An Assessment of Daily Production and Significance of Thyroidal Secretion of 3,3′,5′-Triiodothyronine (Reverse T_3) in Man

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ABSTRACT While 3,3′,5′-triiodothyronine (reverse T_3, rT_3) 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 (T_3) and thyroxine (T_4). Thus, it was noted that rT_3 is cleared from human serum at a much faster rate than are T_3 and T_4; the mean (±SE) MCR of rT_3 was 76.7±5.4 liters/day in 10 subjects, whereas MCR-T_3 and MCR-T_4 in 8 of them were 26.0±2.2 liters/day and 1.02±0.06 liters/day, respectively. Therefore, even though the mean serum concentration of rT_3, 48±2.8 ng/100 ml, was much lower than that (128±6.7 ng/100 ml) of T_3, the mean PR-rT_3 (36.5±2.8 μg/day) and the mean PR-T_3 (33.5±3.7 μg/day) were similar; in comparison, the mean serum concentration and PR of T_4 were 8.6±0.5 μg/100 ml and 87.0±3.9 μg/day, respectively. These data and those on the relative proportion of rT_3, T_3, and T_4 in 10 thyroid glands were used to assess the significance of the contribution of thyroidal secretion to PR-rT_3 and PR-T_3. It was estimated that whereas thyroidal secretion may account for about 23.8% of serum T_3 (or PR-T_3), it may account for only about 2.5% of serum rT_3 (or PR-rT_3). Since peripheral metabolism of T_4 is the only known source of rT_3 and T_3 other than the thyroidal secretion, it could be calculated that as much as 73.0 μg or 84% of daily PR-T_4 may normally be metabolized by monodeiodination either to T_3 or to rT_3.

MCR and PR of various iodothyronines were also examined in five cases with hepatic cirrhosis, where, as documented previously, serum rT_3 may be elevated while serum T_3 is diminished. The mean MCR-rT_3 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-T_3 (26.7 liters/day) and the mean MCR-T_4 (1.19 liters/day) did not differ from those (*vide supra*) in normal subjects. Distinct from changes in MCRs, the mean PR-rT_3 (33.0 μg/day) was similar to, and the mean PR-T_3 (10.1 μg/day) and the mean PR-T_4 (66.4 μg/day) were much less than, the corresponding value in normal subjects. Furthermore, while the ratio of PR-rT_3 and PR-T_4 (rT_3/T_4) in individual patients was either supranormal or normal, the ratio of PR-T_3 and PR-T_4 (T_3/T_4) was clearly subnormal. The various data suggest that: (*a*) just as in the case of T_3, the thyroid gland is a relatively minor source of rT_3; peripheral metabolism of T_4 is apparently its major source; (*b*) the bulk of T_4 metabolized daily is monodeiodinated to T_3 or to rT_3; (*c*) monodeiodination may be an obligatory step in metabolism of T_4; (*d*) monodeiodination of T_4 to rT_3 is maintained normal or is increased in hepatic cirrhosis at a time when monodeiodination of T_4 to T_3 is decreased.

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INTRODUCTION

Like 3,5,3′-triiodothyronine (T_3),[1] 3,3′,5′-triiodothyronine (reverse T_3, rT_3) has recently been identified in human thyroglobulin and in serum of hypothyroid patients and euthyroid subjects receiving treatment with thyroxine (T_4) (1, 2). While considerable data are available in case of T_3 (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 T_3, rT_3, 3,3′,5′-triiodothyronine; T_2, 3,3′-L-diiodothyronine; T_3, 3,5,3′-triiodothyronine; T_4, 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_2 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	rT₃ MCR	rT₃ Production rate	rT₃ Production rate	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 T_3 and T_4, only data up to 36 h were employed to calculate MCR of rT_3; this was done because radioactive rT_3 remaining in serum after 36 h was very small, i.e., less than 0.05% of the dose of radioactive rT_3. 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 rT_3, T_3, and T_4 in Patients with

Case	Age	Sex	Body weight	rT₃ Serum concn*	rT₃ MCR	rT₃ MCR	rT₃ Production rate	rT₃ Production rate	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).

Rate of rT_3, T_3, and T_4 in Euthyroid Subjects

T_3				T_4				
MCR		Production rate		Serum concn*	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 rT_3, T_3, and T_4 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 rT_3, T_3, and T_4 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±0.6%[2] of rT_3, 86.8±0.60% of T_3 and 90.5±0.74% of T_4. The fraction of rT_3 and T_3 precipitated by TCA were similar but both of these values were modestly lower than that in case of T_4 ($P < 0.05$). The radioactivity remaining in the precipitate after ethanol-extraction was 1.2±0.03% for rT_3, 2.3±

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

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

T_3				T_4				
MCR		Production rate		Serum concn	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.

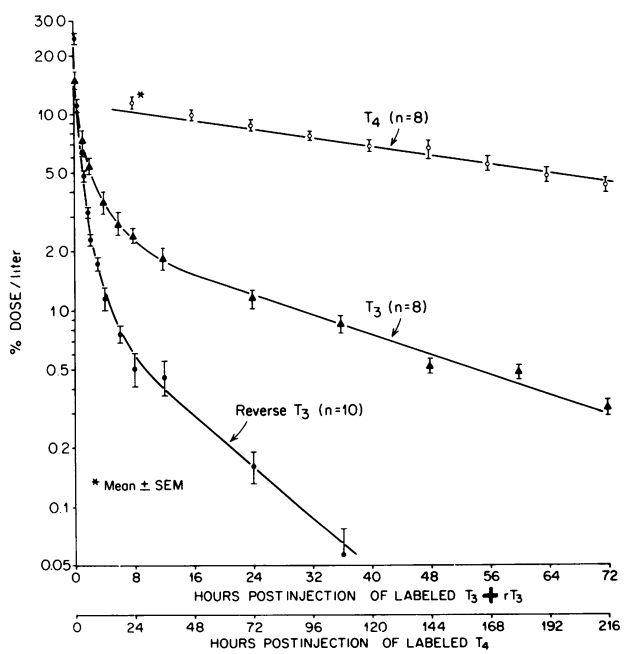

FIGURE 1 Disappearance from serum of TCA-precipitable and ethanol-extractable radioactivity, presumably representing iodothyronines, after injection of radioactive rT_3, T_3, and T_4 intravenously to euthyroid subjects; the data on mean±SE and the percent dose per liter are plotted against time in hours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

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);

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

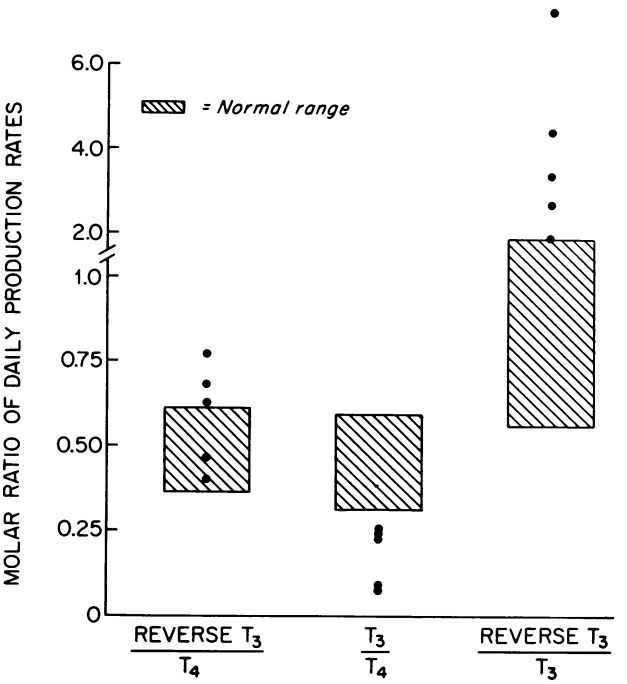

FIGURE 2 Abnormalities in relative production of various thyroid hormones (rT_3, T_3, and T_4) in hepatic cirrhosis. Data are presented as molar ratios of production rates of various iodothyronines.

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-rT$_3$, PR-T$_3$ in patients with hepatic cirrhosis was clearly subnormal; another recent study has also described low PR-T$_3$ in hepatic cirrhosis (25). PR-T$_3$ was low in patients in this study whether it was examined as such (Table II) or in relation to PR-T$_4$ (Fig. 2). The distinctive change in PR-T$_3$ compared to PR-rT$_3$ was clearly evident when the ratio of PR-rT$_3$ to PR-T$_3$ was examined. Thus, it was clear that production of rT$_3$ relative to T$_3$ is increased markedly in hepatic cirrhosis (Fig. 2). Since most of T$_3$ as well as rT$_3$ originate from peripheral metabolism of T$_4$, the present study suggests that the pattern of monodeiodination is so altered in hepatic cirrhosis that conversion of T$_4$ to T$_3$ is decreased, while that to rT$_3$ is maintained normal or is increased. Similar situation has also been observed in normal fetus in whom serum T$_3$ is less than, and serum rT$_3$ is much higher than the adult levels (8). These observations suggest the possibility that alterations in monodeiodination of T$_4$ observed in hepatic cirrhosis and the fetus may also be the explanation in other situations where serum T$_3$ is low and rT$_3$ is high (9, 36, 38).

The routes of metabolism of T$_4$ appear to be somewhat different in patients with hepatic cirrhosis than in normal subjects. Thus, of 66 μg of T$_4$ produced daily (Table II), 38.5 μg ($33 \times 0.975 \times 1.195 = 38.5$) or about 57.9% can be accounted for as monodeiodinated to rT$_3$, while another 9.2 μg ($10.1 \times 0.762 \times 1.195 = 9.2$) or about 13.9% can be seen as monodeiodinated to T$_3$. The remaining approximately 28% ($100 - 57.9 - 13.9 = 28.2\%$) of PR-T$_4$ appears to be metabolized via routes other than T$_3$ or rT$_3$ 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 T$_4$ metabolism, the pathway of conversion of T$_4$ to tetraiodothyroacetic acid or another side chain derivative of T$_4$ may be accelerated in a systemic illness such as hepatic cirrhosis.

The mechanism responsible for the abnormalities in T$_4$ metabolism in hepatic cirrhosis is unclear. This information would probably become available after the mechanisms of conversion of T$_4$ to T$_3$ and to rT$_3$ and other derivatives have been elucidated. Meanwhile, it is interesting to note that serum rT$_3$ as well as T$_3$ return to or towards normal after refeeding of patients with protein-calorie malnutrition (9) and starvation (2). Improvement in low serum T$_3$ has also been observed after recovery of liver function in patients with alcoholic hepatitis (25). These findings suggests that the abnormality of T$_4$ 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 apparent shunting of T$_4$ from conversion to highly potent T$_3$ (39) to the production of calorigenically inactive rT$_3$ (40, 41) or other metabolites of T$_4$ 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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