagnosis of Graves' disease was based on the findings of diffuse goiter, elevated serum T_3 and T_4 concentrations, infiltrative ophthalmology, and/or increased 24-hour thyroid radioiodine uptake. The clinical diagnosis of hypothyroidism was confirmed by diminished serum T_4 concentration and elevated serum thyroid-stimulating hormone (TSH) concentration. Serum was also collected from 21 female subjects, 20 to 40 years of age, who had hypothyroidism and were rendered clinically and biochemically euthyroid with T_4 replacement; some of them (n = 11) were also taking oral contraceptives. Sera were also collected from 25 pregnant women, 20 to 40 years of age, with 15 to 40 weeks gestation, and from the cord blood of 16 full-term fetuses at the time of vaginal delivery. Amniotic fluid samples were obtained from pregnant subjects at 15 to 20 weeks (n = 7) or 35 to 38 weeks gestation (n = 3). Random (typically morning) samples of urine were obtained from 19 normal subjects, 20 to 62 years of age. The study was approved by the Institutional Review Board (IRB) at UCLA.

Competitive Protein Binding Assay of T_4

The competitive protein binding assay (CPBA) of T_4 was used, as described by Murphy and Pattee,[15] to study the relative binding of iodothyronines and their sulfoconjugates to human serum thyroxine binding globulin (TBG). The binding of ^{125}-I-T_4 to TBG in normal serum (diluted 1/32 in 0.075 mol/L barbital buffer, pH 8.6) was studied in the absence and presence of several concentrations of various nonradioactive iodothyronines and their sulfoconjugates; free and bound T_4 were separated using ion exchange resin, Amberlite IRA-400 (Mallinckrodt Chemical Works, St Louis, MO). The binding of ^{125}I-T_4 to TBG in the absence of stable iodothyronines was arbitrarily expressed as 100%, and all binding data in the presence of various iodothyronines were expressed as percent of this (100%) value. The percent ^{125}I-T_4 bound in the presence of various concentrations of iodothyronines studied were plotted on a semilogarithmic plot. The relative potency of the binding of iodothyronines to TBG was inversely related to the amount of iodothyronines required to cause a 50% displacement in the binding of ^{125}I-T_4 to TBG.

Table 1. Relative Reactivities of Various Thyroid Hormone Derivatives and Sodium Sulfate (Na_2SO_4) With T_2S-Binding Antibody

Compound	Relative Reactivity (arbitrary value if L-T_2S = 100)
T_4S	3.1
T_3S	0.81
rT_3S	55.0
3′-T_4S	5.3
T_4	<0.04
T_3	<0.03
rT_3	0.08
3,3′-T_2	0.10
3′-T_1	0.03
Na_2SO_4	<0.00001

Reagents

3,3′-T_2, 3-T_1 were generously provided by the late Dr Paul Block, Jr, River Research, Toledo, OH. Other thyronines and their derivatives, chlorosulfonic acid, N,N-dimethylformamide, 1-ethyl-3 (3-dimethylaminopropyl) carbodiimide, BSA, and Sephadex LH-20 were purchased from Sigma Chemical. Radioactive iodine (^{125}I) was purchased from New England Nuclear (Boston, MA).

Statistical Analyses

The data in the various groups are presented as the mean ± SE. Analysis of variance was used for comparison among various groups. If significant differences were observed, the means were compared by Student's 2-tailed *t* test for unpaired variates.[16] $P < .05$ was considered statistically significant.

RESULTS

Specificity and Sensitivity

Table 1 shows the data on the relative reactivities of various thyroid hormone derivatives based on the dose that caused an approximately 50% inhibition of the binding of [^{125}I]T_2S to anti-T_2S. Reverse T_3 sulfate (rT3S) crossreacted most (55%) with T_2S binding site on the anti-T_2S antibody followed by 3′-T_1S (5.1%), T_4S (3.1%), and T_3S (0.81%). Other thyroid analogues and sulfates crossreacted with anti-T_2S antibody with a potency less than 0.1% that of T_2S.

As shown in Fig 1, the dose response curve of 3,3′-T_2S RIA was essentially linear between 100 and 10,000 pg. Assay sensitivity, defined as the dose of 3,3′-T_2S that significantly ($P <$ .05) displaced ^{125}I-3,3′-T_2S from anti-T_2S antibody, varied between 10 and 30 pg in different assays.

Comparison of Samples With Standards

Fig 1 compares the dose-response curves of inhibition of the binding of ^{125}I-3,3′-T_2S to anti-T_2S antibody produced by ethanol extracts of some serum and urine specimens diluted in 63% ethanol. The final ethanol concentration was constant (19%) at each point. The various dose response curves were essentially parallel.

Recovery

The recovery of nonradioactve T_2S added to charcoal-treated human serum and to 2 normal human sera in concentrations ranging from 200 to 1,000 ng/dL in 12 experiments averaged

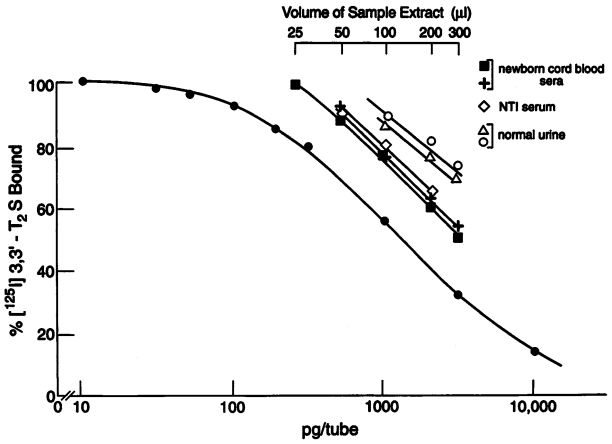

Fig 1. Dose-response curves. Inhibition of the binding of L-[^{125}-I] 3,3′-T_2S to anti-L-3,3′-T_2S antibody by increasing quantities of T_2S and increasing volumes of ethanol extracts of serum from 1 patient with NTI and 2 newborns and urine of 2 normal subjects is shown in a semilogarithmic plot.

96% ± 4.1%. Serum T_2S concentrations in specimens studied were not adjusted for recovery.

Reproducibility

The mean coefficient of variation (CV = SD/mean × 100) for serum samples varying in T_2S concentration from 200 to 800 ng/dL, each of which was tested in duplicate in the same assay, was 9%. The interassay variability was tested by assaying in duplicate in 2 assays 10 serum samples with T_2S concentrations ranging between 110 and 490 ng/dL. The mean interassay CV was 13%.

T_2S Concentrations in Health and Disease

Table 2 presents the data on mean serum T_2S concentrations in normal subjects and patients with various degrees of thyroid dysfunction. The distribution in serum T_2S levels in various groups studied is shown in Fig 2. The mean serum T_2S concentration in 36 healthy euthyroid subjects was 0.86 nmol/L (52 ng/dL). The mean serum T_2S concentration in 10 hyperthyroid patients of 2.2 nmol/L (132 ng/dL) was significantly ($P < .05$) higher than the corresponding mean value in normal subjects. However, the mean serum T_2S in 11 hypothyroid patients, 0.73 nmol/L (44 ng/dL), did not differ significantly from the normal mean value. The mean serum T_2S concentration of 6.0 nmol/L (366 ng/dL) in 16 patients with systemic nonthyroidal illnesses was clearly higher than the corresponding normal value ($P < .005$). Interestingly, we observed highest serum T_2S concentrations in the newborn cord blood serum. Thus, the mean serum T_2S concentration of 18 nmol/L (1,116 ng/dL) in 16 newborn cord blood was markedly higher than the mean value in normal adults ($P < .001$). Serum T_2S concentration was also elevated in pregnancy, but much less so than in cord blood serum. Thus, the mean serum T_2S concentration of 2.7 nmol/L (165 ng/dL) in 25 pregnant women with a 15- to 40-week gestation was significantly ($P < .001$) higher than corresponding value in normal adult subjects, and it was markedly lower than that (18 nmol/L) in the newborn cord blood serum. The mean serum T_2S value in the second trimester of pregnancy did not differ significantly from that in the third trimester of pregnancy. The mean serum T_2S of 0.94 nmol/L (57 ng/dL) in 10 euthyroid women receiving T_4 replacement therapy was comparable to that in normal adult subjects. However, the mean serum T_2S concentration of 2.0 nmol/L (118 ng/dL) in similarly euthyroid 11 women treated with T_4 replacement and oral contraceptives was clearly supranormal ($P < .02$), and it did not differ appreciably from the corresponding mean value in pregnant women.

T_2S concentration was also measured in amniotic fluid samples. It averaged (mean ± SEM) 12.5 ± 2.7 nmol/L (756 ± 166 ng/dL) in 7 samples obtained at 15 to 20 weeks gestation, and it decreased significantly ($P < .02$) to 3.3 ± 1.3 nmol/L (201 ± 80 ng/dL, n = 3) at 35 to 38 weeks gestation.

We also measured urinary excretion of T_2S in relation to creatinine in random urine samples of 19 normal subjects. The mean (± SEM) urinary excretion of T_2S was 10.9 ± 1.3 nmol/g (6.6 ± 0.77 µg/g) creatinine.

Relative Binding of Iodothyronines and Their Sulfoconjugates to TBG

Fig 3 describes the data on the displacement of ^{125}I-T_4 from human TBG by various iodothyronines and their sulfoconjugates. Based on the weight of iodothyronine that decreased the binding of tracer-T_4 to TBG by about 50%, T_4 bound to TBG most avidly and 3'-T_1S bound to TBG least avidly. 3,3′-T_2S bound to TBG with a potency only about 7.6% that of T_4. Among sulfoconjugates of iodothyronines, T_3S bound to TBG most avidly with a potency about 86% that of T_4.

DISCUSSION

We have described a sensitive, practical, and reproducible RIA for the measurement of 3,3′-T_2S in ethanol extracts of human serum, amniotic fluid, and urine. Our anti-T_2S antiserum crossreacted substantially (55%) with 3,3′,5′-T_3 sulfate (rT_3S). Interestingly, however, it turns out that rT_3S circulates in normal human serum in concentrations only about 1/20th that of 3,3′-T_2S (0.86 *v* 0.040 nmol/L or 52 *v* 2.6 ng/dL, vide supra, Wu et al[4]). Similarly, human amniotic fluid concentra-

Table 2. Serum T_2S Concentration in Various Thyroidal States

Group	No.	T_2S Concentration (nmol/L)	*P**
Normal subjects	36	0.86 ± 0.59	
Hyperthyroid	10	2.2 ± 0.06	<.05
Hypothyroid	11	0.73 ± 0.10	NS
Nonthyroidal illness	16	6.0 ± 1.5	<.005
Pregnancy	25	2.7 ± 0.32	<.001
Women treated with T_4 replacement	10	0.94 ± 0.10	NS
Women treated with T_4 replacement and oral contraceptives	11	2.0 ± 0.38	<.02
Newborn (fetal cord serum)	16	18 ± 2.5	<.001

NOTE. Data are mean ± SEM.
Abbreviation: NS, not significant.
*cf normal subjects.

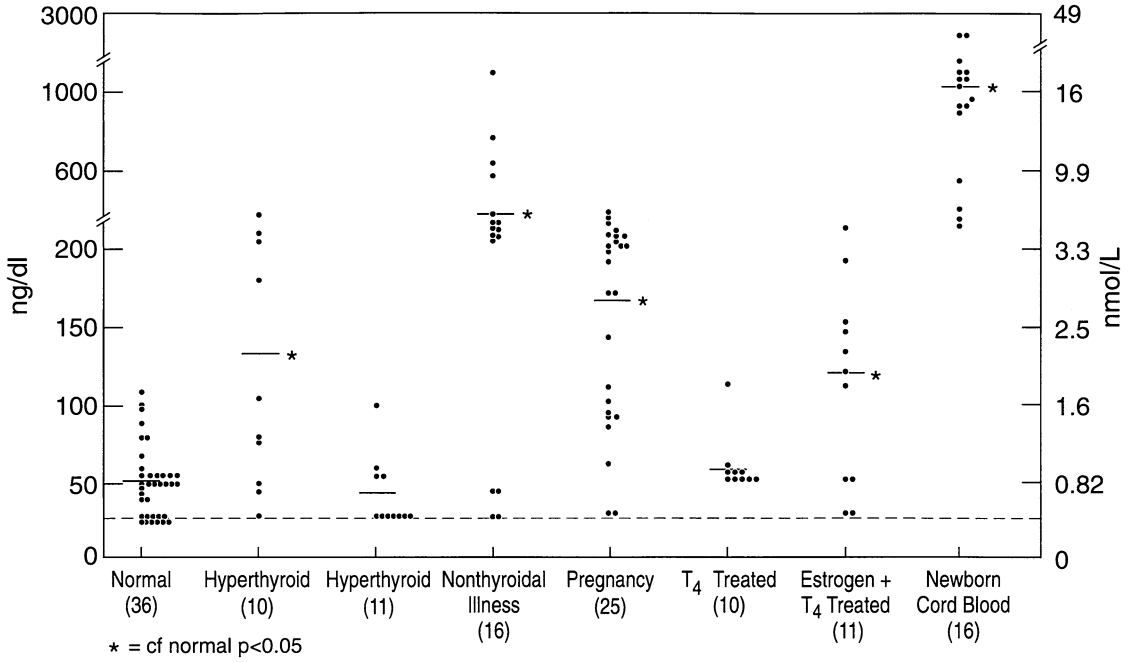

Fig 2. Serum 3,3′-T_2S concentrations in normal subjects, hyperthyroid patients, hypothyroid patients, patients with NTI, pregnant women (15 to 40 weeks gestation), women treated with T_4 replacement (T_4-treated), women treated with T_4 replacement and oral contraceptives (Estrogen and T_4-treated), and cord blood of newborns. The results are expressed as nanograms/deciliter and nanomolars. The horizontal bars represent mean values. The dotted line represents the detection threshold of the T_2S RIA. The numbers of subjects included in each group are indicated in parenthesis.

tion of rT_3S is less than 10% that of 3,3′-T_2S (vide supra, Wu et al[4]). Therefore, it is unlikely that crossreaction of rT_3S in our 3,3′-T_2S RIA significantly influenced the results of 3,3′-T_2S measured in this study. 3′-T_1S, T_4S, and T_3S crossreacted 5%, 3%, and 0.8%, respectively, in our assay. No data are available at present on serum or amniotic fluid concentrations of 3′-T_1S, and therefore we are unable to judge its influence on measurements of 3,3′-T_2S in this study. However, human serum concentrations of T_4S and T_3S average, respectively, only about 2.5% and 10% those of 3,3′-T_2S and are therefore unlikely to influence the values of T_2S measured by our T_2S RIA. Similarly, particularly abundant iodothyronines in human serum, ie, T_4 and T_3, are unlikely to significantly affect 3,3′-T_2S values described in this study because they crossreact minimally ($T_4 < 0.04\%$; $T_3 < 0.03\%$) in our 3′3-T_2S RIA.

Serum concentration of 3,3′-T_2S has been measured once previously.[17] The mean serum T_2S value of 0.17 nmol/L observed in a group of young (19 to 35 years) female subjects was lower than the normal mean value of 0.85 nmol/L noted in this study. The basis for the difference is not known, but methodologic differences and those in the RIA standards may have contributed to it. Additionally, there may have been a role in this difference of factors, such as fewer number (14 *v* 36), younger age (19 to 35 years *v* 18 to 73 years), and female only sex of subjects studied in the previous study.

Our data demonstrating increased serum T_2S levels in hyperthyroidism, pregnancy, and neonatal life and normal T_2S levels in hypothyroidism are similar to those in a previous preliminary report published in abstract form.[17] Additionally, we studied and found elevated serum T_2S in levels in nonthyroid illnesses and in women taking estrogen (Table 2). There was a rather wide variation in serum T_2S levels in patients with systemic nonthyroid illnesses (Fig 2). This observation seemed related more to the severity of the illness than its type or the organ system involved.

Circulating 3,3′-T_2S may derive from sulfoconjugation of 3,3′-T_2 generated either from monodeiodination of triiodothyronines (T_3 and rT_3) or from monodeiodination of T_3S (in the inner ring) and rT_3S (in the outer ring).[18-20] The type I iodothyronine 5′-monodeiodinase (5′-MDI) is selenoprotein that is abundant in the thyroid, the liver, and the kidney. It monodeiodinates iodothyronines ($rT_3 > T_4 > T_3$) in the outer ring to generate 3,3′-T_2 from rT_3 and T_3 fromT_4. Interestingly, however, this enzyme preferentially monodeiodinates sulfoconjugates of iodothyronines in the inner ring of the molecule; this reaction generates 3,3′-T_2S from T_3S and rT_3S from T_4S. Another deiodinase, ie, the type III 5-monodeiiodinase (5-MDIII) deiodinates iodothyronines ($T_3 > T_4$) and their metabolites in the inner ring of the molecule. Recent studies suggest that the 5-MDIII, also a selenoprotein, is most abundant in placenta, central nervous system, and skin.[21]

Available data do not indicate that 3,3′-T_2 or 3,3′-T_2S are secreted by the thyroid. Therefore, increased serum concentration of T_2S in Graves' hyperthyroidism probably reflects increased availability of substrates (eg, 3,3′-T_2, T_3S, rT_3S) that can be metabolized to generate T_2S. However, similar consideration does not explain the observed normal, rather than expected low, serum T_2S levels in hypothyroidism. The differ-

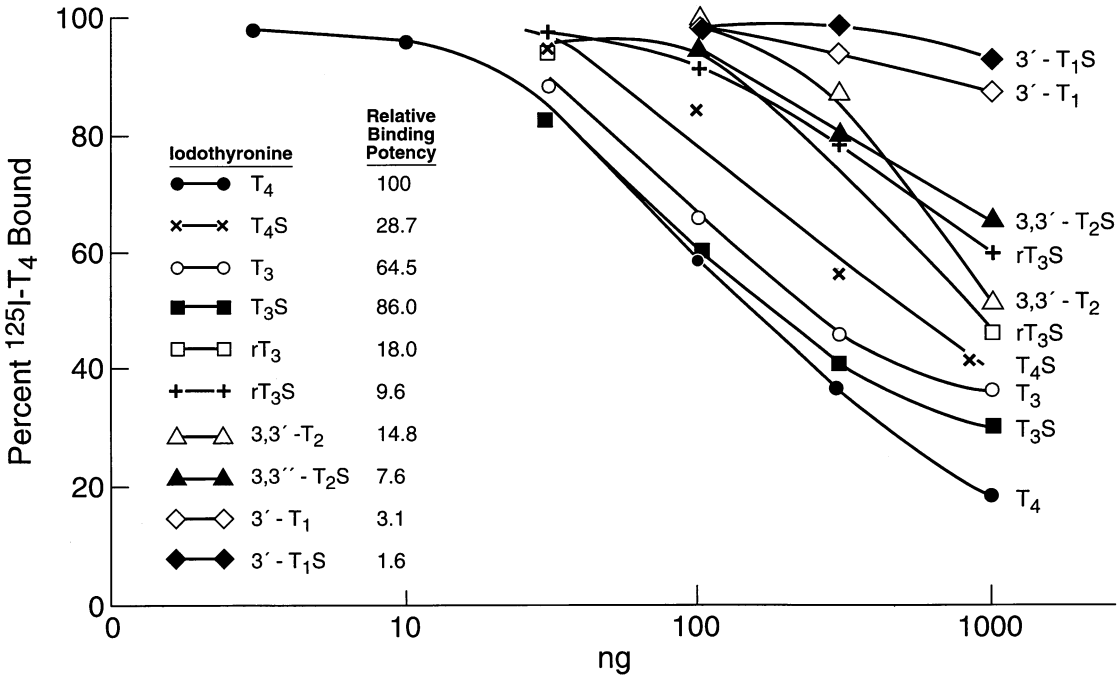

Fig 3. Dose-response curves. Inhibition of the binding of L-[^{125}I] T_4 to human serum TBG by increasing quantities of T_4, T_3, 3, 3′,5′-triiodothyronine (reverse T_3, rT_3), 3, 3′-diiothyronine (T_2), 3′-monoiodothyronine (3′-T_1) and their sulfosubjugates (T_4S, T_3S, rT_3S, 3, 3′-T_2S, and 3′-T_1S, respectively) is shown in a semilogarithmic plot.

ence could be related to decreased metabolic clearance rate of 3,3′-T_2S in hypothyroidism. However, no data are available at this time in favor of or against this explanation.

The activity of the 5′-MDI is decreased in the fetus and the newborn and in NTI. The finding of markedly increased serum T_2S levels in these conditions suggests that low 5′-MDI activity is associated with decreased clearance of 3,3′-T_2S. However, the possibility of increased production of T_2S in those conditions cannot be excluded; a previous study has demonstrated increasing serum T_2S levels with increasing gestational age in fetal sheep.[22]

The reason why the serum concentrations of 3,3′-T_2S is increased in pregnancy is not clear, but a number of factors may be involved. These may include increased production of 3,3′-T_2S in pregnancy, its decreased clearance, transplacental transport of fetal 3,3′-T_2S to the maternal compartment, or a combination of these factors. Pregnancy is associated with increased production of estrogen, which is known to increase serum concentration of TBG. It does so by increasing sialylation of TBG, which in turn, leads to a decrease in its metabolic clearance rate. Our finding that female subjects taking oral contraceptives had elevated serum 3,3′-T_2S concentration supports a possible role of estrogen in increased T_2S concentration in pregnancy. However, the basis for this effect of estrogen remains unclear. TBG binds 3,3′-T_2S rather poorly (Fig 3). Therefore, it is difficult to envision a major diminution in its metabolic clearance rate (MCR) in pregnancy, but some reduction in MCR-T_2S remains a possibility. It seems likely that estrogen enhances sulfation of iodothyronines, and that this mechanism is an important factor contributing to high serum 3,3′-T_2S during pregnancy and treatment with estrogen. Estrogen sulfotransferase has been demonstrated to facilitate sulfation of 3,3′-T_2 and other iodothyronines.[23] Transplacental passage of fetal 3,3′-T_2S to maternal compartment is another possible contributor to high serum T_2S levels in pregnancy, and this has been demonstrated in sheep.[24,25] However, the finding that serum T_2S concentration in female subjects taking oral contraceptives was elevated similarly to pregnancy suggests that the contribution of fetal 3,3′-T_2S to high human maternal serum 3,3′-T_2S levels is small. Previous studies have suggested that the bulk of the fetal iodothyronines transported across the human placenta are converted to "compound W," apparently a metabolite of 3,3′-T_2S.[5] The compound W crossreacts well with T_2S binding sites on some anti-3,3′-T_2S antibodies and RIA of T_2S with such an antibody demonstrates a gradual increase in compound W levels with increasing duration of pregnancy.[5] We did not find an appreciable gestational age-related increase in serum 3,3′T_2S levels in pregnancy. The basis for the difference in observation is not known, but it suggests that compound W does not react well with our anti-3,3′-T_2S antibody. Our data suggest that it is important to use a specific anticompound W antibody to examine the utility of measuring maternal serum compound W levels as a potential marker of fetal thyroid function and that the measurement of serum T_2S may not be useful for this purpose.

The biologic function of 3,3′-T_2S, if any, is not known at this time. Sulfoconjugation of 4'-hydroxyl group in iodothyronines has generally been considered a mechanism for inactivation and excretion of these compounds.[26] Sulfoconjugation of iodothyronines may also provide a substrate reservoir for biologi-

cally active iodothyronines.[27,28] Recently, 3,3′-T_2S has been demonstrated to stimulate mitochondrial respiration in several rat tissues.[29] The possibility that this and other T_4 metabolites may play a physiologic role in certain stages of life cannot be excluded. Additionally, sulfoconjugation of iodothyronines facilitates their transfer from the fetus to the mother, and their subsequent deiodination by iodothyronine deiodinases may help recover iodine that will be important for synthesis of new thyroid hormones.[8]

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