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Metabolism of 3,5,3′-Triiodothyronine Sulfate by Tissues of the Fetal Rat: A Consideration of the Role of Desulfation of 3,5,3′-Triiodothyronine Sulfate as a Source of T_3

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ABSTRACT. We have recently demonstrated that serum concentration of 3,5,3′-triiodothyronine sulfate (T_3S) is markedly elevated in the human newborn at a time when serum 3,5,3′-triiodothyronine (T_3) is very low. The present study explores the ability of maternal (19–21 d pregnant) and near-term fetal Sprague-Dawley rat tissues to *1*) monodeiodinate T_3S and T_3 in both the outer and the inner ring and *2*) desulfate T_3S to T_3. Maternal liver microsomes metabolized T_3S exceedingly efficiently (compare fetus $p < 0.05$). Eighty percent or more of T_3S was consumed during its incubation with 360 μg/mL microsomes for 2 h. The majority of the consumption of T_3S by adult liver microsomes occurred by its 5′-monodeiodination to I^-; little inner-ring monodeiodination to 3,3′-diiodothyronine was demonstrable. In fetal liver microsomes, however, over 75% of the substrate T_3S remained unchanged after a 2-h incubation. T_3 was metabolized similarly moderately by fetal and maternal liver microsomes. Brain microsomes metabolized T_3S poorly in both the mother and the fetus. Over 90% of substrate T_3S remained unchanged after a 2-h incubation in each case. Interestingly, brain microsomes metabolized T_3 more rapidly than T_3S ($p < 0.05$). In the fetus, desulfation of T_3S to T_3 was clearly evident only in microsomes from the liver and the brain; in the adult, it was plentiful in many tissues. Fetal liver and brain tissues metabolize T_3S poorly, and both actively desulfate T_3S to T_3. These data and those indicating high serum T_3S in the fetus suggest that T_3S is a local source of T_3 in critical tissues in the fetus and possibly in adults with the low T_3 syndrome. (*Pediatr Res* 31: 541–544, 1992)

Abbreviations

T_4, thyroxine
rT_3, 3,3′5′-triiodothyronine
T_3, 3,5,3′-triiodothyronine
T_3S, 3,5,3′-triiodothyronine sulfate
3,3′-T_2, 3,3′-diiodothyronine
3,3′-T_2S, 3,3′-diiodothyronine sulfate

T_4 is the predominant secretory product of the thyroid gland in man and experimental animals. The majority of T_4 produced daily is metabolized in extrathyroidal tissues either by 5′-monodeiodination in the outer (phenolic) ring to a much (~3–5×) more active iodothyronine, T_3, or by 5-monodeiodination in the inner (tyrosyl) ring to a calorigenically inactive iodothyronine, rT_3 (reverse T_3). Over 75% of T_3 and 95% of rT_3 produced daily in man derive from extrathyroidal metabolism of T_4, and the rest is secreted by the thyroid. Several recent studies have examined the enzymic processes that activate (or inactivate) T_4 to T_3 (or rT_3). It is clear that these processes are carefully regulated by the activity of several monodeiodinase enzymes serving to generate an optimal supply of thyroid hormone for the tissues (1–3). T_3 and rT_3, like T_4, are mainly metabolized by sequential monodeiodination in the outer and inner ring of the molecules until a completely deiodinated molecule, thyronine, is produced and excreted (4, 5).

Besides monodeiodination, thyroid hormones are also metabolized by conjugation of phenolic hydroxyl with either glucuronic acid or sulfate (3, Fig. 1). Such conjugation of compounds has generally been considered a means to inactivate and facilitate biliary and urinary excretion of hydrophobic aglycons (6). Sulfate conjugation of iodothyronines has evoked much interest recently (7–12). Thus, studies have shown a close interaction between sulfation and deiodination in a manner that may modulate the availability of the active thyroid hormone, T_3, in the tissues. Thus, sulfation of the phenolic hydroxyl of the iodothyronines (Fig. 1) is associated with a marked enhancement of both their outer and inner ring monodeiodination by the type I monodeiodinase in the liver (7–11). Additionally, sulfate conjugates of T_3 (T_3S) have now been measured in serum of man and experimental animals, and substantial increases have been observed in the fetus and newborn, in adults with hyperthyroidism and systemic illness, and in adults after administration of drugs that inhibit 5′-monodeiodination of iodothyronines (9, 10, 13). Increased serum T_3S concentration in fetal sheep and human newborn cord blood has been especially intriguing. A recent study has demonstrated desulfation of T_3S to T_3 by microsomes from some tissues and isolated hepatocytes of the adult rat (14). The present study was undertaken to gather insight into the metabolism of T_3S and T_3 in the fetus and the influence of changes in this system on thyroid hormone economy in fetal life.

MATERIALS AND METHODS

Reagents. Chlorosulfonic acid, N,N dimethylformamide, and LH-20 Sephadex were purchased from Sigma Chemical Company, St. Louis, MO. Radioactive (^{125}I-labeled) T_3 was purchased from New England Nuclear, Boston, MA; its sp act was approximately 1200 mCi/mg. Radioactive T_3S was prepared by reacting ^{125}I-T_3 overnight with a mixture of chlorosulfonic acid and N,N-dimethylformamide (1/4, vol/vol) at 0°C using the method of

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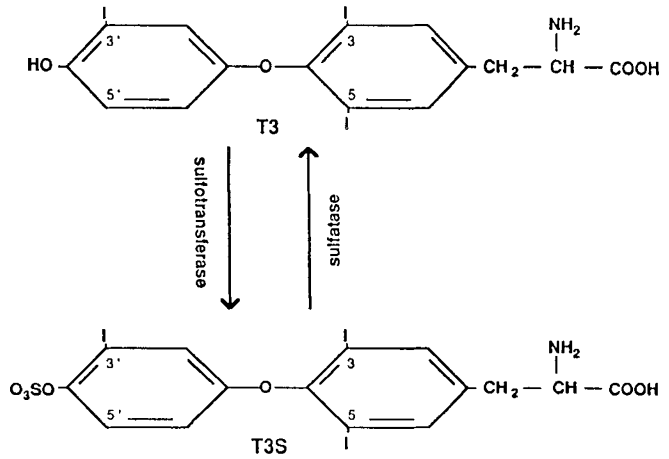

Fig. 1. The conversion of T_3 to T_3S by sulfotransferase in the tissues and the conversion of T_3S back to the parent hormone T_3 by sulfatases in the tissues is shown.

Mol and Visser (15). The reaction mixture was diluted with four volumes of cold (0°C) water and applied to a small (~1-mL) column of LH-20 Sephadex. The column was washed sequentially with 4 × 2 mL fractions of 0.1 N hydrochloric acid, 7 × 2 mL fractions of water, and 4 × 2 mL fractions of 1 N ammonia in ethanol. T_3S was eluted with water.

Tissues. Fetal and maternal tissues were obtained from 19- to 21-d pregnant Sprague-Dawley rats weighing about 320 g. Tissues were homogenized in 50 mM phosphate buffer (pH 7.4) containing 1 mM EDTA and 0.4 mM phenylmethyl-sulfonylfluoride (Sigma Chemical Co.); microsomes were prepared as previously described (16) and suspended by sonication (Branson Sonic Power, Danbury, CT).

Study of T_3 and T_3S metabolism. Deiodination. Microsomes from liver, cerebral cortex, and placenta (0.25–1000 μg protein/mL, final volume 0.5 mL) were incubated in 0.1 M Tris buffer with ^{125}I-T_3 (or ^{125}I-T_3S, sp act 1200 mCi/mg, ~0.6 nM) as substrate and DTT (10 mM) as a sulfhydryl donor-cofactor for 5 min to 4 h at 37°C.

Outer ring (5'-) monodeiodination of the outer ring (3' or 5') labeled ^{125}I-T_3 (or ^{125}I-T_3S) to $^{125}I^-$ by microsomes was studied at both pH 7.4 and 8.0; the data were essentially similar. After an incubation for 15 min to 4 h, the reaction was stopped by addition of 100 μL of 5% BSA to 200 μL of sample followed by 200 μL of 10% trichloroacetic acid. The mixture was centrifuged, and $^{125}I^-$ in the supernatant was quantified as described previously (17).

The inner ring (5-) monodeiodination of ^{125}I-T_3 (or ^{125}I-T_3S) to 3,3'-T_2 (or ^{125}I-3,3'-T_2S) by microsomes was studied both at pH 7.4 and 8.0; the data were essentially similar. After incubation, two volumes of ethanol were added to an aliquot of the reaction mixture and the mixture was centrifuged. ^{125}I-3,3'-T_2 was quantified in 75 μL of the supernatant by incubation with a highly specific rabbit anti-3,3'-T_2 antibody for 16 h at 4°C in a total volume of 1.0 mL; the antibody-bound ^{125}I-3,3'-T_2 was precipitated with a goat anti-rabbit gamma globulin (18). When ^{125}I-T_3S was the substrate, ^{125}I-3,3'-T_2S produced during incubation was hydrolyzed to 3,3'-T_2 by addition of 600 μL of 1.0 M HCl to 150 μL of sample, followed by heating at 80°C for 1 h (15). Subsequently, the pH of the mixture was adjusted to 7.4 and ^{125}I-3,3'-T_2 was quantified by binding to anti-3,3'-T_2 antibody (see above).

Radioactive T_3 remaining after incubation was quantified by incubating overnight at 4°C 75 μL of an ethanol extract (see above) of the reaction mixture with a rabbit anti-T_3 antibody. This was followed by addition of goat anti-rabbit gamma globulin to precipitate antibody bound ^{125}I-T_3 (19). The results were expressed as the percentage of ^{125}I-T_3 added to the reaction mixture in the beginning of the experiment. When ^{125}I-T_3S was the substrate, its amount remaining after incubation was determined similarly except a highly specific rabbit anti-T_3S antibody was used in place of anti-T_3 antibody. The anti-T_3S antibody cross-reacted less than 0.01% with T_4, T_3, rT_3, or 3,3'-T_2 (13).

Desulfation. Desulfation of T_3S to T_3 by various tissues was studied by incubating microsomes (200 μg protein) with nonradioactive T_3S (0.1–2.0 nmol) in 0.1 M sodium phosphate buffer, pH 8.0, for 2 h at 37°C (final incubation volume, 200 μL). The reaction was stopped by adding 100 μL of 2.5% BSA followed by two volumes (600 μL) of ethanol. The mixture was centrifuged and T_3 was measured by RIA of 100 μL of the supernatant as described previously (19). Each assay included two tubes (zero incubation tubes), which were handled as described above except that microsomes were added at the end of incubation immediately before extraction with ethanol. T_3 measured in these tubes was considered present in T_3S or that apparent from cross-reaction of T_3S in T_3 RIA. This amount of apparent T_3 was subtracted from T_3 measured in test specimens to determine the amount of T_3 produced as a result of incubation of microsomes with T_3S. Pilot studies using liver microsomes had demonstrated that the desulfation is linear *1*) with 25–400 μg of microsomal protein, *2*) between 15 min and 4 h of incubation period, and *3*) with 100–1500 pmol of substrate. Additionally, the hepatic microsomal desulfation activity was greater at 37°C than at 25 or 4°C, and it was inhibited by 0.15 mM or more of DTT. Similar data were obtained using maternal and fetal brain and maternal skeletal muscle. Studies using radioactive ($^{125}I^-$) T_3 had indicated that there is little (<10%) loss of T_3 generated during incubation under the above-mentioned conditions.

RESULTS

The metabolism of T_3 and T_3S by hepatic microsomes of the maternal and the fetal rat is shown in Figure 2. Maternal liver microsomes metabolized T_3S exceedingly efficiently. Some 80% or more of T_3S was consumed during its incubation with 360 μg/mL microsomes for 2 h. The majority of the consumption of T_3S by adult rat liver microsomes was explained by outer-ring

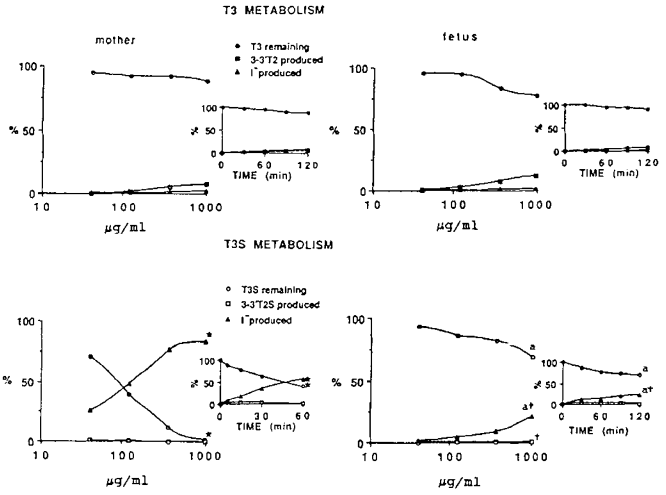

Fig. 2. Metabolism of T_3 and T_3S by liver microsomes of maternal and fetal rat. The effect of tissue protein concentration at 2 h of incubation and the effect of the duration of incubation in the presence of 1 mg/mL protein (*inset*) were studied. Results are the means of two closely agreeing experiments using pooled fetal tissues, each from 40–50 fetuses, and individual maternal tissues. The results were expressed as % of ^{125}I-T_3 (or ^{125}I-T_3S) added to the incubation mixture. Statistical evaluation of the differences in the metabolism of ^{125}I-T_3 and ^{125}I-T_3S by maternal and/or fetal tissues was performed by the analysis of covariance. Mother *vs* fetus, T_3S metabolism, *a*, $p < 0.05$; T_3 *vs* T_3S metabolism in the mother or the fetus, †, $p \leq 0.05$; *, $p \leq 0.01$.

(5′-) monodeiodination with generation of iodide. Little 3,3′-T_2S was demonstrable at the end of incubation. This was the case whether the studies were conducted with an incubation period of 5 min or 2 h. Unlike maternal liver microsomes, however, fetal liver microsomes metabolized T_3S poorly and a large proportion (>75%) of T_3S remained unchanged after a 2-h incubation with fetal liver microsomes (compare mother, $p < 0.05$). Reduced metabolism of T_3S by fetal liver compared with adult liver was mainly a result of its severely reduced outer-ring monodeiodination; the inner-ring metabolism of T_3S was similarly low between the fetus and adult.

T_3 was metabolized similarly moderately by fetal and maternal liver microsomes (Fig. 2). Thus, ~90% and ~80% of ^{125}I-T_3 was still unchanged after a 2-h incubation with 1000 μg/mL microsomes from maternal and fetal liver, respectively. In both cases, 3,3′-T_2 was the predominant product of deiodination (7 and 12%, respectively), whereas $^{125}I^-$ liberated was less than 3%.

Figure 3 shows the data on the metabolism of T_3S and T_3 by cerebral cortical microsomes of the maternal and fetal rat. T_3S was poorly metabolized by both maternal and fetal tissue. Some 90% or more of substrate T_3S was still intact after a 2-h incubation with cerebral cortical microsomes of the adult or the fetus. Interestingly, however, these tissues metabolized T_3 more rapidly than T_3S ($p < 0.05$), both in the inner ring (to 3,3′-T_2) and the outer ring (to I^-). Thus, after a 2-h incubation with 360 μg/mL maternal microsomes, only ~23% of ^{125}I-T_3 remained unchanged, whereas ~37% was monodeiodinated to ^{125}I-3,3′T_2 and ~5% to $^{125}I^-$. Under the same conditions, the corresponding values for fetal microsomes were 13, 46, and 10% respectively. In both maternal and fetal tissues, a substantial proportion of the 3,3′-T_2 generated during incubation had been deiodinated further in the inner ring to 3′-monoiodothyronine, and this reaction accounted for ~30–35% of the starting T_3.

The deiodination of ^{125}I-T_3 and ^{125}I-T_3S by rat placental microsomes, another tissue rich in 5-monodeiodinase (20), was also studied. Some 80% or more of ^{125}I-T_3 was consumed during its incubation with 60 μg/mL microsomes for 2 h, mainly by inner-ring deiodination to ^{125}I-3,3′T_2 (~60%) and to a lesser degree by outer-ring deiodination to ^{125}I-(~20%). Conversely, more than 90% of T_3S was still unchanged after a 2-h incubation with 540 μg/mL microsomes (compare T_3, $p < 0.05$).

The T_3S to T_3 desulfation activity, as determined by incubation of 250 pmol of T_3S with 200 μg of protein of microsomes from pooled tissues of the maternal rat for 2 h at 37°C, was (pmol·h^{-1}·mg $protein^{-1}$) 55 for liver, 32 for kidney, 24 for skeletal muscle, 4.9 for spleen, and 2.1 for cerebral cortex. T_3S desulfation activity was not studied in maternal intestine because the presence of bacterial contamination might influence the results or in fetal spleen because sufficient material was not available. The T_3S to T_3 desulfation activity (pmol·h^{-1}·mg $protein^{-1}$) determined using microsomes (~200 μg of protein) from pooled tissues of the fetal rat was 3.1 for cerebral cortex, 1.4 for liver, <0.3 for kidney, <0.3 for skeletal muscle, and <0.3 for intestine.

Figure 4 shows the Lineweaver-Burk plots (21) of the data on the conversion of T_3S to T_3 by liver and cerebral cortical microsomes from the mother and a pooled (n = 40–50) tissue from the fetal rat. The mean (± SEM) of the T_3S desulfation activity in fetal liver [224 ± 44 μM (n = three tissue pools)] was significantly higher than that in maternal liver [40 ± 19 μM (n = 3)] ($p < 0.02$). The V_{max} of the activity was lower in the fetus than in the adults, but the difference was not statistically significant [1000 ± 154 *versus* 2308 ± 774 pmol of T_3 produced·h^{-1}·mg $protein^{-1}$ ($0.05 > p < 0.1$)]. The Km and V_{max} of T_3S desulfation activity in fetal cerebral cortex were both significantly ($p < 0.05$) higher than the corresponding values in maternal cerebral cortex [Km, 627 ± 163 (n = three tissue pools) *versus* 88 ± 8.6 (n = 3); V_{max}, 905 ± 135 *versus* 283 ± 7.5 pmol of T_3 produced·h^{-1}·mg $protein^{-1}$]. The Km and V_{max} of the T_3S to T_3 desulfation activity was also studied in the maternal skeletal muscle, and the values were comparable to those in the liver (Km, 27 μM; V_{max}, 337 pmol of T_3 produced·h^{-1}·mg $protein^{-1}$, n = 2). However, little or no activity was detected in the case of the fetal skeletal muscle.

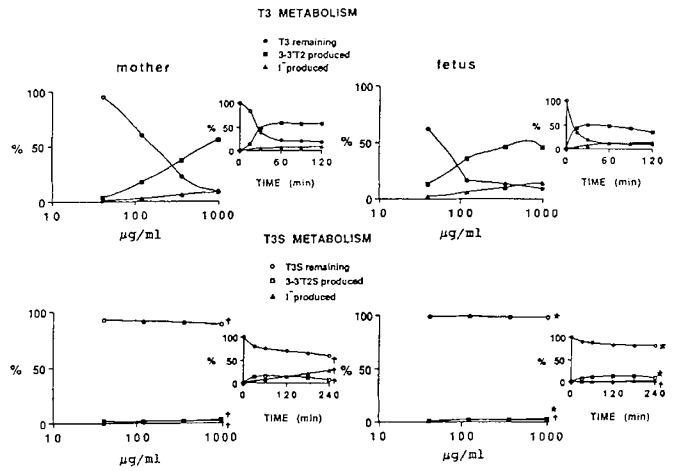

Fig. 3. Metabolism of T_3 and T_3S by cerebral cortical microsomes of maternal and fetal rat. The effect of tissue protein concentration and duration of incubation were studied. The sources of tissues, conditions of incubation, and expression of the results were the same as described in Figure 2. T_3 *vs* T_3S metabolism in the mother or the fetus, †, $p < 0.05$; *, $p \leq 0.01$.

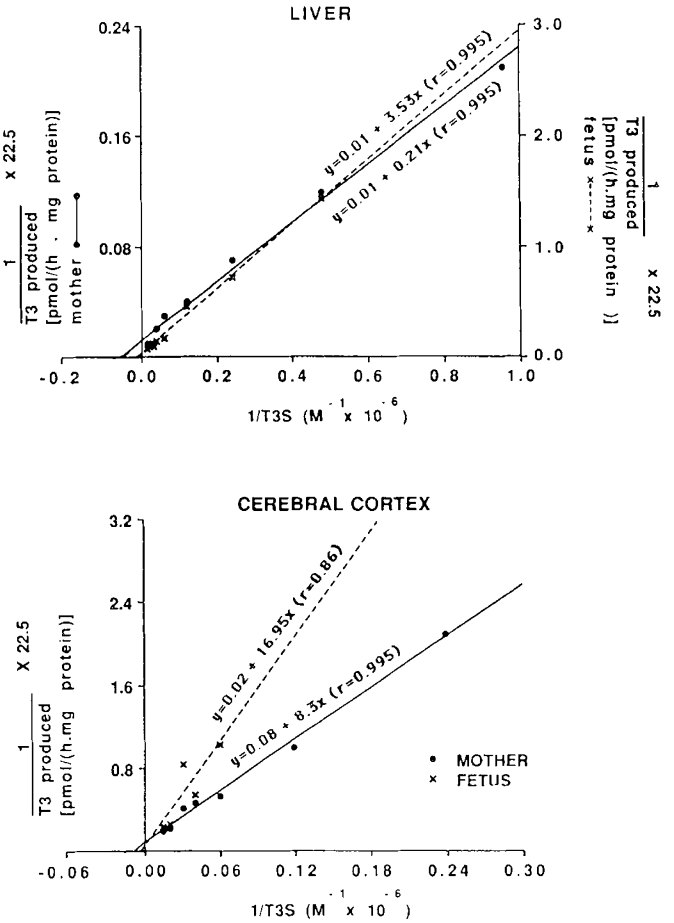

Fig. 4. Double reciprocal plots of the rate of desulfation of T_3S to T_3 by hepatic and cerebral cortical microsomes of the maternal and fetal rat. The conditions of incubation and the method of measurement of T_3 generated during incubation of the tissue with substrate (T_3S) are described in Materials and Methods. The data in one maternal rat and a pooled sample of 40–50 fetal rats are shown.

DISCUSSION

Thyroid hormone is critical for growth and development of the fetus. It is especially important for optimal development, differentiation, and functioning of the CNS (22). However, the extrathyroidal generation rate and serum concentration of the most active thyroid hormone, T_3, are reduced in the fetus as they are in adults with nonthyroidal systemic illnesses (euthyroid sickness syndrome) or complete fasting (23–25). It has been suggested that the reduced serum T_3 and T_3 production rate in the above-mentioned conditions are part of a homeostatic arrangement serving to reduce catabolism and promote optimal anabolism.

Accordingly, fetal tissues are very active in degrading T_3 by inner-ring deiodination, whereas the rate of T_3 production by outer-ring deiodination of T_4 is strongly reduced compared with the adults. It is possible that increased T_3 5-monodeiodination in the placenta contributes to its function as a barrier to the transport of active thyroid hormones from the mother to the fetus (20).

Our data show that, unlike T_3, T_3S is poorly deiodinated by fetal tissues, both in the outer and in the inner ring. These findings are compatible with the high levels of T_3S measured in fetal serum. We also demonstrated that microsomes from both adult and fetal tissues are able to desulfate T_3S to generate T_3. T_3S is considered to have no biologic activity per se, and in normal adults it is quickly deiodinated. Therefore, sulfation of T_3 appears to be a pathway facilitating deiodination in normal adults in whom the necessary amount of thyroid hormone (T_3) to the tissues derives from 5′-monodeiodination of T_4 (to T_3). The role of the sulfation pathway might, however, be different in certain situations in which serum T_3 and T_3 production rates are reduced, *e.g.* in fetal life and systemic nonthyroidal illnesses. Our data suggest that, by creating differences in the metabolism of T_3S and T_3 and by creating higher serum levels of T_3S in the fetus than in the adult, nature has devised a means by which most fetal tissues are exposed to subnormal levels of the highly active and catabolic thyroid hormone, T_3, whereas tissues in which T_3 is critical for growth, development, and/or differentiation, *e.g.* CNS, receive the hormone when needed as a result of their ability to generate it locally by desulfation of T_3S. More rapid metabolism of T_3 than T_3S by fetal tissues may facilitate this selectivity in the supply of T_3 to the tissues. T_3S is considered to have little or no TSH-suppressive activity (12); therefore, its high serum levels would have little or no adverse influence on the growth and development of the fetal thyroid. Clearly, further work is necessary to support or refute this hypothesis. However, it appears likely, as in the case of monodeiodination of T_4 to more active T_3 or less active rT_3, that a relative balance in the ability of tissues to sulfate thyroid hormone(s) or desulfate their sulfoconjugates to active hormone is an important control mechanism that regulates, in a selective manner, the supply of thyroid hormones to different tissues and perhaps even different parts of a tissue.

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