The Role of Sulfhydryl Groups on the Impaired Hepatic 3',3,5-Triiodothyronine Generation from Thyroxine in the Hypothyroid, Starved, Fetal, and Neonatal Rodent

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A B S T R A C T The role of nonprotein sulfhydryl groups (NPSH) in the decreased in vitro hepatic 3',3,5-triiodothyronine (T_3) generation from thyroxine (T_4) in the starved, hypothyroid, fetal and 1- to 4-d-old neonatal rat and dwarf mouse was assessed. NPSH were measured in fresh 25% liver homogenates prepared in 0.1 M PO_4/10 mM EDTA buffer. As compared with values in adult male rats, NPSH concentration was decreased in the 2-d-starved (1.1±0.04 (mean±SE) vs. 2.2±0.15 mmol/250 g wet liver weight, $P < 0.001$), fetal (1.0±0.04 vs. 3.2±0.08, $P < 0.001$), 1-d-old neonatal (1.1±0.03 vs. 2.1±0.04, $P < 0.001$), and hypothyroid (thyroidectomized 60 d) (1.4±0.06 vs. 2.2±0.15 $P < 0.001$) rat. NPSH were also decreased in the hypothyroid, hypopituitary dwarf mouse as compared with values in their normal litter mates (1.3±0.03 vs. 2.0 ±0.2, $P < 0.01$). Chronic administration of T_3 (0.5 μg/ 100 g body wt per d) markedly increased hepatic T_3 generation from T_4 in the thyroidectomized rat and in the dwarf mouse to values similar to those observed in the normal rodent without affecting NPSH concentration. In contrast, T_3 administration to the starved rat did not alter either hepatic T_3 generation from T_4 or NPSH. Reduced glutathione concentration was also markedly decreased in the starved rat (fed; 1.05±0.075 mmol/ 250 g wet tissue vs. starved 0.38±0.02, $P < 0.001$). Dithiothreitol (DTT), a thiol reducing agent, increased hepatic T_3 generation from T_4 in the normal adult male rat by 45±5% in six experiments. When compared to DTT-stimulated control homogenates, the addition of DTT completely restored hepatic T_3 generation in starved rats, partially restored T_3 generation in 1- and

4-d-old neonates, but had little or no effect in the fetal and hypothyroid rat and dwarf mouse. Liver homogenates stored for 6 mo at −20°C lost their capacity to generate T_3 from T_4. NPSH concentrations in the frozen homogenates decreased progressively with increasing storage and were absent by 6 mo. 5'-Deiodinase activity correlated with NPSH concentration in the stored homogenates ($r = 0.95$, $P < 0.005$). Addition of DTT partially restored hepatic T_3 generation in the frozen homogenate. It is concluded that NPSH are important for the action of the liver 5'-deiodinase. The decreased hepatic T_3 generation in the starved rat is associated with decreased NPSH but not with a decrease in the absolute quantity of 5'-deiodinase because provision of sulfhydryl groups restored hepatic T_3 generation to normal. In contrast, the decreased hepatic T_3 generation in the adult hypothyroid rodent and in the fetal rat is probably due to a decrease in the enzyme concentration per se. In the 1- and 4-d neonatal rat, the decrease in hepatic T_3 generation is secondary to a decrease in NPSH and the deiodinating enzyme.

INTRODUCTION

Considerable progress has recently been made in defining the factors that regulate the metabolism and cellular action of the thyroid hormones. In both man and animal, the more active thyroid hormone, 3',3,5-triiodothyronine (T_3),[1] is primarily derived from the 5'-deiodination of thyroxine (T_4) in peripheral tissues (1–5). The noncalorigenic product of T_4 monodeiodination 3,3'-5'-triiodothyronine (reverse T_3, rT_3),

[1] *Abbreviations used in this paper*: DTT, dithiothreitol; dw/dw, dwarf mouse; H-T_3, hepatic T_3 generation from T_4; NPSH, nonprotein sulfhydryl groups; T_4, thyroxine; T_3, 3',3,5-triiodothyronine; rT_3, 3,3',5'-triiodothyronine; Tx, thyroidectomized.

is generated from inner ring deiodination. A decrease in the serum concentration of T_3 (6–10) and the production of T_3 from T_4 (11–13) have been demonstrated in man under a variety of circumstances. An increase in serum rT_3 concentration almost always occurs in these states (9, 10, 14, 15), probably due to a decrease in rT_3 clearance rather than enhanced rT_3 production (11, 16, 17). In animals, the in vitro conversion of T_4 to T_3 was first reported by Larson et al. (18) in 1955 in rat kidney slices, but the significance of these findings was not immediately recognized. The conclusive demonstration of in vivo peripheral conversion of T_4 to T_3 in man (1) revived interest in various aspects of the peripheral metabolism of T_4. Many workers have demonstrated that different tissues actively deiodinate T_4 to T_3 (19–24), but liver and kidney are the most active (25–33). The conversion is enzymatic, because it is temperature, pH, and substrate concentration dependent (29–33). Compounds such as iopanoic acid, propylthiouracil, and rT_3 have been demonstrated to be potent inhibitors of T_4 to T_3 conversion in vitro (29, 33, 34) and in vivo (35–37). Visser et al. (38) first reported the importance of sulfhydryl groups in the conversion of T_4 to T_3 because thiol protective agents such as glutathione or dithiothreitol (DTT) added in vitro enhanced 5'-deiodinating enzyme activity, whereas thiol oxidizing agents abolished it. These observations have recently been confirmed (39, 40).

It has also been reported that conversion of T_4 to T_3 by liver homogenates from starved (27, 30, 32), hypothyroid (32, 41, 42), fetal, and neonatal rats (43) and fetal sheep (44) is markedly decreased. In vivo administration of carbohydrate (27, 32) and protein, but not fat (32), markedly enhances T_4 to T_3 conversion by liver homogenate from starved rats, whereas T_4 or T_3 replacement therapy restores the decreased hepatic T_3 generation observed in hypothyroidism (32, 41). The mechanisms responsible for the decreased enzymatic activity in these various conditions are essentially unknown. However, decreased cofactor activity in the cytosol has been postulated to reduce 5'-monodeiodinase activity in fasting and a decrease in both enzyme and cofactor activity in hypothyroidism (41).

The present studies were carried out in an attempt to further elucidate the mechanisms responsible for the decreased in vitro hepatic conversion of T_4 to T_3 in the starved, hypothyroid, and perinatal rat and in the hypothyroid, dwarf mouse.

METHODS

Adult male rats and fetuses and pups from timed pregnant Sprague-Dawley rats (Charles River Breeding Laboratories, Wilmington, Mass.) were used. Six male rats were housed per wire mesh cage, and pregnant females were housed individually in breeding cages. On the 2nd postnatal day, all litters were culled to eight pups to ensure equal nutrition for the experimental animals. Snell dwarf mice (dw/dw), an inbred colony originally obtained from Jackson Breeding Laboratories, Bar Harbor, Maine, were also studied. This rodent is congenitally hypothyroid as a result of a rudimentary pituitary gland. Dwarf mice were weaned at 6–8 wk, and four housed per breeding cage. Normal litter mates (+/?) were weaned at 3 wk and used as controls in all studies employing the dw/dw mouse. Unless otherwise stated, all animals were fed Purina Formulab Chow (Ralston Purina Co., St. Louis, Mo.) and tap water ad lib. To avoid possible diurnal variations in nonprotein sulfhydryl groups (NPSH), all animals were sacrificed between 8 and 10 a.m. Rooms were lighted from 7 a.m. to 6 p.m., and room temperature maintained at $21\pm1°C$. All chemicals were obtained from Sigma Chemical Co., St. Louis, Mo.

Starved rats. Groups of 5–10 male rats aged 55–65 d were fasted for 2 d before decapitation. Livers were quickly perfused with ice-cold 0.1 M PO_4–10 mM EDTA buffer pH 7.4 to remove excess blood, and then homogenized in iced 0.1 M PO_4–10 mM EDTA buffer as described in detail elsewhere (32). Fed control rats were sacrificed alternately with starved animals, and livers collected in the same manner. To determine the effect of physiologic doses of T_4 or T_3 on hepatic T_3 generation from T_4 (H-T_3) and hepatic NPSH groups in 2-d starved and fed rats, groups of 5–8 starved or fed rats were injected i.p. with T_4 (1.5 μg/100 g per d), T_3 (0.5 μg/100 g per d), or diluent for 10 d and sacrificed 15 h after the last injection.

Thyroidectomized rats. All thyroidectomized (Tx) animals and appropriate controls were given 1% calcium gluconate water ad lib. 10, 60, and 120 d post-Tx, groups of 4–7 rats were sacrificed together with age-matched, normal males, and the livers quickly removed. The effect of T_4 or T_3 replacement on H-T_3 and the effects of T_3 replacement on hepatic NPSH groups were assessed. Groups of 5–7 rats, Tx for 10, 60, or 120 d, were injected i.p. with T_4 (1.5 μg/100 g per d), T_3 (0.5 μg/100 g per d), or diluent for 10 d and sacrificed 15 h after the last injection.

Dwarf mice. Groups of 16 dw/dw mice and 16 normal siblings were injected with physiologic replacement doses of T_4 (1.5 μg/100 g per d), T_3 (0.5 μg/100 g per d), or diluent for 14 d and sacrificed 15 h after the last injection. Livers were quickly removed and pooled in groups of four, homogenized, and analyzed for hepatic NPSH groups. H-T_3 was also determined.

Fetal and neonatal rats. Livers from 19-d gestation fetuses and 1-, 4-, and 7-d neonatal rats were collected as previously described (43). Six male rats (55–65 d) were sacrificed with each fetal or neonatal age group and served as the control for each experiment.

Homogenate preparation and in vitro T_4 deiodination. Fresh liver homogenates for the T_4 deiodination experiments were prepared as previously described (32, 43). The term homogenate refers to the supernatant preparation obtained after homogenizing the livers with 3 vol of iced buffer followed by centrifugation of 800 g for 10 min at 4°C. Because it was found that the rate of T_3 generation from T_4 is linear for the first 30 min of incubation, the T_3 generated (picogram per milligram protein) was determined at 15 min. In the studies with DTT, quadruplicate 1-ml samples were pipetted from each fresh, ice-cold homogenate pool (except the fetal and dwarf mouse pools where small volumes allowed only duplicate samples) and 0.5 μg/ml T_4 added. DTT was then added to half the tubes in a final concentration of 4 mM DTT and the same volume of buffer added to the remaining control tubes. The final volume of the incubate was 1.1 ml. The unstoppered tubes were quickly vortexed and then incubated in a water bath at 37°C for 15 min before stopping the deiodination reaction by placing the tube in iced water and quickly

adding 2 ml of iced 95% ethanol. After storing at −20°C for 1 h, the tubes were centrifuged at 1,000 g for 20 min at 4°C, and the supernate collected and assayed for T_3 content. Blanks for DTT-treated and control homogenates were treated as above except that 0.5 µg of T_4/ml homogenate was added immediately before ethanol extraction. The effect of reduced glutathione (8 mM) on T_3 generation from T_4 was also studied.

The effect of various concentrations of DTT on H-T_3 was evaluated. Homogenate pools from normal, starved, 1-d neonatal, and Tx rats and dw/dw mice and their normal siblings were incubated in triplicate with 0, 0.5, 1, 4, and 8 mM DTT. To further examine any nonspecific effect of DTT on in vitro conversion, T_4 and 4 mM DTT were added to liver homogenate that had been heated at 56°C for 1 h, and the quantity of T_3 generated over 2 h at 37°C determined. In another experiment, 0.5 µg T_4/ml and 4 mM DTT were added to ice-cold homogenates, and T_3 generation determined at 5 min intervals for 30 min. The T_3 present in the ethanol extract was measured by radioimmunoassay and expressed as picograms T_3 per milligram protein. The protein content of the homogenate was measured calorimetrically using Biuret reagent.

Hepatic NPSH groups. The concentration of NPSH was measured in all homogenate pools using minor modifications of the method described by Sedlak and Lindsay (45). Duplicate 0.5-ml samples of ice-cold homogenate were diluted to 5 ml with 20 mM EDTA and vortexed. 4 ml of ice-cold, distilled, deionized water and 1 ml 50% trichloroacetic acid (TCA) were added to 5 ml diluted homogenate, the tubes stoppered, placed in crushed ice, and mixed intermittently for 15 min. Specimens were then centrifuged at 1,000 g for 15 min at 4°C, 2 ml supernate added to 4 ml 0.4 M Tris hydroxymethyl aminomethane buffer (pH 8.9), the tubes stoppered and mixed. The NPSH concentration was then determined colorimetrically at 412 nm within 3 min of adding 0.1 ml 5,5'-dithiobis-2 nitro benzoic acid. Results were compared with those obtained from prepared standards. Because T_4 monodeiodination was found to be dependent upon the molar concentration of NPSH, and 25% liver homogenate was employed, the NPSH concentration was expressed as millimoles per 250 g wet liver weight.

To determine the effect of prolonged incubation at 37°C on NPSH, homogenates were obtained from 10 rats, and the NPSH concentration determined after 0, 15, 60, and 120 min incubation.

We have previously demonstrated that storage of homogenates at −20°C results in a marked loss of deiodinating activity (32). NPSH concentrations were measured in homogenates obtained from 3-, 7-, and 17-d-old rats and stored at −20°C for 6 mo to determine whether loss of NPSH was related to this loss of deiodinating activity. T_4 to T_3 conversion by these homogenates was then studied with and without added DTT. In another study, T_3 generation from T_4 and NPSH concentrations were determined in groups of homogenates stored from 1 to 40 wk at −20°C.

Hepatic GSSG and GSH. The concentrations of GSSG and GSH were measured in hepatic homogenate from 2-d starved and fed rats by the fluorometric method of Hissin and Hilf (46). The livers were immediately homogenized with a Teflon pestle (Du Pont Co., Willmington, Del.) in 15 ml of ice-cold 0.1 M PO_4–0.005 M EDTA buffer (pH 8.0), GSSG and GSH measured immediately, and results expressed as millimoles GSSG or GSH per 250 g wet liver weight.

Statistical analysis. All results were analyzed by the Student's t test.

RESULTS

Effect of preheating and incubation at 0°C on DTT stimulation of H-T_3. Preheating at 56°C for 1 h com-

pletely inhibited T_4 to T_3 conversion by liver homogenates. The addition of DTT to these preheated homogenates did not induce conversion. Similarly, conversion was not observed in homogenates maintained at 0°C after the addition of T_4 substrate with or without DTT.

Effect of incubation and storage on NPSH concentration. The concentration of NPSH did not decrease during the first 15 min of incubation, which was the length of time employed throughout these experiments (control, 2.16±0.032 [mean±SE] mmol/250 g wet tissue [n = 10] vs. 2.11±0.026 [n = 10]). A small but significant decrease in NPSH occurred within 1 h (1.93±0.026 [n = 10], $P < 0.001$) and was more marked by 2 h (1.73±0.033 [n = 10], $P < 0.001$). Thus, NPSH did not change during the initial incubation period when conversion is linear, but did decrease at 1 and 2 h incubation when conversion rate decreases (32).

After 6 mo storage, the NPSH concentration was undetectable in liver homogenates from 3-, 7-, and 17-d-old rats. Conversion of T_4 to T_3 was absent in homogenates obtained from the 3- and 7-d-old rats, and markedly reduced in the 17-d rat liver homogenate (Fig. 1). Addition of DTT to these frozen, stored homogenates strikingly increased T_3 generation from T_4, particularly in homogenates obtained from the older rats in which the addition of DTT increased conversion from 22±4.5 pg T_3/mg protein to values approximating those obtained in fresh homogenates (fresh, 295±12.2 pg T_3/mg protein vs. frozen plus DTT 216±19). There was a positive correlation between NPSH concentration and H-T_3 in homogenates stored for 1–40 wk (Fig. 2).

Effect of hypothyroidism on H-T_3 and NPSH concentrations. The presence of hypothyroidism for 60 or 120 d resulted in a marked decrease in H-T_3 (80% less than control, $P < 0.001$). NPSH groups were also de-
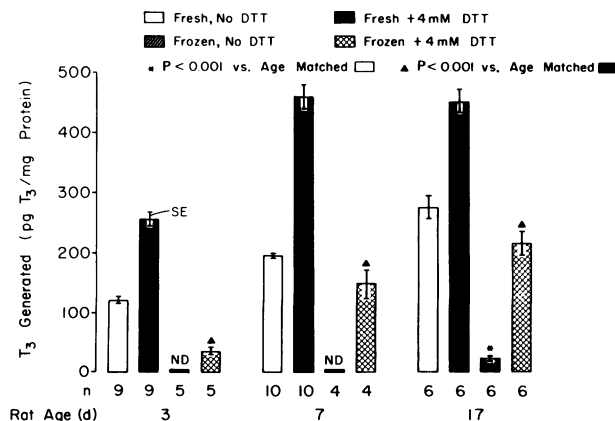

FIGURE 1 Effect of DTT on in vitro H-T_3 in neonatal homogenates frozen for 6 mo at −20°C. Homogenates were incubated with 0.5 µg T_4/1 ml homogenate at 37°C for 15 min. n denotes the number of rats in each group, or in the case of neonatal rats <12 d of age, the number of homogenate pools in each group. Significance calculated by the Student's t test.

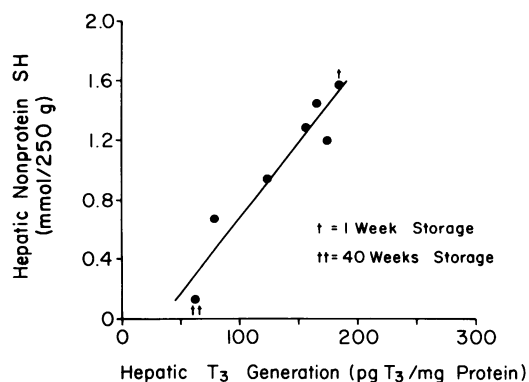FIGURE 2 Effect of storage (−20°C) on hepatic NPSH groups vs. H-T$_3$. Each solid circle represents the mean of four to six homogenate pools stored for variable periods of time from 1 to 40 wk. For other details, see Fig. 1. Linear regression, r = 0.95, $P < 0.005$.

creased in these hypothyroid rats (Table I). The administration of physiologic replacement doses of T$_3$ or T$_4$ for 10 d to rats hypothyroid for 60 d completely restored the decreased H-T$_3$ (Fig. 3). However, the concentration of NPSH groups was not affected by T$_3$ treatment (Table I). In another experiment, short-term hypothyroidism (10 d) resulted in a 70% decrease in H-T$_3$ ($P < 0.001$) but no decrease in NPSH groups (Table I).

To determine whether the decreased NPSH groups were related to the decreased conversion of T$_4$ to T$_3$, DTT was added to liver homogenates from hypothyroid rats and induced only small increases in H-T$_3$, values far below those obtained in normal rat liver homogenates with or without DTT (Fig. 3). The addition of various molar concentrations of DTT increased T$_3$ generation far less in homogenates from hypothyroid rats as compared with homogenates from normal rats (Fig. 4).

To exclude the possibility that altered serum concentrations of pituitary hormones (growth hormone and thyroid-stimulating hormone) induced by hypothyroidism (47) might be involved in the decreased H-T$_3$ and hepatic NPSH groups, the hypopituitary, hypothyroid dw/dw mouse was studied. H-T$_3$ in the dw/dw mouse was essentially undetectable (Fig. 5). Addition of various molar concentrations of DTT to dw/dw liver homogenates did not enhance H-T$_3$, but significantly increased H-T$_3$ in their normal siblings (Figs. 4 and 5). T$_4$ administration to the dw/dw mouse for 14 d significantly increased H-T$_3$, but values were still less than H-T$_3$ in T$_4$-treated or untreated normal siblings. In contrast, T$_3$ replacement for 14 d almost restored H-T$_3$ to values observed in the normal siblings (Fig. 5). Liver NPSH groups were significantly decreased in dw/dw mice as compared with their normal siblings (1.3±0.08 vs. 1.8±0.12 mmol/250 g, $P < 0.02$). T$_4$ or T$_3$ replace-

TABLE I

Effect of Starvation, Rat Age, and Hypothyroidism on Hepatic NPSH Groups in the Rodent

Expt. No.	Hepatic NPSH						P Value Student's t test
	Experimental group			Control group			
	mmol/250 g wet wt						
1	Starved 2 d	(4)*	1.14±0.04‡	Fed	(4)	2.19±0.12	<0.001§
2	19-d fetal	(6)	0.97±0.04		(6)	3.23±0.08	<0.001
3	1-d neonate	(10)	1.07±0.02		(6)	2.05±0.04	<0.001
4	4-d neonate	(12)	1.35±0.05	55–65-d male	(6)	2.33±0.05	<0.001
5	5-d neonate	(12)	1.44±0.06		(6)	2.18±0.04	<0.001
6	7-d neonate	(10)	1.82±0.05		(6)	2.60±0.02	<0.001
7	Tx for						
	10 d	(6)	2.44±0.05	Normal male	(4)	2.33±0.04	NS
8	60 d	(4)	1.43±0.06	Normal male	(4)	2.22±0.15	<0.005
	60 d + T$_3$‖	(4)	1.60±0.05	Normal male + T$_3$‖	(5)	2.18±0.14	<0.01
	120 d	(5)	1.07±0.13	Normal male	(4)	2.22±0.15	<0.001
9	Dwarf mouse	(4)	1.28±0.04	Normal sibling	(4)	1.83±0.12	<0.005
	+ T$_4$¶	(4)	1.15±0.01	+ T$_4$¶	(4)	1.94±0.03	<0.001
	+ T$_3$‖	(4)	1.24±0.09	+ T$_3$‖	(4)	1.60±0.06	<0.02

* Numbers in parentheses represent the number of animals in each group or the number of separate homogenate pools.
‡ Mean±SE.
§ Experimental group vs. control group.
‖ 0.5 μg T$_3$/100 g daily for 10 d (Tx) or 14 d (dwarf).
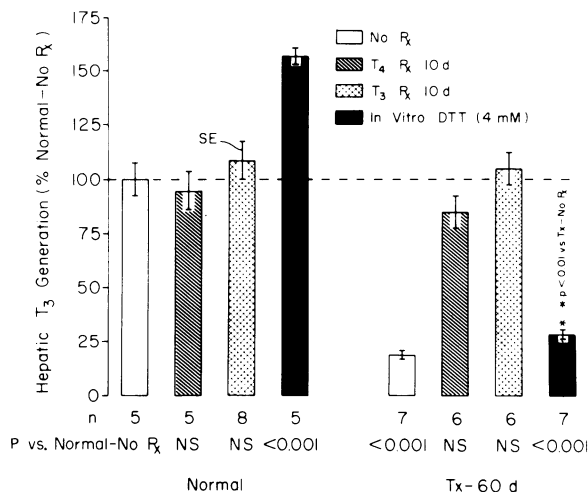¶ 1.5 μg T$_4$/100 g daily for 14 d (dwarf).

FIGURE 3 Effect of thyroidectomy (Tx) and T_4 (1.5 μg/100 g per d, i.p.) or T_3 (0.5 μg/100 g per d, i.p.) treatment and in vitro addition of DTT on H-T_3 in the rat. For other details, see Fig. 1.

FIGURE 5 Effect of T_4 (1.5 μg/100 g per d, s.c.) or T_3 (0.5 μg/100 g per d, s.c.) administration and in vitro addition of DTT on H-T_3 in the normal and hypopituitary, hypothyroid dwarf mouse. For other details, see Fig. 1.

ment therapy had no effect on hepatic NPSH groups (Table I).

Effect of age on H-T_3 and NPSH concentration. The quantity of T_3 generated by perinatal rat liver homogenates is compared with that obtained in 60-d adult male rats (Fig. 6). The 19-d fetal liver generated very little T_3. Addition of DTT had only a minimal stimulatory effect and did not increase H-T_3 to values observed in adult rat homogenates with or without DTT. As previously reported (43), a gradual age-related increase in T_3 generation was observed, reaching adult control values by 7 d. Addition of DTT to liver homogenates from 1-, 4-, and 7-d neonatal rats markedly enhanced T_3 generation from T_4 but when compared with DTT-stimulated homogenates from 60-d-old male rats, T_3 generation re-
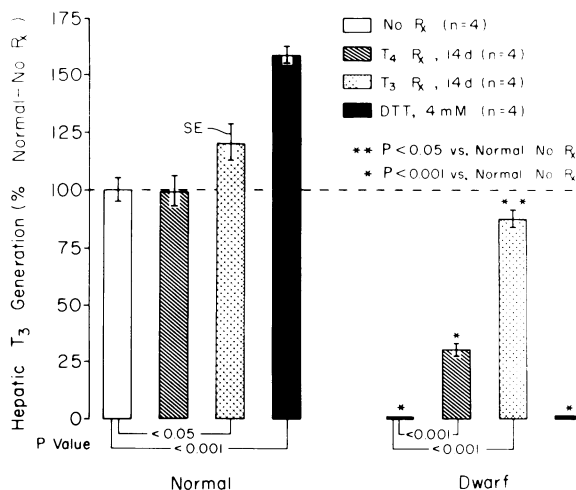
mained significantly lower until day 7. The concentration of NPSH groups was markedly decreased in the fetal rat liver and gradually increased with age (Table I). Thus, the gradual increase in H-T_3 with age was accompanied by an increase in NPSH groups and in the stimulatory effect of DTT on T_3 generation.

Effect of starvation on hepatic NPSH groups and on the effect of DTT on H-T_3. As previously reported (32), 2 d starvation decreased H-T_3 by 47%, and the administration of physiologic doses of T_4 or T_3 during starvation did not enhance T_3 generation from T_4 (Fig. 7). Hepatic NPSH groups were significantly decreased in the 2-d starved rat and remained so during T_3 treatment (Fig. 7, Table I). The addition of DTT to liver
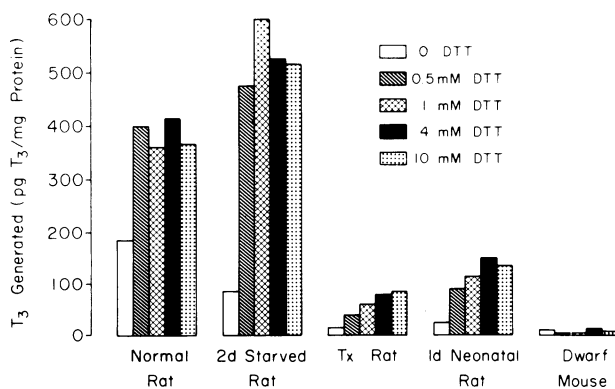

FIGURE 4 Effect of various concentrations of DTT on H-T_3 in the normal, 2-d starved, Tx (60 d), and 1-d neonatal rat and dwarf mouse. Each bar represents the mean of triplicate samples from the appropriate homogenate pool. For other details, see Fig. 1.
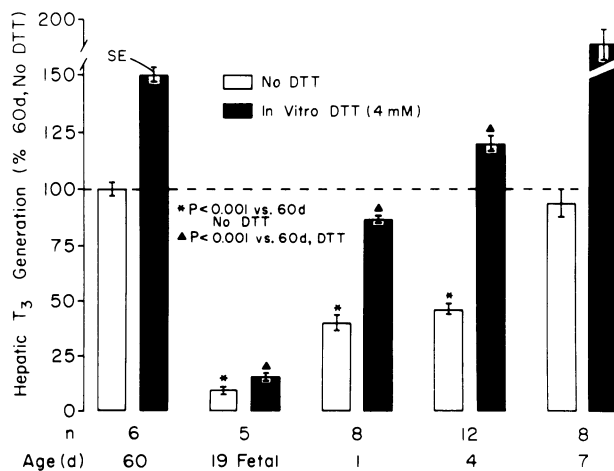

FIGURE 6 Effect of age on DTT stimulation of in vitro H-T_3 in the rat. For other details, see Fig. 1.
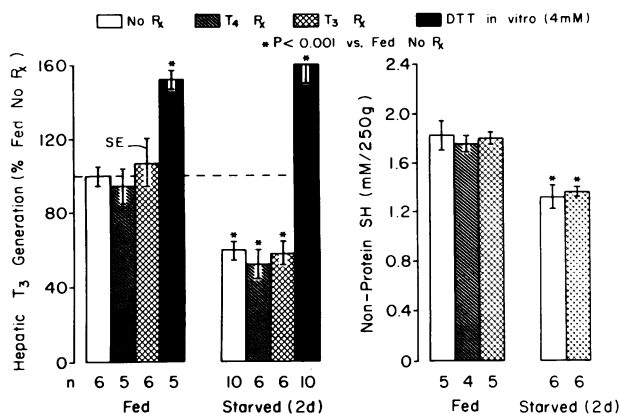
FIGURE 7 Effect of starvation and T_4 (1.5 μg/100 g per d, i.p. × 10 d) or T_3 (0.5 μg/100 g per d, i.p. × 10 d) administration on hepatic NPSH groups and DTT stimulation of H-T_3. For other details, see Fig. 1.

homogenates from starved and fed rats induced greater increases in T_3 generation in homogenates from starved rats than from fed rats, and the DTT-stimulated H-T_3 values were similar in both groups. The addition of GSH to hepatic homogenates from starved rats restored H-T_3.

GSH and GSSG concentrations were also assessed in the liver cytosol from six fed and six starved rats. The concentration of GSH was markedly decreased in the liver cytosol from the starved rats (fed, 1.05±0.075 vs. starved, 0.38±0.02 mmol/250 g; $P < 0.001$). Liver cytosol GSSG was also markedly decreased in the starved rat (fed, 0.1±0.01 vs. starved, 0.048±0.002 mmol/250 g; $P < 0.001$). This was not unexpected because glutathione is the major contributor of NPSH groups in the cystosol. GSH:GSSG ratio was similar in hepatic cytosol from starved and fed rats.

DISCUSSION

The present studies demonstrate that different mechanisms are responsible for the decrease in H-T_3 in the starved, hypothyroid, and perinatal rat. Decreases in the serum concentrations of T_4 and T_3 and in the hepatic concentration of NPSH groups are found in all three states, but their role in the decreased H-T_3 appears to be different. In hypothyroidism, the in vitro generation of T_3 by liver homogenates was fully restored by the in vivo administration of thyroid hormones without increasing hepatic NPSH groups, whereas the addition of DTT, a thiol protective agent, had little (hypothyroid rat) or no (dw/dw mouse) effect on T_3 generation. These observations strongly suggest that the decreased in vitro hepatic conversion of T_4 to T_3 in hypothyroidism is primarily because of a decrease in the quantity of the outer ring deiodinase itself and not to a lack of cofactor(s). It is evident, therefore, that thyroid hormones

regulate the peripheral conversion of T_4 to T_3 by inducing the synthesis or preventing the degradation of the deiodinating enzyme or a yet unidentified cofactor or by a combination of these possibilities. The total absence of hepatic T_4 to T_3 conversion in the more severely hypothyroid, hypopituitary dwarf mouse and its dramatic increase after thyroid hormone replacement therapy also demonstrate the important role of thyroid hormones in regulating this process and suggest that the enhancement of enzyme activity is due to the direct action of the thyroid hormones rather than to alterations in the secretion of pituitary hormones.

The factors responsible for the decrease in hepatic conversion of T_4 to T_3 associated with starvation appear to be different. The administration of physiologic doses of T_4 and T_3, sufficient to restore the decreased serum T_4 and T_3 concentrations induced by starvation (32, 41), had no effect on the decreased H-T_3. Moreover, in contrast to hypothyroidism, the addition of DTT to the hepatic homogenate completely restored conversion of T_4 to T_3, even though hepatic NPSH groups were decreased in both starvation and hypothyroidism. These findings suggest that during starvation the 5'-deiodinase is present in sufficient quantity but is relatively inactive due to conformational changes, to lack of coenzyme availability, or to both. A decrease in compounds supplying sulfhydryl groups, such as GSH, resulting in a decreased quantity of reduced enzyme, may be responsible, at least in part, for the decreased conversion of T_4 to T_3. Glutathione is also important in the metabolism of peptide hormones such as insulin. It has been reported that the activity of the insulin-degrading enzyme in the liver, glutathione insulin transhydrogenase, varies with the availability of hepatic glutathione (48).

Although the finding of decreased glutathione concentration in the liver of starved rats may be important in the observed decrease in T_4 to T_3 conversion, the precise mechanism remains unclear. One possible explanation is shown in Fig. 8. During starvation, the lack of glucose substrate and decreased concentrations of glucose-6-phosphate dehydrogenase and malate dehydrogenase (49, 50) result in a decrease in cytosol NADPH. NADPH in the soluble cytoplasm of the liver is primarily generated by the hexosemonophosphate shunt and by conversion of L-malate to pyruvate by the NADP malate dehydrogenase. NADPH is decreased in the cytosol of starved rats (unpublished observations), and glucose and amino acid, but not fat administration, restores H-T_3 generation in the starved rat (32), presumably by increasing the quantity of cytosolic NADPH or its rate of generation. Decreased concentration of NADPH may, therefore, decrease the activity of the converting enzyme directly or decrease the rate of generation of the already decreased quantity of GSH. This is in agreement with the findings of Balsam and
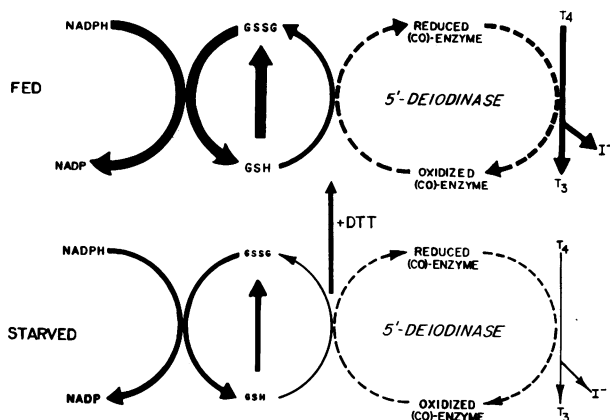
FIGURE 8 Proposed scheme depicting the influence of co-factors, NADPH, and GSH on T_4 deiodination to T_3 in the fed and starved state. The varying concentrations in GSH are represented by changes in the letter size. Rates of change represented by arrow size. Addition of DTT or GSH to homogenates from starved animals replenishes concentrations of reduced sulfhydryl groups and enhances 5'-deiodinase activity, presumably by reduction of the S-S bonds of the enzyme 5'-deiodinase, and restores T_4 to T_3 conversion.

Ingbar (51) which suggest that the addition of NADPH restored the decreased hepatic conversion of T_4 to T_3 by the starved rat liver microsomes. Thus, the action of NADPH may be similar to that of DTT, reducing the protein S-S bonds of the enzyme or coenzyme (52), either directly or indirectly by maintaining glutathione in the reduced state.

The fetal rat liver homogenate generated very little T_3 from T_4, and the concentration of NPSH groups was markedly decreased. The addition of DTT resulted in only a small increase in H-T_3, similar to results obtained in the hypothyroid rat, suggesting that reduced H-T_3 is primarily due to a decrease in 5'-deiodinase concentration. An increase in enzyme activity, NPSH groups, and the response to DTT were observed with increasing age, indicating an increase in both cofactor(s) and deiodinating enzyme. When these studies were completed, others reported that the decreased T_3 generation from T_4 observed in the fetal sheep liver homogenate was restored to normal when DTT was added (39). Thus, a decrease in NPSH groups and not enzyme concentration was suggested as the primary event in the decreased T_3 generation in the fetal sheep. The present findings in the fetal rat differ from those in the fetal sheep in that addition of DTT did not restore H-T_3 to normal, suggesting a decrease in 5'-deiodinase concentration. The findings in the 4-d-old neonatal rat resemble those in the fetal sheep. Differences observed in the younger perinatal rat and sheep are probably due to the delayed maturation of 5'-deiodinase in the rat.

The importance of NPSH in protecting the T_4 5'-monodeiodinase activity is further strengthened by the

finding that the loss of NPSH groups induced by prolonged storage at $-20°C$ paralleled the loss of enzyme activity. The addition of sulfhydryl groups to these previously stored homogenates markedly enhanced the conversion of T_4 to T_3. Finally, the decrease in NPSH groups observed during the incubation period was accompanied by a decrease in enzyme activity.

Although the metabolic significance of the present findings remains uncertain, it is possible that the action of T_4 is amplified or attenuated by its conversion to metabolites such as T_3 or rT_3. The NADPH-glutathione cycle and its regulation by specific nutriments may play an important role in the conversion of T_4 to T_3 and, therefore, the metabolic action of the thyroid hormones. The present studies also indicate that the thyroid hormones themselves modulate the 5'-deiodinase and provide a mechanism of regulating the conversion of T_4 to its more active metabolite, T_3.

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