0013-7227/78/1035-1759$02.00/0
Endocrinology

Vol. 103, No. 5
Printed in U.S.A.

The Effect of Thyroidectomy, Hypophysectomy, and Hormone Replacement on the Formation of Triiodothyronine from Thyroxine in Rat Liver and Kidney*

ALAN BALSAM, FRANKLIN SEXTON, AND SIDNEY H. INGBAR

Thorndike Laboratory, Harvard Medical School, and Department of Medicine, Beth Israel Hospital, Boston, Massachusetts 02215

ABSTRACT. Studies were performed to assess the effects of thyroidectomy and hypophysectomy on the overall metabolism of [^{125}I]T_4 and its conversion to [^{125}I]T_3 in slices of rat liver and kidney. Within 2 days after thyroidectomy a slight reduction was evident in the generation of [^{125}I]T_3 from [^{125}I]T_4 in both liver and kidney, and 31 days after surgery the activity of this process in these tissues was markedly decreased. Thyroidectomy was associated with diminished disappearance of [^{125}I]T_4, decreased generation of [^{125}I]iodide and, in kidney, decreased formation of [^{125}I]tetraiodothyroacetic acid. Each of these changes in the metabolism of [^{125}I]T_4 in liver and kidney of thyroidectomized animals was reversed by administration of replacement doses of T_3 for 10 days.

Hypophysectomy was similarly associated with diminished conversion of [^{125}I]T_4 to [^{125}I]T_3 that was slight 2 days postoperatively and became more pronounced 31 days after surgery. In liver and kidney from hypophysectomized animals, the degradation of [^{125}I]T_4 was significantly reduced and the generation of labeled iodide was unchanged, whereas in kidney, the formation of [^{125}I]tetraiodothyroacetic acid was markedly reduced. These abnormalities in T_4 metabolism in tissues from hypophysectomized animals were completely corrected by hormone replacement with T_4. No independent or additional effect was noted when animals were given replacement doses of adrenocortical or gonadal steroids.

The present direct studies of the conversion of T_4 to T_3 in rat liver and kidney *in vitro* suggest that the impairment in this process observed after thyroidectomy or after hypophysectomy is related to deficiency of thyroid hormone. Moreover, decreased conversion of [^{125}I]T_4 to [^{125}I]T_3 in hypothyroidism, at least after thyroidectomy, could not be explained by a more rapid disappearance of [^{125}I]T_3 formed, because the metabolism of [^{125}I]T_3 was instead markedly decreased in similar preparations after removal of the thyroid. (*Endocrinology* **103:** 1759, 1978)

IT IS generally agreed that, in man, monodeiodination of T_4 in peripheral tissues is the major source of the T_3 present in plasma (1, 2). A variety of abnormal states and pharmacological agents would appear to impair this process, since they are accompanied by or induce pronounced lowering of the serum T_3 concentration. Among these are severe acute and chronic illness (3, 4); operative stress (5, 6); caloric, especially carbohydrate, deprivation (7–13); and such drugs as propylthiouracil (PTU) (14–17), propranolol (18), amiodarone (19), certain x-ray contrast media (20, 21), and dexamethasone (22–24). In the case of starvation and cirrhosis, the decreased serum T_3 concentration has been shown to result from decreased production of T_3, rather than increased metabolic clearance of the hormone (25–28), although rates of conversion of T_4 to T_3 have not been measured directly.

We have recently reported observations which strongly suggest that the conversion of T_4 to T_3 in slices or homogenates of rat liver affords a reliable model for this process in man, since inhibition of the generation of T_3 from T_4 by starvation, dexamethasone, PTU, and amiodarone could readily be demonstrated in this system (29). We now extend our observations with this model to a consideration of the effects of thyroidectomy and hypophysectomy, and of hormonal treatment thereof, on the *in vitro* conversion of T_4 to T_3 in rat liver and kidney. A portion of this work had been presented in abstract form (30).

Received January 30, 1978.

Address reprint requests to: Dr. Alan Balsam, Division of Endocrinology, Beth Israel Hospital, 330 Brookline Ave., Boston, Massachusetts 02215.

* This research was supported in part by Research Grant AM8416 from the NIAMDD and by Grant RR-01032 from the General Clinical Research Centers Program of the Division of Research Resources, NIH, Bethesda, Md.

Materials and Methods

Animals and diet

Intact, thyroidectomized, and hypophysectomized male Sprague-Dawley rats weighing 150–200 g were purchased from Charles River Breeding Laboratories (Wilmington, MA) and were either provided with a diet consisting of pelleted laboratory chow and drinking water *ad libitum,* or were totally deprived of food for 48 h before sacrifice, as indicated below.

Labeled and unlabeled hormones

[^{125}I]T_4 (SA 50–70 μCi/μg) and L-[^{125}I]T_3 (SA, 50–70 μCi/μg) were purchased from Abbott Laboratories (North Chicago, IL). Crystalline T_4, T_3, corticosterone, deoxycorticosterone acetate (DOCA), and testosterone were purchased from Sigma Chemical Company (St. Louis, MO).

Hormonal replacement

Studies in thyroidectomized and hypophysectomized animals were usually begun 2–3 weeks after surgery, during which period marked impairment of growth was noted in both groups. Thereafter, thyroidectomized rats received daily sc injections of approximate physiological replacement doses of either T_4 (1.5 μg/100 g BW) or T_3 (0.5 μg/100 g BW) (31), or appropriate diluent for 7–12 days before sacrifice. Hypophysectomized rats received daily sc injections in peanut oil (μg/100 g BW) of replacement doses of testosterone, 1.5; corticosterone, 100; DOCA, 1; T_4, 1.5; or all of these hormones together for 10 days before sacrifice (32). Intact or hypophysectomized control rats received daily injections of peanut oil alone.

Preparation and incubation of tissue slices

Slices of liver and kidney cortex of uniform thickness weighing approximately 250 and 100 mg, respectively, were prepared with the aid of a Stadie-Riggs microtome. Slices from control and experimental animals were trimmed and matched according to weight before incubation. A single slice from each animal was then suspended in 2 ml Krebs-Ringer-phosphate (KRP) buffer, pH 7.4, enriched with [^{125}I]T_4 (1 μCi/ml, 0.020 μg/ml) or [^{125}I]T_3 (1.3 μCi/ml, 0.026 μg/ml) and was incubated in room air at 37 C for 4 h. At least four vessels for each experimental group were studied in each experiment. At the end of incubations, vessels were plunged into crushed ice and slices were then homogenized in their own media. A portion of the homogenate was combined with outdated blood bank plasma (1:2, v/v) and frozen until subsequently analyzed.

In each experiment, two types of control vessels were employed, one incubated at 37 C and containing no tissue and the other containing tissue slices, but incubated at 0 C. Values for the percentage of the several labeled products of [^{125}I]T_4 or [^{125}I]T_3 metabolism that were generated in the presence of tissue incubated at 37 C were always corrected by subtracting the percentage value of the corresponding contaminant in the nonmetabolizing controls.

Analysis of reaction products

Frozen homogenates were thawed and mixed thoroughly; 10-μl portions were applied as a spot to the origin of strips of Whatman 3MM chromatography paper together with carrier iodide, T_4, T_3, and, in some instances, tetraiodothyroacetic acid (T_4AC). Strips were then subjected to descending chromatography in a hexane-tertiary amyl alcohol-2 N ammonia (1:10:11) solvent system. Chromatograms were dried and the positions of iodide and iodothyronines were identified by spraying with palladium chloride or by fluorescence under ultraviolet light, respectively. The dried chromatograms were cut into zones corresponding to the origin and carrier compounds and were counted in a γ scintillation counter. The radioactivity in these zones accounted for approximately 98% of the total radioactivity between the origin and the solvent front.

The labeled substrate T_4 was 96% pure as judged by paper chromatography, and contained 0.5–1% of [^{125}I]T_3 and 2–3% of [^{125}I]iodide. The [^{125}I]T_3 substrate contained as a definable contaminant only 2% of [^{125}I]iodide.

The fractional degradation of the labeled T_4 (or T_3) was calculated as the difference between 100 and the percentage of T_4 present in chromatograms of the metabolizing vessels, divided by 100. The fractional generation of each of the products of iodothyronine degradation was calculated as the percentage contribution of that product to the total measured radioactivity, corrected for contamination of the substrate, divided by 100.

Statistical analysis

All experiments were performed at least twice, with good concurrence in the results obtained in replicate studies. Therefore, results for all studies dealing with a particular experimental manipulation were pooled for statistical analysis. In the case of experiments involving only two groups, Student's *t*-test was employed. In experiments involving three or more groups, the presence of significant differ-

ences was established by analysis of variance and Duncan's multiple range test (33) was then employed to evaluate the significance of differences between any two groups.

Results

Slices of rat liver generated the following ^{125}I-labeled products from [^{125}I]T_4: small quantities of chromatographically immobile origin material and compounds having the chromatographic mobility of iodide and of T_3. In kidney, all of these products and, in addition, a ^{125}I-labeled compound with the mobility of carrier [^{125}I]T_4AC, were observed. Discernible ^{125}I-labeled compounds formed by both liver and kidney from [^{125}I]T_3 were only iodide and small quantities of origin material. Formation of labeled origin material during the metabolism of either [^{125}I]T_4 or [^{125}I]T_3 was not materially altered by the experimental manipulations employed, and will not, therefore, be further discussed.[1]

Effect of thyroidectomy (Table 1)

When compared to slices obtained from intact controls, liver slices from rats thyroidectomized 2 days before study displayed a small, but significant, reduction in the generation of [^{125}I]T_3 from [^{125}I]T_4. The generation of [^{125}I]iodide, the degradation of [^{125}I]T_4 and the ratio T_3 generation/T_4 degradation, though lower in livers from thyroidectomized animals than from controls, were not significantly so. Liver slices from rats subjected to thyroidectomy approximately 4 weeks before study generated much less [^{125}I]T_3 from labeled T_4 than did liver slices from intact controls. Slight, insignificant reductions in the rates of [^{125}I]T_4 degradation and [^{125}I]iodide generation were detected in these preparations, but the ratio of T_3 generation to T_4 degradation was markedly and significantly decreased. Kidney slices from rats thyroidectomized 2 days before study revealed no significant changes in any aspect of T_4 metabolism studied. In contrast, all aspects of *in vitro* T_4 metabolism, including T_3 generation, iodide, and T_4AC generation, T_4 degradation, and the ratio of T_3 generation to T_4 degradation, were markedly and significantly decreased in kidney slices from animals thyroidectomized 1 month earlier. Studies conducted in tissues from animals thyroidectomized between 2 and 31 days earlier revealed changes in hormone metabolism intermediate in magnitude (data not shown).

Effect of thyroid hormone replacement (Table 2)

In these experiments, some animals thyroidectomized 1 month before study were given no treatment, whereas others, as well as intact controls, were given T_3 (0.5 μg/100 g BW) for 10 days before sacrifice.

Among the several aspects of T_4 metabolism by liver and kidney slices that were studied differences between untreated thyroidectomized and T_3-treated control animals were almost identical to the differences between thyroidectomized and intact control animals described above (Table 1).

For both liver and kidney, administration of T_3 to thyroidectomized animals restored values of each of the several functions studied to ones that were not significantly different from those found in T_3-treated intact controls.

Studies of the effects of T_4 replacement during the last 10 days of a 1-month period after thyroidectomy were conducted only in slices of liver. Here, as was the case with T_3, essentially complete reversal of the diminished T_3 generation from T_4 seen in slices from thyroidectomized animals was observed (data not shown).

Effect of hypophysectomy (Table 3)

As was the case after thyroidectomy, in animals studied 2 days after hypophysectomy, T_3 generation from T_4 was significantly lower in liver, but not kidney, slices from operated animals than in slices of corresponding tissues from intact controls. The remaining functions studied were not materially altered.

In animals studied 1 month after operation, T_3 generation from T_4 was markedly decreased in slices of both liver and kidney. In addition, the remaining indices of T_4 metabolism were decreased in both tissues, usually significantly so.

[1] In Tables 1-4, the percentage generation of origin material accounts for the difference between the [^{125}I]T_4 degraded and the sum of the indicated labeled products generated.

TABLE 1. Effect of thyroidectomy on the metabolism of [^{125}I]T_4 in slices of rat liver and kidney

	No. of animals	[^{125}I]T_4 degradation (A) (% added T_4)	[^{125}I]T_3 generation (B) (% added T_4)	[^{125}I]Iodide generation (C) (% added T_4)	[^{125}I]T_4AC generation (D) (% added T_4)	T_3 generation/T_4 degradation (2B/A)
Liver						
Exp 1						
Control	5	31.6 ± 0.8	4.1 ± 0.3	23.0 ± 0.9		0.27 ± 0.02
2 Days postthyroidectomy	5	27.0 ± 2.4	3.0 ± 0.2[a]	20.1 ± 2.1		0.23 ± 0.02
Exp 2						
Control	12	37.8 ± 3.0	4.9 ± 0.3	25.2 ± 2.0		0.27 ± 0.02
31 Days postthyroidectomy	12	31.3 ± 2.9	1.1 ± 0.2[b]	21.8 ± 2.3		0.11 ± 0.02[b]
Kidney						
Exp 1						
Control	5	41.0 ± 1.5	3.1 ± 0.2	26.9 ± 0.8	4.1 ± 0.3	0.15 ± 0.01
2 Days postthyroidectomy	5	41.9 ± 2.5	2.6 ± 0.2	27.7 ± 1.5	4.1 ± 0.4	0.13 ± 0.01
Exp 2						
Control	8	51.0 ± 2.0	5.3 ± 0.3	28.2 ± 1.4	6.6 ± 0.9	0.21 ± 0.01
31 Days postthyroidectomy	8	31.7 ± 3.4[b]	2.3 ± 0.1[b]	18.0 ± 1.5[b]	3.7 ± 0.6[a]	0.15 ± 0.01[c]

The data in this and subsequent tables represent values for the mean ± SE. The percentage generation of origin material accounts for the difference between the [^{125}I]T_4 degraded and the sum of the indicated labeled products generated.

[a] $P < 0.05$.

[b] $P < 0.001$.

[c] $P < 0.01$.

TABLE 2. Effect of thyroid hormone replacement on the metabolism of [^{125}I]T_4 in slices of liver and kidney from thyroidectomized animals

	No. of animals	[^{125}I]T_4 degradation (A) (% added T_4)	[^{125}I]T_3 generation (B) (% added T_4)	[^{125}I]Iodide generation (C) (% added T_4)	[^{125}I]T_4AC (D) (% added T_4)	T_3 generation/T_4 degradation (2B/A)
Liver						
Control + T_3	4	39.8 ± 3.4	4.3 ± 0.3 (c)	27.1 ± 2.5		0.22 ± 0.02 (a)
Thyroidectomized	4	30.0 ± 6.0	1.4 ± 0.2 (c, c1)	20.9 ± 3.9		0.10 ± 0.02 (a, a1)
Thyroidectomized + T_3	4	37.0 ± 4.8	3.5 ± 0.4 (c1)	25.5 ± 3.0		0.20 ± 0.04 (a1)
Kidney						
Control + T_3	4	46.4 ± 2.6	5.1 ± 0.6 (b)	25.8 ± 1.7	3.8 ± 0.6	0.22 ± 0.04 (a)
Thyroidectomized	4	37.1 ± 5.7	2.4 ± 0.3 (a, b)	19.0 ± 2.8 (b)	2.7 ± 0.5	0.14 ± 0.04 (a)
Thyroidectomized + T_3	4	49.3 ± 2.0	4.5 ± 0.5 (a)	29.8 ± 1.7 (b)	4.0 ± 0.3	0.17 ± 0.01

Letters within parentheses denote P levels determined by Duncan's multiple range testing: (a) and (a1) indicate $P < 0.05$; (b) indicates $P < 0.01$; (c) and (c1) indicate $P < 0.001$. Two groups that differ at a given P level share common letter designations. The percentage generation of origin material accounts for the difference between the [^{125}I]-T_4 degraded and the sum of the indicated labeled products generated.

Effect of hormone replacement in hypophysectomized animals (Table 4)

In replacement experiments, among the several aspects of T_4 metabolism by liver and kidney slices that were studied, including the generation of T_3, differences between hypophysectomized and control animals were very similar to those seen before (Table 3). Hormonal replacement with either testosterone or corticosterone plus DOCA had essentially no effect on any of the aspects of T_4 metabolism studied (data not shown). In contrast, administration of T_4 restored values for T_3 generation and for each of the other functions in both liver and kidney to levels that were not significantly different from those observed in tissues from intact control animals. No addi-

TABLE 3. The effect of hypophysectomy on the metabolism of [^{125}I]T_4 in slices of rat liver and kidney

	Tissue	No. of animals	[^{125}I]T_4 degradation (A) (% added T_4)	[^{125}I]T_3 generation (B) (% added T_4)	[^{125}I]I^- generation (C) (% added T_4)	[^{125}I]T_4 AC generation (D) (% added T_4)	T_3 generation/T_4 degradation (2B/A)
2 Days Posthypophysectomy							
Control	Liver	5	31.6 ± 0.8	4.1 ± 0.3	23.0 ± 0.9		0.26 ± 0.02
Hypophysectomized	Liver	5	30.7 ± 3.7	2.8 ± 0.2[a]	22.6 ± 3.4		0.20 ± 0.03
Control	Kidney	5	41.0 ± 1.5	3.1 ± 0.2	26.9 ± 0.8	4.1 ± 0.3	0.15 ± 0.01
Hypophysectomized	Kidney	5	42.8 ± 2.4	2.6 ± 0.1	26.6 ± 1.2	4.6 ± 0.6	0.12 ± 0.01[b]
31 Days Posthypophysectomy							
Control	Liver	9	24.5 ± 1.5	3.9 ± 0.6	16.4 ± 1.3		0.32 ± 0.04
Hypophysectomy	Liver	9	18.8 ± 1.7[b]	1.0 ± 0.1[c]	13.1 ± 1.6		0.12 ± 0.02[c]
Control	Kidney	4	33.5 ± 4.1	4.1 ± 0.5	13.2 ± 2.0	6.0 ± 0.8	0.25 ± 0.04
Hypophysectomized	Kidney	4	25.0 ± 3.2	1.4 ± 0.1[c]	12.9 ± 2.2	2.6 ± 0.3[b]	0.11 ± 0.01[b]

P values indicate significance of differences between control and hypophysectomized groups. The percentage generation of origin material accounts for the difference between the [^{125}I]T_4 degraded and the sum of the indicated labeled products generated.

[a] $P < 0.05$.

[b] $P < 0.01$.

[c] $P < 0.001$.

TABLE 4. Effect of thyroxine replacement on the metabolism of [^{125}I]T_4 in slices of liver and kidney from hypophysectomized animals

Treatment group	Tissue	No. of Animals	[^{125}I]T_4 degradation (A) (% added T_4)	[^{125}I]T_3 generation (B) (% added T_4)	[^{125}I]I^- generation (C) (% added T_4)	[^{125}I]T_4AC generation (D) (% added T_4)	T_3 generation/T_4 degradation (2B/A)
Intact	Liver	4	22.7 ± 2.5 (a)	4.9 ± 1.2 (b)	12.8 ± 1.2 (a)		0.42 ± 0.06 (b)
Hypox.	Liver	4	14.4 ± 1.6 (a, b)	1.1 ± 0.1 (b, b1)	8.3 ± 1.1 (a, b)		0.16 ± 0.02 (b, b1)
Hypox. + T_4	Liver	4	27.1 ± 1.3 (b)	5.0 ± 0.7 (b1)	16.7 ± 0.7 (b)		0.37 ± 0.04 (b1)
Intact	Kidney	4	33.5 ± 4.1 (a)	4.1 ± 0.5 (b)	12.6 ± 2.0	5.3 ± 0.4 (b)	0.71 ± 0.16 (b)
Hypox.	Kidney	4	25.0 ± 3.2 (a, a1)	1.4 ± 0.1 (b, b1)	12.4 ± 2.3	2.6 ± 0.3 (b, b1)	0.25 ± 0.05 (a, b)
Hypox. + T_4	Kidney	4	34.7 ± 1.8 (a1)	4.7 ± 0.04 (b1)	17.5 ± 2.0	4.4 ± 0.2 (b1)	0.56 ± 0.06 (a)

Letters within parentheses denote *P* levels determined by Duncan's multiple range testing: (a) and (a1) indicate $P < 0.05$; (b) and (b1) indicate $P < 0.01$. Two groups that differ at a given *P* level share common letters designations. The percentage generation of origin material accounts for the difference between the [^{125}I]T_4 degraded and the sum of the indicated labeled products generated.

tional effect was seen when testosterone, corticosterone, and DOCA were added to the daily regimen of T_4 treatment (data not shown).

Effect of thyroidectomy on T_3 metabolism (Table 5)

Both kidney and liver slices from animals thyroidectomized 1 month before study displayed significant decreases in [^{125}I]T_3 degradation and [^{125}I]iodide generation, when compared to corresponding values in tissues from control animals.

Effect of fasting in hypothyroid animals (Fig. 1)

Generation of [^{125}I]T_3 from [^{125}I]T_4 was assessed in intact control animals, animals thyroidectomized 1 month earlier, and animals thyroidectomized but given replacement doses of T_3 for 10 days before sacrifice. In each group, half of the animals were allowed free access to food throughout, whereas the remaining half were completely deprived of food for 48 h before sacrifice. As previously reported (34), fasting of intact animals led to a marked decrease in T_3 generation from T_4 in slices of liver. Again, slices obtained from fed thyroidectomized animals displayed a markedly lower rate of T_3 generation from T_4 than did slices from fed control animals, and activity was significantly decreased still further by fasting. Administration of T_3 to fed thyroidectomized animals again restored T_3 generating activity to the levels seen in intact controls; here too, fasting greatly decreased T_3 generation.

TABLE 5. Effect of thyroidectomy on the metabolism of [^{125}I]T_3 in slices of rat liver and kidney

Treatment group	Tissue	No. of animals	[^{125}I]T_3 degradation (% added T_3)	[^{125}I]I^- generation (% added T_3)
Control	Liver	5	16.4 ± 1.3	13.8 ± 1.0
Thyroidectomized	Liver	5	8.6 ± 1.3[a]	5.2 ± 0.3[a]
Control	Kidney	4	41.5 ± 2.4	33.5 ± 2.5
Thyroidectomized	Kidney	4	21.9 ± 1.1[a]	13.1 ± 0.7[a]

[a] $P < 0.001$ in comparison with control group.

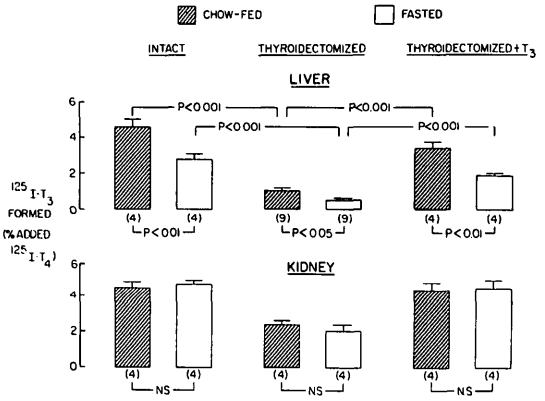

FIG. 1. The effect of a 48-h fast on the generation of [^{125}I]T_3 from [^{125}I]T_4 in slices of liver and kidney from intact and thyroidectomized rats and from thyroidectomized rats given 0.5 μg T_3 daily for 10 days.

Findings in the kidney slices from fed animals with respect to the generation of T_3 from T_4 were similar to those described above, being significantly decreased in slices from thyroidectomized animals and restored to normal by the administration of T_3. As previously reported (34), 48 h of fasting did not affect the generation of T_3 from T_4 in kidney slices from intact animals. Similarly, this period of fasting had no effect in kidney slices from either untreated or treated hypothyroid animals.

Discussion

The present studies demonstrate that the metabolism of [^{125}I]T_4 by slices of rat liver and kidney is greatly altered by thyroidectomy or by hypophysectomy. These effects are apparently due to lack of thyroid hormone, since they can be reversed in both thyroidectomized and hypophysectomized animals by the administration of replacement doses of thyroid hormone whereas gonadal and adrenocortical steroids are without effect. The effect of thyroidectomy and hypophysectomy of principal interest was the impairment of [^{125}I]T_3 generation from [^{125}I]T_4 that these ablations produced, an effect that was minimal or moderate by 2 days after operation, but was pronounced 3–4 weeks later.

Accompanying these changes in the generation of T_3 from T_4 were reductions in both the overall rate of [^{125}I]T_4 degradation and the rate of [^{125}I]iodide generation. In the case of kidney slices from both thyroidectomized and hypophysectomized animals, [^{125}I]T_4AC formation was also signficantly reduced. In addition, after both types of surgical ablation, and in both liver and kidney, the ratio of T_3 generation to T_4 degradation was significantly decreased. This finding suggests that alternate pathways of T_4 metabolism were inhibited to a lesser extent than was the 5′-monodeiodination that leads to the formation of T_3. As with the generation of T_3 from T_4, all of the other abnormalities in T_4 metabolism described above were reversed by the administration of thyroid hormone.

The decreased apparent generation of T_3 from T_4 in liver and kidney associated with hypothyroidism, at least after thyroidectomy, cannot be ascribed to an enhanced rate of T_3 degradation, since the thyroidectomy was shown to impair the overall degradation of T_3 in these tissues.

The present findings regarding the effects of hypothyroidism on T_4 metabolism *in vitro* appear to accord well with certain of the findings previously reported, but not with certain others. In both animals and man, overall clearance and deiodination of T_4 are retarded in association with hypothyroidism (35–38), and these abnormalities are reversed by treatment with thyroid hormone (39, 40). What appears difficult to explain in the light of our findings are reports which suggest that the fractional peripheral conversion of T_4 to T_3 is increased

in patients with hypothyroidism (39–41). Possible explanations for this apparent discrepancy must presently be speculative, but would include the following: the technical difficulty and complexity of the *in vivo* measurements; the possibility that complete equilibrium between intracellularly generated and exogenously administered T_3 was not achieved during hypothyroidism (31); the possibility that tissues other than liver and kidney contribute substantially to T_3 generation from T_4 *in vivo* and that their activity is increased by hypothyroidism; and the seemingly unlikely possibility that the differences are species-related.

On the other hand, our data are entirely in accord with several direct studies of T_4 converison to T_3 by rat liver *in vitro,* currently reported in abstract form (42–44). In each of these studies, liver homogenate from thyroidectomized animals was found to generate markedly less T_3 from stable T_4, as judged from RIA, than liver homogenate from intact control animals did. This defect was specifically related to a deficiency of thyroid hormone *in vivo,* since it was further observed that the capacity of liver from thyroidectomized animals to form T_3 from T_4 returned toward normal in a dose-dependent manner as the animals were treated with T_4 (44).

In man, the diverse pathophysiological conditions (4, 5, 8–10) and pharmacological agents (17, 19–21, 23) cited that appear to inhibit the conversion of T_4 to T_3 are usually associated with decreased serum T_3 concentrations and reciprocally increased serum rT_3 concentrations. Although it is uncertain whether these effects are entirely independent of one another or are in some ways related, it has been shown that rT_3 is a potent competitor of T_4 conversion to T_3 in rat liver homogenate (45). Hence, it has been suggested (44, 45) that an increase in the concentration of rT_3 at the locus of T_4 to T_3 converison might in itself explain the reduction of T_3 generation from T_4 that occurs in conditions in which serum rT_3 concentration is elevated. The findings of the present studies would appear, however, to exclude this effect, at least as regards the influence of fasting in the rat. Thus, we have demonstrated that fasting is associated with a further decrease in the already low T_3-generating activity of liver from the untreated hypothyroid rat. Moreover, the inhibitory effect of fasting was also seen in livers of thyroidectomized animals given replacement doses of T_3. Since all of these studies were conducted in animals thyroidectomized 1 month earlier, it seems clear that regardless of whether T_3 replacement was administered there would have been essentially no T_4 available to give rise to rT_3, and, hence, no means by which the rT_3 concentration in plasma or tissues could have been increased by fasting.

Acknowledgment

Data analysis was performed, in part, on the PROPHET system, a national computer resource sponsored by the Chemical/Biological Information Handling Program, NIH.

References

1. Ingbar, S. H., and L. E. Braverman, Active form of the thyroid hormone, *Ann Rev Med* **26:** 443, 1975.
2. Utiger, R. D., Serum triiodothyronine in man, *Ann Rev Med* **25:** 289, 1974.
3. Bermudez, F., M. I. Surks, and J. H. Oppenheimer, High incidence of decreased serum triiodothyronine concentration in patients with nonthyroidal disease, *J Clin Endocrinol Metab* **41:** 27, 1975.
4. Chopra, I. J., U. Chopra, S. R. Smith, M. Reza, and D. H. Solomon, Reciprocal changes in serum concentrations of 3,3′5′-triiodothyronine (reverse T_3) and 3,3′,5-triiodothyronine (T_3) in systemic illnesses, *J Clin Endocrinol Metab* **41:** 1043, 1975.
5. Burr, W. A., E. G. Blach, R. S. Griffiths, R. Hoffenberg, H. Meinhold, and K. W. Wenzel, Serum triiodothyronine and reverse triiodothyronine concentrations after surgical operation, *Lancet* **2:** 1277, 1975.
6. Brandt, M., H. Kehlet, J. M. Hansen, and L. Skovsted, Serum triiodothyronine and surgery, *Lancet* **1:** 491, 1976.
7. Portnay, G. I., J. T. O'Brian, J. Bush, A. G. Vagenakis, F. Azizi, R. A. Arky, S. H. Ingbar, and L. E. Braverman, The effect of starvation on the concentration and binding of thyroxine and triiodothyronine in serum and on the response to TRH, *J Clin Endocrinol Metab* **39:** 191, 1974.
8. Vagenakis, A. G., A. Burger, G. I. Portnay, M. Rudolph, J. T. O'Brian, F. Azizi, R. A. Arky, P. Nicod, S. H. Ingbar, and L. E. Braverman, Diversion of peripheral thyroxine metabolism from activating to inactivating pathways during complete fasting, *J Clin Endocrinol Metab* **41:** 191, 1975.
9. Chopra, I. J., and S. R. Smith, Circulating thyroid hormones in adult patients with protein-calorie malnutrition, *J Clin Endocrinol Metab* **40:** 221, 1975.

10. Spaulding, S. W, I. J. Chopra, R. S. Sherwin, and S. S. Lyall, Effect of caloric restriction and dietary composition on serum T_3 and reverse T_3 in man, *J Clin Endocrinol Metab* **42:** 197, 1976.
11. Miyai, K., T. Yamamoto, M. Azukizawa, K. Ishibashi, and Y. Kumahara, Serum thyroid hormones and thyrotropin in anorexia nervosa, *J Clin Endocrinol Metab* **40:** 334, 1975.
12. Moshang, T., J. S. Parks, L. Baker, V. Vaidya, R. D. Utiger, A. M. Bongiovanni, and P. J. Snyder, Low serum triiodothyronine in patients with anorexia nervosa, *J Clin Endocrinol Metab* **40:** 470, 1975.
13. Croxson, M. S., and H. K. Ibbertson, Low serum triiodothyronine (T_3) and hypothyroidism in anorexia nervosa, *J Clin Endocrinol Metab* **44:** 167, 1977.
14. Oppenheimer, J. H., H. L. Schwartz, and M. I. Surks, Propylthiouracil inhibits the conversion of L-thyroxine to L-triiodothyronine: an explanation of the antithyroxine effect of propylthiouracil and evidence supporting the concept that triiodothyronine is the active thyroid hormone, *J Clin Invest* **51:** 2493, 1972.
15. Saberi, M., F. H. Sterling, and R. D. Utiger, Reduction in extrathyroidal triiodothyronine production by propylthiouracil in man, *J Clin Invest* **55:** 218, 1975.
16. Geffner, D. L., M. Azukizawa, and J. Hershman, Propylthiouracil blocks extrathyroidal conversion of thyroxine to triiodothyronine and augments thyrotropin secrtion in man, *J Clin Invest* **55:** 224, 1975.
17. Kaplan, M. M., M. Schimmel, and R. D. Utiger, Changes in serum 3,3′,5′-triiodothyronine (reverse T_3) concentrations with altered thyroid hormone secretion and metabolism, *J Clin Endocrinol Metab* **45:** 447, 1977.
18. Wiersinga, W. M., and J. L. Touber, The influence of β-adrenoreceptor blocking agents on plasma thyroxine and triiodothyronine, *J Clin Endocrinol Metab* **45:** 293, 1977.
19. Burger, A., D. Dinichert, P. Nicod, M. Jenny, T. LeMarchand-Beraud, and M. B. Valloton, Effect of amiodarone on serum triiodothyronine, reverse triiodothyronine, thyroxin, and thyrotropin: a drug influencing peripheral metabolism of thyroid hormones, *J Clin Invest* **58:** 255, 1976.
20. Burgi, H., C. Wimpfheimer, A. Burger, W. Zaunbauer, H. Rosler, and T. Lemarchand-Beraud, Changes of circulating thyroxine, triiodothyronine, and reverse triiodothyronine after radiographic contrast agents, *J Clin Endocrinol Metab* **43:** 1203, 1976.
21. Wu, S. Y., I. J. Chopra, D. H. Solomon, and L. R. Bennett, Reduction of serum triiodothyronine (T_3) by ipodate in hyperthyroidism and other states, *Clin Res* **25:** 304A, 1977.
22. Duick, D. S., D. W. Warren, J. T. Nicoloff, C. L. Otis, and M. S. Croxson, Effect of single dose dexamethasone on the concentration of serum triiodothyronine in man, *J Clin Endocrinol Metab* **39:** 1151, 1974.
23. Chopra, I. J., D. E. Williams, J. Orgiazzi, and D. H. Solomon, Opposite effects of dexamethasone on serum concentrations of 3,3′,5′-triiodothyronine (reverse T_3) and 3,3′,5-triiodothyronine (T_3), *J Clin Endocrinol Metab* **41:** 911, 1975.
24. DeGroot, L. J., and K. Hoye, Dexamethasone suppression of serum T_3 and T_4, *J Clin Endocrinol Metab* **42:** 976, 1976.
25. Eisenstein, Z., S. Hagg, A. G. Vagenakis, S. L. Fang, B. Ransil, A. Burger, A. Balsam, L. E. Braverman, and S. H. Ingbar, Effect of starvation on the production and peripheral metabolism of 3,3′,5′-triiodothyronine in euthyroid obese subjects *J Clin Endocrinol Metab* (in press), 1977.
26. Suda, A., C. Pittman, and J. Chambers, Jr., Regulation of 3,5,3′-triiodothyronine (T_3) production during fast and refeeding, Proceedings of American Thyroid Association 53rd meeting, 1977, PT-14 (Abstract).
27. Nomura, S., C. S. Pittman, J. B. Chambers, Jr., M. W. Buck, and T. Shimizu, Reduced peripheral conversion of thyroxine to triiodothyronine in patients with hepatic cirrhosis, *J Clin Invest* **56:** 643, 1975.
28. Chopra, I. J., An assessment of daily production and significance of thyroidal secretion of 3,3′,5′-triiodothyronine (reverse T_3) in man, *J Clin Invest* **58:** 33, 1976.
29. Balsam, A., F. Sexton, and S. H. Ingbar, The influence of fasting, diabetes, and several pharmacological agents on the pathways of thyroxine metabolism in rat liver, *J Clin Invest* **62:** 415, 1978.
30. Balsam, A., F. Sexton, and S. H. Ingbar, Independent inhibitory effects of hypothyroidism and fasting on hepatic conversion of thyroxine (T_4) to triiodothyronine (T_3) in the rat, Program of the 59th annual meeting of the Endocrine Society, 1977, p. 239 (Abstract).
31. Garcia, M. D., F. Escobar del Rey, G. Morreale de Escobar, Thyrotropin-releasing hormone and thyroid hormone interactions on thyrotropin secretion in the rat: lack of inhibiting effects of small doses of triiodo-L-thyronine in the hypothyroid rat, *Endocrinology* **98:** 203, 1976.
32. Bilder, G. E., Activity of the reticuloendothelial system following endocrine manipulation in rats, *J Reticuloendothel Soc* **19:** 11, 1976.
33. Dunnett, C. W., Multiple comparisons, *In* J. W. McArthur and T. Colton (eds.), Statistics in Endocrinology, vol. 1, MIT Press, Cambridge, 1970, p. 86.
34. Balsam, A., F. Sexton, and S. H. Ingbar, In vitro generation of T_3 from T_4 by rat liver: reversible inhibition induced by starvation and diabetes mellitus, Program of the 52nd meeting of the American Thyroid Association, 1976, p. T-16 (Abstract).
35. Ingbar, S. H., and N. Freinkel, Simultaneous estimation of rates of thyroxine degradation and thyroid hormone synthesis, *J Clin Invest* **34:** 808, 1955.
36. Sterling, K., and R. B. Chodos, Radiothyroxine turnover studies in myxedema, thyrotoxicosis and hypermetabolism without endocrine disease, *J Clin Invest* **35:** 806, 1956.
37. Nicoloff, J. T., J. C. Low, J. H. Dussault, and D. A. Fisher, Simultaneous measurement of thyroxine and triiodothyronine peripheral turnover kinetics in man, *J Clin Invest* **51:** 473, 1972.
38. Cullen, J. M., G. F. Doherty, and S. H. Ingbar, The

effect of hypothyroidism and thyrotoxicosis on thyroxine metabolism in the rat, *Endocrinology* **92:** 1028, 1973.

39. Inada, M., K. Kasagi, S. Kurata, Y. Kazama, H. Takayamma, K. Torizuka, M. Fukase, and T. Soma, Estimation of thyroxine and triiodothyronine distribution and of the conversion rate of thyroxine to triiodothyronine in man. *J Clin Invest* **55:** 1337, 1975.
40. Maguire, S. B., A. Dennehy, and M. J. Cullen, The effect of thyrotoxicosis and hypothyroidism on the extrathyroidal conversion of thyroxine to triiodothyronine in man, *In* Thyroid Research, J. Robbins and L. E. Braverman (eds.), American Elsevier, New York, 1975, p. 259.
41. Shimizu, T., C. S. Pittman, J. B. Chambers, Jrs., M. W. Buck and C. C. Thurston, The effect of thyroxine on the peripheral conversion rate of thyroxine to triiodothyronine in man, *In* Thyroid Research, J. Robbins and L. E. Braverman (eds.), American Elsevier, New York, 1975, p. 263.
42. Maeda, M., K. Shizume, H. Uchimura, and S. Nagataki, In vitro generation of triiodothyronine (T_3) and reverse T_3 from graded doses of thyroxine in liver homogenates of rats in varying thyroid states, Program of the 59th annual meeting of the Endocrine Society, 1977, p. 323 (Abstract).
43. Harris, A., S. Fang, L. Braverman and A. Vagenakis, Effect of carbohydrate, protein and fat infusion on hepatic T_3 generation in the fasted rat, Program of the 53rd meeting of the American Thyroid Association, 1977, p. T-13 (Abstract).
44. Grussendorf, M., and M. Hufner, Deiodination of T_4 in liver homogenate of normal and thyroid hormone substituted, thyroidectomized rats, Program of the 8th annual meeting of the European Thyroid Association, Lyon, 1977, p. 32A (Abstract).
45. Chopra, I. J., A study of extrathyroidal conversion of thyroxine (T_4) to 3,3',5-triiodothyronine (T_3) in vitro, *Endocrinology* **101:** 453, 1977.