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On the Mechanism of Impaired *in Vitro* Generation of 3,5,3'-Triiodothyronine from Thyroxine in the Livers of Hypothyroid Rats*

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ABSTRACT. It has been demonstrated previously that in rat liver, the generation of T_3 from T_4 (T_3 neogenesis) is mediated by an enzyme present in the crude microsomal fraction and that the activity of the enzyme is enhanced by cofactors present in the cytosol. The present studies were undertaken to evaluate the respective roles of microsomal and cytosolic factors in producing the impaired hepatic T_3 neogenesis previously shown to occur in hypothyroid rats. To this end, T_3 neogenesis was assessed in mixtures of microsomes and buffer or microsomes and cytosols from intact and thyroidectomized rats in the presence and absence of added cofactors. Regardless of whether studies were conducted with buffer, normal cytosol, or cytosol from hypothyroid animals, microsomes from normal animals consistently generated more T_3 from T_4 than did microsomes from hypothyroid animals. Moreover, although glutathione (GSH) in buffer greatly stimulated T_3 neogenesis in control microsomes, it had little such effect in microsomes from hypothyroid animals. A secondary and less severe abnormality appeared to be present in the hepatic cytosol from hypothyroid rats, since T_3 neogenesis was consistently greater in the presence of control cytosol than when cytosol from hypothyroid animals was used. As judged from studies with control microsomes, the defect in supporting activity of cytosol from hypothyroid animals was abolished by the addition of NADPH or GSH.

The findings indicate that the major factor responsible for defective T_3 neogenesis in the liver of the hypothyroid rat is a decrease in the activity of the 5'-monodeiodinase for T_4 present in the crude hepatic microsomal preparation. It is not clear, however, whether the decrease reflects a change in the concentration of enzyme or a change in its properties leading to decreased responsiveness to stimulatory cofactors. A secondary abnormality in hepatic T_3 neogenesis is evidently a reduction in the supporting activity of the cytosol, possibly reflecting a decrease in the concentrations of NADPH or GSH, or both. (*Endocrinology* **105:** 1115, 1979)

IN PREVIOUS studies (1) we have presented evidence that the generation of T_3 from T_4 by preparations of rat liver *in vitro* reliably reflects the *in vivo* activity of this process, sometimes termed T_3 neogenesis. T_3 neogenesis *in vitro* is impaired in livers obtained from rats that have been starved, made diabetic, or given propylthiouracil (1), and these manipulations have been directly demonstrated in man and animals to reduce the production of T_3 from T_4 (2-5). Moreover, a variety of pharmacological agents, including dexamethasone (6, 7), the antiarrythmic agent, Amiodarone (8), and radiographic contrast media (9, 10), lower serum T_3 concentration in man. These agents, which are presumed to do so by decreasing T_3 neogenesis *in vivo*, have been found to inhibit hepatic T_3 neogenesis *in vitro* (1) (Balsam, A., Z. Eisenstein, F. Sexton, and S. H. Ingbar, unpublished observations).

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Other studies have demonstrated that the hepatic 5'-monodeiodinase that mediates conversion of T_4 to T_3 is mainly associated with the crude microsomal fraction (11, 12), and that cofactors supportive of its activity are present in hepatic cytosol (11, 13, 14). Studies in which microsomes and cytosols from livers of control and experimental rats were variously mixed have made it possible to attribute the defective hepatic T_3 neogenesis associated with a 48-h period of fasting to a cytosolic abnormality (13, 15). In the present studies we have used this technique to explore the nature and locus of the defect in T_3 neogenesis previously shown to be present in livers of hypothyroid rats (16-19). A portion of the results has been presented in abstract form (20).

Materials and Methods

Reagents

All chemicals and labeled thyroid hormones used in these studies were purchased from commercial sources.[1]

[1] Phenolic ring ^{125}I-labeled L-T_4 ([^{125}I]T_4; SA, 50-70 μCi/μg) and L-T_3 ([^{125}I]T_3; SA, 50-75 μCi/μg) were purchased from Abbott Laboratories (North Chicago, IL). Crystalline T_4 and T_3, NADP, isocitric acid, isocitric acid dehydrogenase, and glutathione (GSH) were all purchased from Sigma Chemical Co. (St. Louis, MO).

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Animals and diet

Intact and thyroidectomized adult male Sprague-Dawley rats, weighing 150–175 g, were purchased from Charles River Breeding Laboratoties (Wilmington, MA). Animals were maintained on Agway chow (Agway, Inc., Syracuse, NY) and tap water *ad libitum* for at least 2 weeks before study.

Preparation of tissue fractions

Rats were killed by cervical subluxation and their livers were rapidly excised and weighed. Portions of liver were homogenized (2:5, wt/vol) in phosphate (0.05 M)-sucrose (0.25 M) buffer, pH 7.4, and centrifuged at 10,000 × *g* for 15 min. The pellet was discarded and the supernatant was centrifuged at 105,000 × *g* for 1 h. The resulting supernatant (cytosol) was separated from the sediment by aspiration. The crude microsomal pellet was then washed twice in 20 ml buffer by resuspension and resedimentation at 20,000 × *g*. The washed crude microsomal pellet was then suspended in sucrose-phosphate buffer by gentle homogenization.

Incubation conditions

Aliquots of microsomal suspensions (200 μl; equivalent to 300 mg liver) were added to either 1.6 ml phosphate-sucrose buffer or 1.6 ml cytosol previously enriched with $[^{125}I]T_4$ (1 μCi/ml; 0.020 μg/ml) or $[^{125}I]T_3$ (1.3 μCi/ml; 0.025 μg/ml). Buffer alone (300 μl) or buffer containing additives to be studied was then added to yield a final volume of 2.1 ml. These were incubated at 37 C under N_2 for 3 h. Under these incubation conditions, the formation of $[^{125}I]T_3$ from $[^{125}I]T_4$ has been shown to increase progressively for 4 h. In all experiments, two types of incubation controls containing $[^{125}I]T_4$ were regularly employed, one devoid of tissue fractions which was incubated at 37 C and another containing tissue fractions which was incubated at 0 C. In none of the incubation controls was there detectable degradation of $[^{125}I]T_4$ or generation of $[^{125}I]T_3$. Incubations were terminated by plunging vessels into cracked ice. Measured aliquots of the incubation mixtures were then rapidly withdrawn and mixed with equal volumes of outdated blood bank plasma. The resulting mixtures were stored frozen at −20 C for subsequent analysis by paper chromatography.

Paper chromatography

Reaction mixtures were thawed and thoroughly mixed and 10-μl portions were subjected, along with appropriate carrier compounds, to descending paper chromatography in a hexane-tertiary amyl alcohol-2 N ammonia (1:10:11) solvent system. After chromatograms had been dried, carrier iodothyronines were localized with UV light and iodide (I^-) was identified by means of an 0.1% aqueous palladium chloride spray. Zones corresponding to the origin and carrier compounds were excised and counted in a γ-scintillation counter. Over 98% of the total radioactivity applied to the strips was recovered in these zones. Values for the percent generation of labeled products were corrected for the percent contamination of these products found in nonmetabolizing control vessels and for the percent of $[^{125}I]T_4$ or $[^{125}I]T_3$ present in the initial substrate. As assessed by paper chromatography of the labeled substrates, $[^{125}I]T_4$ was 96–97% pure, containing 0.3–0.5% $[^{125}I]T_3$ and 1–2% $[^{125}I]I^-$ as contaminants, while $[^{125}I]T_3$ was 95% pure and contained 2% $[^{125}I]I^-$ as the only definable contaminant. The coefficient of variation of the chromatographic method for the measurement of the various functions under diverse conditions did not exceed 11%.

Protein measurements

Protein concentrations were measured in cytosols and in microsomes dispersed in 0.1% deoxycholate using the method of Lowry and coworkers (21).

Statistical methods

All experiments were performed three or more times, with close agreement in the results obtained. Hence, data were pooled for statistical analysis. When a single experimental variable was studied, its effect was evaluated with Student's *t* test; when two or more variables were studied, analysis of variance, followed by Duncan's multiple range test, was used to identify significant differences between groups (22).

Results

Products of $[^{125}I]T_4$ and $[^{125}I]T_3$ metabolism

Under all experimental conditions studied, only four labeled products of $[^{125}I]T_4$ metabolism were detected: chromatographically immobile origin material (OM), I^-, labeled material corresponding in migration to 3,5,3′,5′-tetraiodothyroacetic acid, and T_3. Since the deiodination of T_4 leading to the generation of T_3 was the principal focus of these experiments, results concerning the degradation of T_4 and the generation of T_3 and I^- therefrom will be presented. The formation of labeled OM and 3,5,3′,5′-tetraiodothyroacetic acid accounted for only a few percent of the total radioactivity on the final chromatograms and will not be discussed.[2]

In the case of $[^{125}I]T_3$, I^- and trace amounts of OM were the only labeled products detected; labeled 3,5,3′-triiodothyroacetic acid was not seen.

T_3 neogenesis in unsupplemented systems (Table 1)

As previously reported (14, 16), T_4 degradation and T_3 neogenesis were far less when microsomes were incubated in buffer rather than in cytosol; in the present studies, this was true regardless of whether tissue frac-

[2] A complete tabulation of all of the results of the experiments presented in this manuscript together with statistical analyses of the significance of differences between means for all functions measured among all experimental groups have been archived in the National Auxiliary Publication Service (NAPS), document 03523. This information may be ordered from ASIS/NAPS, Microfiche Publications, P.O. Box 3513, Grand Central Station, New York, New York 10017. Remit in advance $3.00 for microfiche copy, or for photocopy, $5.00 for up to 20 pages plus 25¢ for each additional page. Checks should be made payable to Microfiche Publications.

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tions from normal or hypothyroid animals were employed. Nevertheless, in buffer, both T_4 degradation and the generation of T_3 were greater with microsomes from control animals (group A) than with microsomes from hypothyroid animals (group B). In experiments in which cytosols from control or hypothyroid animals were mixed with microsomes from euthyroid controls, the control cytosol (group C) supported both T_4 degradation and T_3 neogenesis to a significantly greater degree than did the experimental cytosol (group D). Significant differences in the stimulatory activity of the two types of cytosol were not seen, however, when microsomes from hypothyroid animals were employed (groups E and F).

TABLE 1. The effect of hypothyroidism on the metabolism of $[^{125}I]T_4$ in mixtures of microsomes and buffer, and microsomes and cytosols from rat liver

Microsome source	Cytosol source	n[a]	$[^{125}I]T_4$ degradation (% added T_4)[b]	$[^{125}I]T_3$ formation (% added T_4)[c]	$[^{125}I]I$ formation (% added T_4)[d]
A. Euthyroid	(Buffer)	12	6.5 ± 0.8	1.2 ± 0.2	3.4 ± 0.5
B. Hypothyroid	(Buffer)	12	3.2 ± 0.3	0.4 ± 0.1	1.7 ± 0.3
C. Euthyroid	Euthyroid	16	15.9 ± 1.2	3.7 ± 0.3	7.5 ± 0.7
D. Euthyroid	Hypothyroid	14	12.2 ± 1.2	2.8 ± 0.3	6.2 ± 0.9
E. Hypothyroid	Euthyroid	8	12.5 ± 0.8	1.7 ± 0.1	5.7 ± 0.8
F. Hypothyroid	Hypothyroid	10	10.4 ± 0.6	1.4 ± 0.2	4.0 ± 0.5

Levels of statistical significance of differences between means among various experimental groups were determined by analysis of variance and Duncan's multiple range test. Analyses are given only for those comparisons considered to be important. Values given are the mean ± SE. Parentheses indicate buffer used in place of cytosol.

[a] Number of separate experiments performed for each experimental group.

[b] NS for D *vs.* F and E *vs.* F; $P < 0.05$ for A *vs.* B and C *vs.* E; $P < 0.01$ for A *vs.* C, A *vs.* D, B *vs.* E, B *vs.* F, C *vs.* D, and C *vs.* F.

[c] NS for E *vs.* F; $P < 0.05$ for A *vs.* B and B *vs.* F; $P < 0.01$ for A *vs.* C, A *vs.* D, B *vs.* E, C *vs.* D, C *vs.* E, C *vs.* F, and D *vs.* F.

[d] NS for A *vs.* B, C *vs.* D, C *vs.* E, and E *vs.* F; $P < 0.05$ for B *vs.* F and D *vs.* F; $P < 0.01$ for A *vs.* C, A *vs.* D, B *vs.* E, and C *vs.* F.

In the presence of control cytosol, control microsomes (group C) degraded significantly more T_4 and generated significantly more T_3 than did microsomes from hypothyroid animals (group E). In the presence of cytosol from hypothyroid animals, T_4 degradation was not significantly greater with control (group D) than with experimental microsomes (group F), but the generation of T_3 and I^- was.

In a series of six experiments, the mean (±SE) protein concentrations (milligrams per g liver) of control and thyroidectomized rat liver microsomes were, respectively, 15.3 ± 0.6 *vs.* 13.4 ± 1.4 (P = NS) and of the corresponding cytosols were, respectively, 70.0 ± 2.9 *vs.* 80.8 ± 2.5 ($P < 0.02$).

Effects of NADPH (Table 2)

In experiments conducted to assess the effects of an NADPH-generating system, differences among the various mixtures of microsomes and cytosols or buffer with respect to T_4 degradation, T_3 neogenesis, and I^- generation in the absence of added NADPH entirely confirmed

TABLE 2. The effect of NADPH on the metabolism of $[^{125}I]T_4$ in mixtures of microsomes and buffer, and microsomes and cytosols from livers of euthyroid and hypothyroid rats

Microsome source	Cytosol source	Cofactor[a]	n[b]	$[^{125}I]T_4$ degradation (% added T_4)[c]	$[^{125}I]T_3$ formation (% added T_4)[d]	$[^{125}I]I$ formation (% added T_4)[e]
A. Euthyroid	(Buffer)		6	7.2 ± 1.2	1.2 ± 0.4	4.0 ± 0.5
B. Euthyroid	(Buffer)	NADPH	6	6.2 ± 0.6	1.9 ± 0.5	3.5 ± 0.6
C. Hypothyroid	(Buffer)		6	3.8 ± 0.5	0.3 ± 0.1	2.5 ± 0.3
D. Hypothyroid	(Buffer)	NADPH	6	3.4 ± 1.1	0.4 ± 0.02	1.9 ± 1.1
E. Euthyroid	Euthyroid		18	15.7 ± 0.9	3.7 ± 0.3	7.9 ± 0.7
F. Euthyroid	Euthyroid	NADPH	10	8.4 ± 1.0	3.4 ± 0.3	4.3 ± 0.7
G. Euthyroid	Hypothyroid		16	13.4 ± 0.9	3.0 ± 0.3	6.9 ± 0.7
H. Euthyroid	Hypothyroid	NADPH	8	11.1 ± 0.8	3.7 ± 0.1	6.2 ± 0.7
I. Hypothyroid	Euthyroid		4	11.1 ± 0.4	1.6 ± 0.1	4.7 ± 0.2
J. Hypothyroid	Euthyroid	NADPH	4	6.5 ± 1.4	1.4 ± 0.1	4.0 ± 1.0
K. Hypothyroid	Hypothyroid		6	8.7 ± 0.9	1.7 ± 0.3	3.7 ± 0.6
L. Hypothyroid	Hypothyroid	NADPH	6	5.7 ± 1.0	1.6 ± 0.4	2.8 ± 0.9

Levels of statistical significance of differences between means among various experimental groups were determined by analysis of variance and Duncan's multiple range test. Analyses are given only for those comparisons considered to be important. Values given are the mean ± SE. Parentheses indicate buffer used in place of cytosol.

[a] NADPH added as an NADPH-generating system: isocitric acid dehydrogenase (1 mg/ml), isocitric acid (20 mM), and NADP (0.1 mM).

[b] Number of separate experiments performed for each experimental group.

[c] NS for A *vs.* B, A *vs.* C, A *vs.* D, B *vs.* D, F *vs.* J, G *vs.* H, I *vs.* J, and K *vs.* L; $P < 0.05$ for E *vs.* G; $P < 0.01$ for E *vs.* F, E *vs.* H, E *vs.* I, E *vs.* J, and H *vs.* L.

[d] NS for A *vs.* B, A *vs.* C, A *vs.* D, B *vs.* D, E *vs.* F, E *vs.* H, I *vs.* J, and K *vs.* L; $P < 0.05$ for E *vs.* G; $P < 0.01$ for E *vs.* I, E *vs.* J, F *vs.* J, G *vs.* H, and H *vs.* L.

[e] NS for A *vs.* B, A *vs.* C, A *vs.* D, B *vs.* D, E *vs.* G, E *vs.* H, F *vs.* J, G *vs.* H, I *vs.* J, and K *vs.* L; $P < 0.05$ for E *vs.* I; $P < 0.01$ for E *vs.* F, E *vs.* J, and H *vs.* L.

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the data presented in Table 1 which has already been discussed.

In mixtures of microsomes and buffer, regardless of the origin of the microsomes, NADPH had no effect on the three major functions assessed (groups A-D). When both microsomes and cytosol from intact animals were employed (group E), NADPH did not affect T_3 neogenesis (group F) but significantly decreased fractional T_4 degradation and I^- generation. In contrast, in mixtures of control microsomes and experimental cytosol (group G), addition of NADPH (group H) restored the decreased generation of T_3 to levels seen in the presence of control cytosol (groups E and F) without significantly affecting either T_4 degradation or I^- generation.

In the presence of microsomes from hypothyroid animals, regardless of the source of the cytosols employed, NADPH had no significant effect on either T_4 degradation or the generation of T_3 or I^- (groups I-L).

In the presence of NADPH, regardless of the source of the cytosol, T_3 neogenesis in control microsomes was always greater than that seen with experimental microsomes (group F *vs.* group J; group H *vs.* group L). In every combination of microsomes and cytosols, the addition of NADPH tended to decrease both T_4 degradation and I^- generation (groups F, H, J, and L).

Effects of GSH (Table 3)

In experiments conducted to assess the effects of GSH (5 mM), differences among the various mixtures of microsomes with cytosols or buffer with respect to T_4 degradation, T_3 neogenesis, and I^- formation in the absence of added GSH, as is seen in Table 1, were again confirmed.

In mixtures of microsomes and buffer (groups A-D), GSH, in contrast to NADPH, markedly influenced T_4 metabolism. In the case of both control and experimental microsomes, the addition of GSH clearly stimulated both T_4 degradation and T_3 neogenesis. Generation of I^- was enhanced in the presence of control and experimental microsomes, but the change in the latter group was not statistically significant. In the presence of GSH, values for these three functions were far higher when control (group B) rather than experimental microsomes (group D) were used.

When both microsomes and cytosol from control animals were employed, GSH significantly stimulated T_3 neogenesis (group E *vs.* group F) but failed to significantly increase the degradation of T_4 or the generation of I^-. When GSH was added to mixtures of control microsomes and experimental cytosol (group H), the decreased level of T_3 neogenesis seen in the absence of GSH (group G) was restored to that seen in the presence of control cytosol (group F).

In the presence of microsomes from hypothyroid animals, regardless of the source of the cytosols employed, GSH slightly increased T_3 neogenesis, T_4 degradation, and I^- generation (groups I-L), but the changes were not significant. In the presence of GSH, regardless of the type of cytosol employed, T_3 neogenesis by control mi-

TABLE 3. The effect of GSH on the metabolism of $[^{125}I]T_4$ in mixtures of microsomes and buffer, and microsomes in cytosols from livers of euthyroid and hypothyroid rats

Microsome source	Cytosol source	Cofactor[a]	n[b]	$[^{125}I]T_4$ degradation (% added T_4)[c]	$[^{125}I]T_3$ formation (% added T_4)[d]	$[^{125}I]I$ formation (% added T_4)[e]
A. Euthyroid	(Buffer)		8	5.5 ± 0.6	1.3 ± 0.4	2.5 ± 0.5
B. Euthyroid	(Buffer)	GSH	8	20.6 ± 2.8	7.1 ± 0.8	11.9 ± 1.6
C. Hypothyroid	(Buffer)		8	2.7 ± 0.5	0.4 ± 0.1	1.3 ± 0.5
D. Hypothyroid	(Buffer)	GSH	8	6.5 ± 0.7	1.7 ± 0.2	3.2 ± 0.4
E. Euthyroid	Euthyroid		16	17.4 ± 0.6	4.3 ± 0.2	9.1 ± 0.5
F. Euthyroid	Euthyroid	GSH	8	19.4 ± 1.2	6.0 ± 0.5	11.1 ± 0.9
G. Euthyroid	Hypothyroid		16	13.6 ± 0.8	3.4 ± 0.3	7.3 ± 0.6
H. Euthyroid	Hypothyroid	GSH	8	16.5 ± 1.6	5.8 ± 0.8	9.0 ± 1.1
I. Hypothyroid	Euthyroid		8	11.2 ± 0.4	2.1 ± 0.2	3.6 ± 0.2
J. Hypothyroid	Euthyroid	GSH	8	12.7 ± 1.3	2.6 ± 0.1	4.7 ± 0.8
K. Hypothyroid	Hypothyroid		8	6.5 ± 0.5	1.3 ± 0.2	2.1 ± 0.2
L. Hypothyroid	Hypothyroid	GSH	8	7.1 ± 0.4	1.8 ± 0.2	2.9 ± 0.2

Levels of statistical significance of differences between means among various experimental groups were determined by analysis of variance and Duncan's multiple range test. Analyses are given only for those comparisons considered to be important. Values given are the mean ± SE. Parentheses indicate buffer used in place of cytosol.

[a] GSH, 5 mM.

[b] Number of separate experiments performed for each experimental group.

[c] NS for A *vs.* C, A *vs.* D, E *vs.* F, F *vs.* H, G *vs.* H, I *vs.* J, and K *vs.* L; $P < 0.05$ for C *vs.* D; $P < 0.01$ for A *vs.* B, B *vs.* D, E *vs.* G, F *vs.* J, H *vs.* L, and J *vs.* L.

[d] NS for A *vs.* C, A *vs.* D, F *vs.* H, I *vs.* J, J *vs.* L, and K *vs.* L; $P < 0.05$ for C *vs.* D and E *vs.* G; $P < 0.01$ for A *vs.* B, B *vs.* D, E *vs.* F, F *vs.* J, G *vs.* H, and H *vs.* L.

[e] NS for A *vs.* C, A *vs.* D, C *vs.* D, F *vs.* H, G *vs.* H, I *vs.* J, J *vs.* L, and K *vs.* L; $P < 0.05$ for E *vs.* F and E *vs.* G; $P < 0.01$ for A *vs.* B, B *vs.* D, F *vs.* J, and H *vs.* L.

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crosomes always greatly exceeded that by experimental microsomes (group F *vs.* group J; group H *vs.* group L).

Effects of cofactors on the metabolism of [^{125}I]T_3

The degradation of added [^{125}I]T_3 was slight and did not exceed 10% of the labeled substrate in any of the reaction mixtures studied. The addition of NADPH to reaction mixtures consistently decreased the percentage of T_3 degraded. These decreases were statistically significant ($P < 0.05$) in the case of control microsomes incubated in buffer (2.4 ± 0.6% *vs.* 1.1 ± 0.3%), control microsomes incubated in control cytosol (7.5 ± 2.0% *vs.* 2.6 ± 0.2%), and experimental microsomes incubated in control cytosol (5.4 ± 1.5% *vs.* 2.1 ± 0.4%). The addition of GSH increased T_3 degradation when added to microsomes incubated in buffer alone, but had no consistent effect in mixtures containing cytosol.

Discussion

The data obtained in this study strongly indicate that the major factor leading to decreased T_3 neogenesis in the liver of hypothyroid rats is a decrease in the intrinsic activity of the enzyme present in the crude microsomal fraction. The following observations support this view. In buffer, T_3 neogenetic activity of control microsomes was greater than that of experimental microsomes, even after enrichment with GSH. Moreover, in the presence of cytosol from either intact or hypothyroid animals, formation of T_3 by control microsomes greatly exceeded that by experimental microsomes, and this was the case even when these mixtures were enriched with NADPH or GSH. Thus, neither unidentified nor known cytosolic cofactors were able to correct the deficient activity found in hepatic microsomes derived from hypothyroid animals.

The data also indicate, however, that an abnormality of a lesser degree exists in the hepatic cytosol of hypothyroid animals. Thus, hepatic cytosol from such animals was consistently and significantly less supportive of T_3 neogenesis by control microsomes than was cytosol from control animals. Not surprisingly, these differences in cytosolic support activity were not evident when the less active microsomes from thyroidectomized animals served as the source of enzyme. Because of this consideration, it seemed necessary to draw conclusions regarding the abnormality in hepatic cytosol of hypothyroid animals from experiments with microsomes derived from livers of control rather than hypothyroid animals.

When control microsomes were employed as a source of enzyme, NADPH and GSH each proved capable of completely restoring the deficient supporting activity of hepatic cytosol from hypothyroid animals, suggesting that it may be deficient in one or both cofactors. This suggestion is consonant with direct measurements, which have shown that hypothyroidism decreases the concentrations of NADPH (23) and GSH (24) in rat liver. We have previously suggested that GSH acts as a direct cofactor, since GSH increased T_3 neogenesis by microsomes suspended in buffer (13, 15). NADPH had no such direct effect but did restore to normal the deficient supporting activity of hepatic cytosol derived from fasted animals (13, 15), in which the concentration of NADPH has been shown to be subnormal (25). These observations led to the suggestion that the action of NADPH is to maintain GSH in the reduced form by serving as a cofactor for the enzyme GSH reductase. The findings in the present study are again consistent with this formulation.

The present investigations provide several lines of evidence to indicate that the differences in T_3 neogenesis among mixtures of control and experimental microsomes and cytosols as well as the effects of NADPH and GSH therein could not be explained by effects on the rate of degradation of T_3. First, in the systems employed, the rate of degradation of T_3 was very low, so that in no combination of experimental variables did less than 90% of added [^{125}I]T_3 remain at the end of incubation periods. Thus, assuming that T_3 generated from T_4 is degraded at a rate similar to that of added T_3, differences in T_3 degradation alone could not explain the several-fold differences in net T_3 neogenesis that were observed among the various experimental groups. Furthermore, in general, those conditions associated with lower T_3 neogenesis were not associated with accelerated T_3 degradation but, to the contrary, were associated with constant or lower rates of T_3 metabolism.

An additional conclusion is suggested by the results of the present study, *i.e.* that the mechanism for T_3 degradation in rat liver is subject to controls different from those of the mechanism for T_3 neogenesis. Although GSH stimulated T_3 degradation by microsomes suspended in buffer, as it did T_3 neogenesis, it did not significantly affect the decreased activity of experimental cytosol with respect to T_3 degradation. Most striking, however, were the effects of NADPH, which consistently decreased the rate of T_3 degradation in all combinations of microsomes and cytosols and did so even when added to microsomes suspended in buffer. Previous data suggest that T_3 metabolism by liver homogenates is directed mainly toward the production of 3,3′-diiodothyronine, involving an inner ring rather than an outer ring deiodination of T_3 (26). If so, then the present data suggest the possibility that NADPH may have a direct inhibitory effect on this process. We have not explored this problem directly by assessing the effect of NADPH on the generation of 3,3′-diiodothyronine from T_3, but the problem appears worthy of further study.

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The results of this and other studies (16–19) indicating the presence of reduced *in vitro* T_3 neogenesis in livers and kidneys of hypothyroid rats appear to be in accord with some previously reported data but not with others. Thus, in both animals and man, metabolic clearance and deiodination of T_4 are decreased in hypothyroidism (27–30), and these abnormalities are reversed by treatment with thyroid hormone (31, 32). However, other studies have suggested that the fractional rate of conversion of T_4 to T_3 in hypothyroid patients is equal to or greater than that in euthyroid subjects (31, 33). The explanations for the apparent discrepancy between the *in vitro* and *in vivo* data are not known but may be related to the fact that in the *in vivo* studies, complete equilibrium between intracellularly generated and exogenously administered T_3 may not have been attained during hypothyroidism, a possibility suggested by the studies of Garcia and coworkers (34). In addition, the reports of a higher fractional rate of T_3 neogenesis in hypothyroid patients than in euthyroid humans may be explained by the lower stable T_4 concentration in the former group, as suggested by the data of Maeda *et al.* (19).

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Ovary Workshop, 1980: "Physiologic Cessation of Ovarian Function"

The biannual Ovary Workshop will be held August 8, 10, and 11, 1980 in Ann Arbor, MI, immediately before the meeting of the Society for the Study of Reproduction (August 12–14, 1980 in Ann Arbor).

Atresia, ovulation, seasonal diapause, luteolysis, and ageing will be the five topics discussed by invited speakers and open only for poster presentations by participants. Persons interested in presenting posters dealing specifically with these topics should submit a single page abstract (single space) headed by title, authors, and affiliation. Indicate in the upper right-hand corner which of the five topics is relevant to the abstract. The original and five copies of the abstract should be sent to Dr. N. B. Schwartz, Department of Biological Sciences, Northwestern University, Evanston, IL 60291, by December 1, 1979.

All persons whose abstracts are accepted *must* bring to the Workshop a short manuscript for publication. Details of the format will be mailed later to authors. Discussion of the talks and posters will be recorded for possible publication with the short manuscripts.

Attendance will be limited by space considerations primarily to those presenting posters of accepted abstracts.

Planning committee: N. B. Schwartz (Chair), C. P. Channing, M. Hunzicker-Dunn, J. Lobotsky, J. R. Lorenzen, J. A. Richards, R. J. Ryan, and G. Ross.

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