An Assessment of Daily Production and Significance of Thyroidal Secretion of 3,3′,5′-Triiodothyronine (Reverse T₃) in Man

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ABSTRACT While 3,3′,5′-triiodothyronine (reverse T₃, rT₃) has been detected both in human serum and in thyroglobulin, no quantitative assessment of its metabolic clearance rate (MCR), production rate (PR), or secretion by the thyroid is yet available. This study examines this information in euthyroid subjects and evaluates it in light of similar information about two other iodothyronines in the thyroid: 3,5,3′-triiodothyronine (T₃) and thyroxine (T₄). Thus, it was noted that rT₃ is cleared from human serum at a much faster rate than are T₃ and T₄; the mean ($\pm$SE) MCR of rT₃ was 76.7$\pm$5.4 liters/day in 10 subjects, whereas MCR-T₃ and MCR-T₄ in 8 of them were 26.0$\pm$2.2 liters/day and 1.02$\pm$0.06 liters/day, respectively. Therefore, even though the mean serum concentration of rT₃, 48$\pm$2.8 ng/100 ml, was much lower than that (128$\pm$6.7 ng/100 ml) of T₃, the mean PR-rT₃ (36.5$\pm$2.8 µg/day) and the mean PR-T₃ (33.5$\pm$3.7 µg/day) were similar; in comparison, the mean serum concentration and PR of T₄ were 8.6$\pm$0.5 µg/100 ml and 87.0$\pm$3.9 µg/day, respectively. These data and those on the relative proportion of rT₃, T₃, and T₄ in 10 thyroid glands were used to assess the significance of the contribution of thyroidal secretion to PR-rT₃ and PR-T₃. It was estimated that whereas thyroidal secretion may account for about 23.8% of serum T₃ (or PR-T₃), it may account for only about 2.5% of serum rT₃ (or PR-rT₃). Since peripheral metabolism of T₄ is the only known source of rT₃ and T₃ other than the thyroidal secretion, it could be calculated that as much as 73.0 µg or 84% of daily PR-T₄ may normally be metabolized by monodeiodination either to T₃ or to rT₃.

MCR and PR of various iodothyronines were also examined in five cases with hepatic cirrhosis, where, as documented previously, serum rT₃ may be elevated while

serum T₃ is diminished. The mean MCR-rT₃ in these cases (41.0 liters/day) was clearly ($P < 0.005$) less than that (76.7 liters/day) in normal subjects. This was the case at a time when the mean MCR-T₃ (26.7 liters/day) and the mean MCR-T₄ (1.19 liters/day) did not differ from those (vide supra) in normal subjects. Distinct from changes in MCRs, the mean PR-rT₃ (33.0 µg/day) was similar to, and the mean PR-T₃ (10.1 µg/day) and the mean PR-T₄ (66.4 µg/day) were much less than, the corresponding value in normal subjects. Furthermore, while the ratio of PR-rT₃ and PR-T₄ (rT₃/T₄) in individual patients was either supranormal or normal, the ratio of PR-T₃ and PR-T₄ (T₃/T₄) was clearly subnormal. The various data suggest that: (a) just as in the case of T₃, the thyroid gland is a relatively minor source of rT₃; peripheral metabolism of T₄ is apparently its major source; (b) the bulk of T₄ metabolized daily is monodeiodinated to T₃ or to rT₃; (c) monodeiodination may be an obligatory step in metabolism of T₄; (d) monodeiodination of T₄ to rT₃ is maintained normal or is increased in hepatic cirrhosis at a time when monodeiodination of T₄ to T₃ is decreased.

INTRODUCTION

Like 3,5,3′-triiodothyronine (T₃),[1] 3,3′,5′-triiodothyronine (reverse T₃, rT₃) has recently been identified in human thyroglobulin and in serum of hypothyroid patients and euthyroid subjects receiving treatment with thyroxine (T₄) (1, 2). While considerable data are available in case of T₃ (3–6), there is a paucity of information about the kinetics of peripheral metabolism and the daily

[1] Abbreviations used in this paper: MCR(s), metabolic clearance rate(s); PR(s), production rate(s); reverse T₃, rT₃, 3,3′,5′-triiodothyronine; T₂, 3,3′-L-diiodothyronine; T₃, 3,5,3′-triiodothyronine; T₄, thyroxine; TCA, trichloroacetic acid.

turnover rate of reverse T_3. Similarly, little information is available regarding the relative significance of the contribution of the thyroidal secretion and the peripheral metabolism of T_4 to serum rT_3 in man. The studies to be described were undertaken to gather information pertaining to these issues and to estimate the extent to which T_4 may normally be metabolized by monodeiodination either to T_3 or to reverse T_3. Additionally, it has recently been reported that serum rT_3 levels may be increased in several situations, e.g., the fetus and the newborn and a variety of systemic illnesses, protein-calorie malnutrition and starvation, where serum concentration and(or) daily production rate of T_3 may be decreased (1, 2, 7–9). In the present study, an attempt has been made to investigate the changes in relative metabolic clearance rates and production rates of T_4, T_3, and rT_3 in one such situation, i.e., hepatic cirrhosis, where serum T_3 and rT_3 may vary in opposite directions.

METHODS

Subjects. Studies were conducted in 15 volunteer subjects, 10 of whom (6 men and 4 women, 19–41-yr-old) were euthyroid healthy subjects and 5 of whom (3 men and 2 women, 35–66-yr-old) were patients with hepatic cirrhosis. The patients with hepatic cirrhosis had a history of chronic alcohol abuse and had presented with clear evidence of chronic liver disfunction. All patients had icterus, spider angiomata, palmar erythema, hepatomegaly, and edema and(or) ascites; their liver function tests revealed decreased serum albumin, elevated serum bilirubin, transaminase, and alkaline phosphate levels, and subnormal prothrombin time. Liver biopsy was performed at a previous hospitalization in four of five cases; histology was consistent with the diagnosis of hepatic cirrhosis in each of these cases. Hepatic scintiscan supported the clinical diagnosis of cirrhosis and portal hypertension in the remaining case.

Hormonal measurements. Serum concentrations of rT_3, T_3, and T_4 were measured by radioimmunoassays described previously (1, 10, 11).

Kinetic studies. Radioactive (^{125}I or ^{131}I) L-T_3 and L-T_4 (sp act 80–100 mCi/mg in each case) were purchased from Industrial Nuclear Co., Inc., St. Louis, Mo.; the chromatographic studies suggested over 95% purity of these radioactive hormones. $[^{125}I]$L-rT_3 (sp act 350–700 mCi/mg), prepared by beta ring radioiodination of 3,3′-L-diiodothyronine (T_2), was obtained from Abbott Laboratories, North Chicago, Ill. Under the conditions employed, iodination of T_2 in 5-position was unlikely (12); radioactive rT_3 and radioactive T_3 were the only detectable products of radioiodination, and these were carefully separated by Mr. Bill J. Green of Abbott Laboratories by a modification of the Sephadex column chromatographic method of Lissitzky et al. (13). While the iodination procedure was unlikely to yield radioactive T_3 and T_4 from nonradioactive T_2, contamination of radioactive rT_3 with radioactive T_3 and T_4 could occur by exchange labeling of nonradioactive T_3 and T_4 contaminating nonradioactive T_2. Therefore, the cross-reaction of nonradioactive T_2 in T_3 and T_4 immunoassays was studied. T_2 cross reacted 2.2% in T_4 immunoassay and 3.0% in T_3 immunoassay. Since at least part of the cross-reaction

of T_2 in T_3 and T_4 immunoassays must be due to true cross-reaction as opposed to contamination with T_3 and T_4, these studies suggested that contamination of radioactive rT_3 with radioactive T_3 or T_4 would be small, i.e., less than 3%. The extent of contamination of $[^{125}I]rT_3$ with $[^{125}I]T_3$ was also examined by the study of the binding of $[^{125}I]rT_3$ to a highly specific T_3 antibody prepared by immunization of rabbits with T_3-human serum albumin conjugate. An amount of anti-T_3 which could bind 80% of approximately 20,000 cpm of $[^{125}I]T_3$ (sp act approximately 100 mCi/mg) bound only 0.9% of about 20,000 cpm of $[^{125}I]rT_3$ (sp act 350 mCi/mg). Contamination of $[^{125}I]rT_3$ with $[^{125}I]T_4$ could not be studied similarly by using anti-T_4, because the available anti-T_4 was prepared by immunization of rabbits with thyroglobulin, which, besides T_4, also contained rT_3; previous studies had revealed the presence of significant concentrations of rT_3 binding sites in the available anti-T_4. The composition of $[^{125}I]rT_3$ was also studied by paper chromatography for 16 h at 37°C using hexane, tertiary amyl alcohol, and 2 N ammonium hydroxide (vol/vol, 1 : 5 : 6); rT_3, T_4, and T_3 were separated quite clearly by this procedure. However, radioautography of the paper chromatogram revealed only one band of radioactivity corresponding to the rT_3 region. Besides other iodothyronines, radioiodide could be another contaminant of radioactive rT_3 as well as of radioactive iodothyronines studied. However, the effect of any radioiodide contaminating labeled iodothyronines on the kinetics of iodothyronines would be minimized by the technique used for processing of specimens (*vide infra*).

The metabolic clearance rates (MCRs) of the various iodothyronines rT_3, T_3, and T_4 were studied by the method employing single intravenous injection of the radioactive hormone. Two or three blood samples were obtained during a 12–24-h period before administration of radioactive hormones for measurement of serum concentrations of rT_3, T_3, and T_4. Tracer doses, 30–50 μCi each of radioactive rT_3, T_3, and(or) T_4 diluted in sterile 2% human albumin solution were then injected intravenously. The schedule of injections of radioactive iodothyronines was as follows: (a) $[^{125}I]rT_3$ and $[^{131}I]T_3$ simultaneously first and $[^{125}I]T_4$ 72 h later in four euthyroid subjects (case 1, 2, 4, and 7, Table I) and five patients with hepatic cirrhosis (Table II); (b) $[^{125}I]rT_3$ first and $[^{125}I]T_3$ and $[^{131}I]T_4$ simultaneously 72 h later in case 3 and 4; (c) $[^{125}I]rT_3$ and $[^{131}I]T_4$ simultaneously and no T_3 in case 5 and 8; (d) $[^{125}I]rT_3$ and $[^{131}I]T_3$ simultaneously and no T_4 in case 6 and 10. For study of MCR of rT_3 and T_3, blood samples were obtained at 10–15-min intervals until 1 h; at 30–60 min-intervals until 6 h; at 2–4-h intervals until 12 h; and at 8–12-h intervals until 72 h. For study of MCR of T_4, blood samples were obtained one or two times daily until 9 to 11 days after the injection. Thyroid uptake of radioiodide liberated from metabolism of iodothyronines was minimized by oral administration of either Lugol's iodine or saturated solution of potassium iodide, five drops three times daily throughout the study. Urine samples were collected during the 1st day of study in some subjects to estimate the amount of radioactivity excreted during first 24 h after injection of each iodothyronine (rT_3, T_3, and T_4). Aliquots of injected radioactive iodothyronines, diluted in human albumin solution, were kept in a refrigerator (4°C) to serve as reference standards. 500-μl aliquots of serum samples were precipitated with two volumes of 10% trichloroacetic acid (TCA) and washed twice with two volumes of 5% TCA to separate the radioiodide from radioactive iodothyronines and

TABLE I

Serum Concentration, MCR, and Turnover

Case	Age	Sex	Body weight	rT₃ Serum concn*	rT₃ MCR liters/day	rT₃ MCR liters/day/70 kg	rT₃ Production rate μg/day	rT₃ Production rate μg/day/70 kg	T₃ Serum concn*
			kg	*ng/100 ml*	*liters/day*	*liters/day/70 kg*	*μg/day*	*μg/day/70 kg*	*ng/100 ml*
1	19	M	61.4	50	92.9	106	46.4	52.9	82.0
2	19	M	63.4	42	93.7	103	39.4	43.5	128
3	33	M	64.7	44	95.2	103	41.9	45.3	158
4	32	M	72.4	45	97.8	94.5	44.0	42.5	150
5	25	M	67.9	62	59.4	61.2	36.8	37.9	128
6	41	M	164.5	39	61.0	26.0	23.8	10.1	130
7	24	F	47.5	54	82.4	121	44.5	65.6	114
8	19	F	56.6	62	62.4	77.2	38.7	47.9	118
9	23	F	50.4	41	56.5	78.5	23.2	32.4	125
10	31	F	104.7	40	66.1	44.2	26.4	17.7	142
Mean	27			75.3	48	76.7	81.5	36.5	39.5	128
SEM	2.3			11.1	2.8	5.40	9.55	2.80	5.1	6.70

* Mean of two or three consecutive samples tested in duplicate.

radioactive iodoproteins in serum. Radioactivity in iodoproteins was determined by repeated extraction of TCA precipitates with three volumes of 95% ethanol in a manner similar to that described by Oppenheimer, Surks, and coworkers (14, 15). The radioactivity in the iodoproteins was subtracted from that in TCA precipitates and the remaining radioactivity was assumed to represent the concentration of radioiodothyronine at each period of study. Aliquots of standards were added to the zero-time serum sample or a pooled serum of hospitalized patients and processed in a manner identical to that employed for test samples. All radioactive samples were counted to statistical accuracy of ±2% in dual-channel gamma counter (Nuclear-Chicago Corp., Des Plaines, Ill.). The counts in serum were expressed as percent of dose per liter of serum. MCRs were calculated from the area under the curve of disappearance of radioactive iodothyronine from serum by the noncompartmental integral approach described previously by Oppenheimer, Surks, and coworkers (14–16). While data from all points of study were employed to calculate MCR of T₃ and T₄, only data up to 36 h were employed to calculate MCR of rT₃; this was done because radioactive rT₃ remaining in serum after 36 h was very small, i.e., less than 0.05% of the dose of radioactive rT₃. Turnover or production rates (PRs) were calculated by multiplying the MCR (liters/day) with the concentration (μg/liter) of the corresponding iodothyronine tested before the injection of radioactivity.

TABLE II

Serum Concentration, MCR, and Production Rate of rT₃, T₃, and T₄ in Patients with

Case	Age	Sex	Body weight	rT₃ Serum concn*	rT₃ MCR liters/day	rT₃ MCR liters/day/70 kg	rT₃ Production rate μg/day	rT₃ Production rate μg/day/70 kg	T₃ Serum concn
			kg	*ng/100 ml*	*liters/day*	*liters/day/70 kg*	*μg/day*	*μg/day/70 kg*	*ng/100 ml*
11	35	F	67.9	76	49.3	50.8	37.5	38.7	58
12	58	F	43.0	86	16.1	26.2	13.9	21.0	22
13	65	M	83.0	86	41.0	34.6	35.6	30.0	48
14	66	M	62.2	80	59.6	67.0	47.7	53.7	24
15	66	M	81.7	77	39.1	33.5	30.1	25.8	41
Mean‡	58			67.6	81	41.0	42.4	33.0	33.8	39
SEM	5.9			7.30	2.1	7.20	7.34	5.50	5.75	6.9
P	<0.001			NS	<0.001	<0.005	<0.02	NS	NS	<0.001

* Mean of two or three consecutive samples tested in duplicate.
‡ Compare corresponding value in normal subjects (Table I).

T₃				Serum concn*	T₄			
MCR		Production rate			MCR		Production rate	
liters/day	liters/day/ 70 kg	μg/day	μg/day/kg	μg/100 ml	liters/day	liters/day/ 70 kg	μg/day	μg/day/ 70 kg
29.6	33.7	24.3	27.7	7.6	1.22	1.39	92.7	106
25.5	28.2	32.6	36.0	8.5	1.00	1.10	85.3	94.2
19.4	21.0	30.6	33.1	10	0.97	1.05	97.4	105
35.3	34.1	52.9	51.2	8.2	1.29	1.25	106	103
—	—	—	—	7.2	1.06	1.09	76.3	78.7
32.1	13.7	42.4	18.0	7.6	—	—	—	—
19.7	29.0	22.5	33.2	12	0.69	1.01	85.4	126
—	—	—	—	7.6	0.99	1.22	75.2	93.0
19.4	27.1	24.2	33.8	8.3	0.94	1.31	78.0	109
27.3	18.3	38.8	26.0	8.3	—	—	—	—
26.0	25.6	33.5	32.4	8.6	1.02	1.18	87.0	102
2.20	2.58	3.70	3.38	0.50	0.06	0.05	3.90	4.86

Thyroid glands. Thyroid glands were obtained at the time of autopsy of 10 apparently euthyroid subjects. Thyroid glands were homogenized, and an aliquot hydrolyzed with Pronase (Calbiochem, La Jolla, Calif.) under previously described conditions (17, 18). The hydrolysates were extracted with 2 volumes of 95% ethanol and rT₃, T₃, and T₄ were determined by radioimmunoassays as described previously (1, 18, 19).

RESULTS

Radioactive iodothyronines in serum of euthyroid subjects. To compare the efficiency of TCA precipitation and ethanol extraction of various iodothyronines from serum, about 10,000 cpm of radioactive rT₃, T₃, and T₄ were added to 0.5 ml-aliquots of four sera and sera were processed as described above in the Methods. TCA precipitates contained $88.2 \pm 0.6\%$[a] of rT₃, $86.8 \pm 0.60\%$ of T₃ and $90.5 \pm 0.74\%$ of T₄. The fraction of rT₃ and T₃ precipitated by TCA were similar but both of these values were modestly lower than that in case of T₄ ($P < 0.05$). The radioactivity remaining in the precipitate after ethanol-extraction was $1.2 \pm 0.03\%$ for rT₃, $2.3 \pm$

[a] Data expressed as mean±SE here and elsewhere unless stated otherwise.

Hepatic Cirrhosis and Comparison of the Values with those in Normal Subjects

T₃				Serum concn	T₄			
MCR		Production rate			MCR		Production rate	
liters/day	liters/day/ 70 kg	μg/day	μg/day/kg	μg/100 ml	liters/day	liters/day/ 70 kg	μg/day	μg/day/ 70 kg
24.4	25.2	14.0	14.4	6.3	1.04	1.07	65.5	67.5
14.4	23.5	3.10	5.10	4.3	0.96	1.57	41.3	70.0
22.1	18.6	10.6	8.90	6.1	0.90	0.76	54.9	46.3
27.1	30.5	6.50	7.30	6.0	1.53	1.72	91.8	103.2
45.7	39.2	16.1	13.8	5.1	1.54	1.32	78.5	67.3
26.7	27.4	10.1	9.90	5.6	1.19	1.29	66.4	70.9
5.10	3.51	2.4	1.82	0.38	0.14	0.17	8.80	9.15
NS	NS	<0.001	<0.001	<0.001	NS	NS	<0.025	<0.001

0.10% for T_3, and 1.2±0.06% for T_4. The values in case of rT_3 and T_4 were similar; the value in case of T_3 was, however, higher than those of rT_3 and T_3 ($P < 0.05$). These studies indicated that TCA precipitation and ethanol extraction of various iodothyronines yields results which are similar but not identical. Therefore, with a view to minimize the effects of these (albeit small) differences in extractability of iodothyronines on kinetic studies, the standards were processed in a manner identical to that employed for the test specimens.

The relative rates of disappearance from serum of TCA-precipitable and ethanol-extractable radioactivity, presumably representing the iodothyronines, after injection of radioactive rT_3, T_3, and T_4 are shown in Fig. 1; the data on the mean percent of dose per liter of serum of various iodothyronines have been plotted against time on a semilogarithmic plot. Reverse T_3 disappeared from serum at a much faster rate than did T_3 and T_4; more than 98% of radioactive rT_3 left the serum by 5 h of the injection of radioactive rT_3.

Urine studies. Urinary excretion of radioactivity was examined in two cases given radioactive rT_3. The radioactivity excreted in urine was 40.8 and 32.1%, respectively, at 8 h, and 77.0 and 59.7%, respectively, at 24 h after the injection. In contrast, the radioactivity

excreted in urine during the first 24 h after injection of radioactive T_3 was 32.6 and 32.8%, respectively in two cases. Urinary excretion of radioactivity was measured in four cases after injection of radioactive T_4; the values at 24 h varied between 5.1 and 8.7% (mean, 6.9%). To examine whether the radioactivity excreted in urine after injection of radioactive rT_3 or T_3 represented intact hormone or its metabolic product, e.g., radioiodide, aliquots of urine were mixed with an equal volume of pooled normal serum, incubated for 30 min at room temperature, and precipitated with 10% TCA to separate protein-bound radioactivity. Less than 5% of radioactivity could be recovered in the precipitate in each case studied and the remaining (over 95%) remained in the supernate. These findings suggested that urinary radioactivity was probably merely a degradation product and not the intact hormone per se.

MCRs and PRs of thyroid hormones in euthyroid man. The data on serum concentration, MCR, and PR of rT_3, T_3, and T_4 in euthyroid subjects under study are presented in Table I. The mean serum concentration of rT_3 was 48 ng/100 ml (range, 39–62); the mean MCR of rT_3 was 76.7 liter/day (range, 56.5–97.8); and the mean PR of rT_3 was 36.5 μg/day (range, 23.2–46.4). The mean serum concentration of T_3 was 128 ng/100 ml (range, 82–158), whereas the mean MCR was 26.0 liters/day (range 19.4–35.3), and the mean PR was 33.5 μg/day (range, 22.5–52.9). The mean serum concentration of T_4 was 8.6 μg/100 ml (range, 7.2–12), whereas the mean MCR was 1.02 liters day (range, 0.69 to 1.29), and the mean PR was 87 μg/day (range, 75.2–106).

Relative proportions of rT_3, T_3, and T_4 in the thyroid. To assess the significance of the thyroidal secretion of rT_3 and T_3 relative to T_4 in euthyroid man, the relative proportions of rT_3, T_3, and T_4 were examined in homogenates of 10 thyroid glands. The mean T_4 content of the thyroid was found to be 351.5±48.1 μg/g wet weight. The mean molar ratio of the contents of T_4 and rT_3 (T_4/rT_3) was 75.5±0.10 (range, 33.5–121.6) and the mean molar ratio of the contents of T_4 and T_3 (T_4/T_3) was 9.3±0.66 (range, 6.7–13.0).

Thyroidal contribution to serum concentration (or daily PR) of rT_3 and T_3. The significance of the contribution of thyroidal rT_3 secretion to PR-rT_3 in euthyroid man was assessed by estimating the approximate thyroidal contribution by using the data on MCR-rT_3, MCR-T_4, serum T_4, and rT_3/T_4 ratio in the thyroid in calculations similar to those described previously for T_3 (20, 21); it was assumed in these calculations that rT_3 and T_4 are secreted in the proportions in which they exist in the thyroid. These calculations suggest that thyroidal secretion which results in a mean serum T_4 of 8.6 μg/100 ml in euthyroid man (Table I) adds only 1.2 ng/100 ml to rT_3 in serum (or 0.92 μg/day to PR-rT_3);
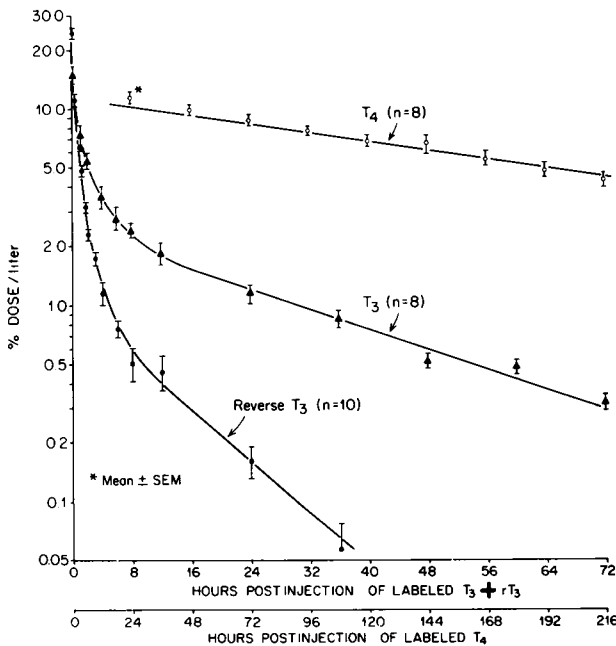

FIGURE 1 Disappearance from serum of TCA-precipitable and ethanol-extractable radioactivity, presumably representing iodothyronines, after injection of radioactive rT_3, T_3, and T_4 intravenously to euthyroid subjects; the data on mean±SE and the percent dose per liter are plotted against time in hours on a semilogarithmic plot.

this amount of rT$_3$ is only about 2.5% of that (48 ng/100 ml) actually measured in serum. These analyses suggest that the bulk, as much as 97.5% (100 − 2.5 = 97.5) of serum rT$_3$, derives from peripheral metabolism of T$_4$.

The significance of the contribution of thyroidal T$_3$ secretion to PR-T$_3$ in euthyroid man was assessed by using the data on MCR-T$_3$, MCR-T$_4$, serum T$_4$, and T$_3$/T$_4$ in the thyroid in calculations similar to those used for rT$_3$. The calculations suggest that thyroidal secretion which results in a mean serum T$_4$ of 8.6 µg/100 ml may add about 30.4 ng T$_3$ to 100 ml of serum (or 7.9 µg/day to PR-T$_3$); this amount of T$_3$ is about 23.8% of that (128 ng/100 ml) actually measured in serum. These analyses suggest that majority of T$_3$ in serum, about 76.2% (100 − 23.8 = 76.2), derives from peripheral metabolism of T$_4$.

Metabolism of T$_4$ by monodeiodination. From data on daily PRs of rT$_3$, T$_3$, and T$_4$ in euthyroid man (Table I) and the assessment of thyroidal secretion of rT$_3$ and T$_3$, the amount of T$_4$ metabolized by monodeiodination either to rT$_3$ or to T$_3$ were estimated. Thus, of the 33.5 µg of T$_3$ produced daily about 76.2% or 25.5 µg appeared to be produced from T$_4$ and of the 36.5 µg of rT$_3$ produced daily all but 2.5% or 35.6 µg appeared to derive from T$_4$. Accounting for the difference in the molecular weights of T$_3$ and rT$_3$ vs. T$_4$, it could be calculated that about 73.0 µg ([25.5 + 35.6] × 1.195 = 73.0; where 25.5 and 35.6 are amounts T$_3$ and rT$_3$ estimated to be produced daily from T$_4$ and 1.195 is the ratio of molecular weights of T$_4$ and T$_3$ or rT$_3$) or 84% (73/87 = 0.84) of daily PR-T$_4$ (87 µg/day, Table I) may be used in production of either T$_3$ or rT$_3$ in normal man.

MCRs and PRs of thyroid hormones in hepatic cirrhosis. The data on serum concentration and MCR and PR of rT$_3$, T$_3$, and T$_4$ in five cases with hepatic cirrhosis are shown in Table II. The mean serum rT$_3$ concentration (81 vs. 48 ng/100 ml, $P < 0.001$) in these cases was clearly higher, whereas the mean serum T$_3$ (39 vs. 128 ng/100 ml, $P < 0.001$) and the mean serum T$_4$ (5.6 vs. 8.6 µg/100 ml, $P < 0.001$) were clearly lower than the corresponding value in normal subjects. The mean MCR-rT$_3$ (41.0 vs. 76.7 liters/day, $P < 0.005$) was significantly less than normal, whereas the mean MCR-T$_3$ (26.7 vs. 26.0 liters/day) and the mean MCR-T$_4$ (1.19 vs. 1.02 liters/day) did not differ significantly from normal (Table I). The mean PR-rT$_3$ (33.0 vs. 36.5 µg/day) in these cases was similar to, whereas the mean PR-T$_3$ (10.1 vs. 33.5 µg/day, $P < 0.005$), and the mean PR-T$_4$ (66.4 vs. 87.0 µg/day, $P < 0.025$) were significantly lower than the corresponding normal value.

An examination of the relationship between PRs of rT$_3$, T$_3$, and T$_4$ in hepatic cirrhosis is presented in Fig. 2. The mean molar ratio of PR-rT$_3$ and PR-T$_4$ (rT$_3$/T$_4$) of 0.59±0.07 in hepatic cirrhosis patients was similar to
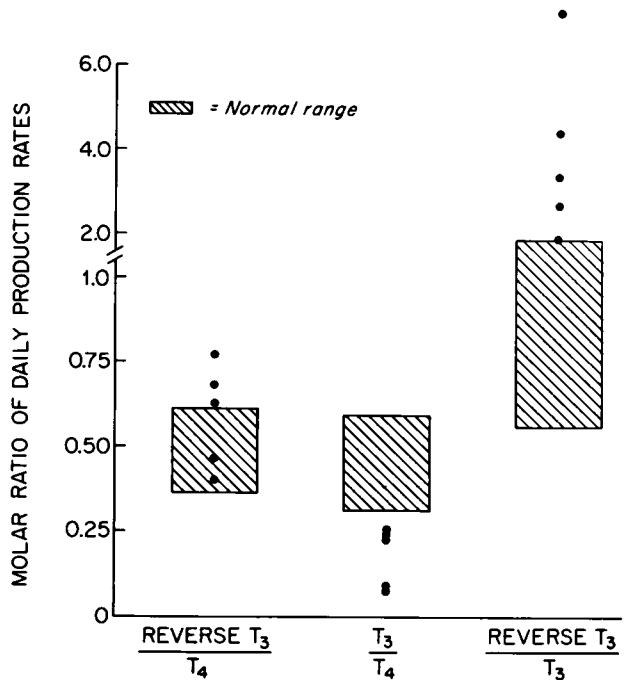

FIGURE 2 Abnormalities in relative production of various thyroid hormones (rT$_3$, T$_3$, and T$_4$) in hepatic cirrhosis. Data are presented as molar ratios of production rates of various iodothyronines.

that (0.54±0.03) in normal subjects. On the other hand, the mean molar ratio of PR-T$_3$ and PR-T$_4$ (T$_3$/T$_4$) of 0.18±0.04 was significantly ($P < 0.005$) lower than that (0.40±0.04) in normal subjects. The mean molar ratio of PR-rT$_3$ and PR-T$_3$ (rT$_3$/T$_3$) of 3.95±0.95 was much higher than that (1.19±0.19) in normal subjects ($P < 0.02$).

DISCUSSION

The present study indicates that daily PR of rT$_3$ (36.5 µg/day) may normally be equal to or more than that of T$_3$ (33.5 µg/day). There are no other data available at present to compare with the PR-rT$_3$ observed in this study. However, some information is available on two individual components of PR-rT$_3$, i.e., serum concentration and MCR. Thus, serum concentrations of rT$_3$ similar to those measured in this study have also been observed by a different radioimmunoassay (2). Furthermore, the rate of disappearance of radioactive rT$_3$ in man has been examined in a previous study (22). The data were similar to those reported here in indicating that rT$_3$ disappears very rapidly from human circulation and that most (> 50%) of the radioactivity administered appears as iodide in urine within 24 h of the injection (22). However, quantitative assessment of MCR of rT$_3$ has not been reported in this (or any other) previous

study. Calculation of MCR has been conducted in this study by an integral noncompartmental approach which is considered more appropriate for measurement of MCR of rapidly disappearing iodothyronines than the conventional single-compartmental technique (14–16). Some assessment of the validity of measurements of MCR-rT$_3$ is also provided by the data on MCR of other, extensively studied, iodothyronines, T$_4$ and T$_3$; these data were obtained by methods similar to those used for MCR of rT$_3$. Thus, the mean normal MCR of T$_4$, 1.02 liters/day, and mean normal MCR-T$_3$, 26.0 liters/day, observed in this study are quite similar to those (22–27 liters/day for T$_3$ and 1.0–1.3 liters/day for T$_4$) observed in previous studies that used various different techniques for measurement of MCR but where the possibility of incorporation of radioactive hormone into iodoproteins was taken into account (4–6, 16, 23–25).

In addition to MCRs and PRs, the present study has examined the contribution of thyroidal secretion to PR of rT$_3$ and T$_3$. Thus, it appears that whereas thyroidal secretion may contribute about 23.8% of T$_3$ produced daily, it contributes only about 2.5% of rT$_3$ produced daily. Furthermore, since 3,3'-T$_2$ cross reacts significantly in rT$_3$ radioimmunoassay (1) and since the possibility that 3,3'-T$_2$ may be secreted by the thyroid cannot be excluded, it seems possible that even the present low estimate of contribution of thyroidal secretion to PR-rT$_3$ (2.5%) may be an overestimate. Meanwhile, since thyroidal secretion and peripheral metabolism of T$_4$ are the only two known sources of rT$_3$ and T$_3$ (1, 2), the present study suggests that about 76.2% of PR-T$_3$ and 97.5% of PR-rT$_3$ may be derived from peripheral monodeiodination of T$_4$. These estimates must, however, be considered approximations because: (a) the calculations have been based on mean values of various parameters, and appreciable variations were evident in individual cases in each of the various parameters of study; (b) multiple measurements are required to arrive at above estimates, and each measurement, although reasonably accurate, is not flawless; (c) the data on relative proportions of iodothyronines in the thyroid gland and those on kinetics of various iodothyronines had to be obtained in different subjects; and (d) it is assumed in above calculations that rT$_3$, T$_3$, and T$_4$ are secreted in the proportions in which they exist in the thyroid; the estimates of the significance of thyroidal secretion would have to be modified if subsequent studies were to show that T$_3$ and(or) rT$_3$ are secreted preferentially. In any case, while there are these limitations, there are also reasons to consider the above estimates of relative contributions of thyroidal secretion and T$_4$ metabolism to PR-T$_3$- and PR-rT$_3$ sufficiently reliable to allow deduction as to the routes of metabolism of T$_4$. Thus, the significance of the contribution of the peripheral metabolism of T$_4$ to PR-T$_3$ has been evaluated in several studies using different methods

(5, 15, 20, 26, 27), and in each case it has been estimated that peripheral conversion of T$_4$ to T$_3$ may contribute about 22–24 μg of T$_3$. Since recent studies using T$_3$ radioimmunoassay indicate that daily euthyroid PR-T$_3$ may only be about 28–36 μg/day (6, 28) the results of the present study agree with the previous studies in indicating that peripheral metabolism of T$_4$ must normally be a major source of T$_3$; studies in sheep have also led to a similar conclusion (29).

Little information is currently available about the contribution of thyroidal secretion or peripheral metabolism of T$_4$ to PR-rT$_3$. However, the findings that serum rT$_3$ concentrations in subjects receiving exogenous T$_4$ are similar to or higher than those in normal subjects (1, 2) suggests that T$_4$ metabolism must be a major source of rT$_3$. Furthermore, previous studies (7) indicating that administration of thyrotropin results in little or no increase in serum rT$_3$ at a time when serum T$_3$ and T$_4$ are increased suggest that thyroidal secretion must normally be a poor source of rT$_3$. Additionally, detailed studies in sheep have provided data that agree with those in this study by indicating that thyroidal secretion may contribute rT$_3$ only to an extent of about 3% of daily PR-rT$_3$ (8).

Several previous studies suggest that about 85% of T$_4$ produced daily is metabolized by deiodination (26, 30–33); the rest is apparently lost as T$_4$ or its conjugates (32) or is metabolized by deamination and decarboxylation of alanine side chain to tetraiodothyroacetic acid (34). It has also been demonstrated that deiodination of T$_4$ can occur both in the phenolic and in the tyrosyl ring of the T$_4$ molecule (1, 35). The present study suggests that the majority of T$_4$ metabolized daily is monodeiodinated either to T$_3$ or rT$_3$; thus, one can account for almost 84% of daily PR-T$_4$ as being monodeiodinated to T$_3$ or rT$_3$. It appears that monodeiodination may be an obligatory step in deiodination of T$_4$.

Recent studies indicate that serum rT$_3$ is increased in several situations, e.g., hepatic cirrhosis, chronic renal failure, acute febrile illness, protein-calorie malnutrition (9), and starvation (2) where serum T$_3$ is decreased. In this study, MCR and PR of various iodothyronines were examined in one of these conditions, i.e. hepatic cirrhosis. The findings reflect several important alterations in iodothyronine metabolism in this disease. Thus, the data suggest that increase in serum rT$_3$ in hepatic cirrhosis must be influenced importantly by the decrease in MCR of rT$_3$ (Table II); the mean daily PR-rT$_3$ was similar to that in normal subjects. However, the daily production of rT$_3$ relative to T$_4$ was high in some cases (Fig. 2). This was the case because PR-T$_4$ was low. Others have also observed low normal or low PR-T$_4$ in hepatic cirrhosis (25, 36, 37); beside the illness, the higher age of patients than normal subjects may have also contributed to diminished PR-T$_4$ observed

in hepatic cirrhosis patients studied here. As against PR-rT₃, PR-T₃ in patients with hepatic cirrhosis was clearly subnormal; another recent study has also described low PR-T₃ in hepatic cirrhosis (25). PR-T₃ was low in patients in this study whether it was examined as such (Table II) or in relation to PR-T₄ (Fig. 2). The distinctive change in PR-T₃ compared to PR-rT₃ was clearly evident when the ratio of PR-rT₃ to PR-T₃ was examined. Thus, it was clear that production of rT₃ relative to T₃ is increased markedly in hepatic cirrhosis (Fig. 2). Since most of T₃ as well as rT₃ originate from peripheral metabolism of T₄, the present study suggests that the pattern of monodeiodination is so altered in hepatic cirrhosis that conversion of T₄ to T₃ is decreased, while that to rT₃ is maintained normal or is increased. Similar situation has also been observed in normal fetus in whom serum T₃ is less than, and serum rT₃ is much higher than the adult levels (8). These observations suggest the possibility that alterations in monodeiodination of T₄ observed in hepatic cirrhosis and the fetus may also be the explanation in other situations where serum T₃ is low and rT₃ is high (9, 36, 38).

The routes of metabolism of T₄ appear to be somewhat different in patients with hepatic cirrhosis than in normal subjects. Thus, of 66 µg of T₄ produced daily (Table II), 38.5 µg (33 × 0.975 × 1.195 = 38.5) or about 57.9% can be accounted for as monodeiodinated to rT₃, while another 9.2 µg (10.1 × 0.762 ×1.195 = 9.2) or about 13.9% can be seen as monodeiodinated to T₃. The remaining approximately 28% (100 − 57.9 − 13.9 = 28.2%) of PR-T₄ appears to be metabolized via routes other than T₃ or rT₃ in patients with hepatic cirrhosis compared to about 16% in normal subjects. Since tetraiodothyroacetic acid has recently been demonstrated in human serum (34), it seems possible that in addition to the above-mentioned abnormalities in T₄ metabolism, the pathway of conversion of T₄ to tetraiodothyroacetic acid or another side chain derivative of T₄ may be accelerated in a systemic illness such as hepatic cirrhosis.

The mechanism responsible for the abnormalities in T₄ metabolism in hepatic cirrhosis is unclear. This information would probably become available after the mechanisms of conversion of T₄ to T₃ and to rT₃ and other derivatives have been elucidated. Meanwhile, it is interesting to note that serum rT₃ as well as T₃ return to or towards normal after refeeding of patients with protein-calorie malnutrition (9) and starvation (2). Improvement in low serum T₃ has also been observed after recovery of liver function in patients with alcoholic hepatitis (25). These findings suggests that the abnormality of T₄ metabolism in illness and starvation is frequently reversible.

The biological purpose, if any, of the alteration in iodothyronine metabolism in hepatic cirrhosis is not clear at this time. It seems possible, however, that the ap-

parent shunting of T₄ from conversion to highly potent T₃ (39) to the production of calorigenically inactive rT₃ (40, 41) or other metabolites of T₄ such as tetraiodothyroacetic acid (34, 42) may reflect a defense reaction of the body intended to safeguard against excessive metabolic stimulation during illness. Obviously, much further study will be needed before this or an alternative possibility can be established.

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