Estimation of Thyroxine and Triiodothyronine Distribution and of the Conversion Rate of Thyroxine to Triiodothyronine in Man

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ABSTRACT Studies on peripheral metabolism of simultaneously administered ^{125}I-labeled L-thyroxine ([^{125}I]T_4) and ^{131}I-labeled L-triiodothyronine ([^{131}I]T_3) were performed in five normal subjects, in four patients with untreated hypothyroidism, and in 3 hypothyroid patients made euthyroid by the administration of T_4. The fractional turnover rate (λ_{03}) of thyroid hormones irreversibly leaving the site of degradation and the volumes of pool 1 (serum, V_1), of pool 2 (interstitial fluid, V_2), and of pool 3 (all tissues, V_3) were obtained by using a three-compartment analysis. In addition to the turnover studies, the ratios for the in vivo T_4 to T_3 conversion were determined by paper chromatographic study in sera obtained 4, 7, and 10 days after the injection. The rate (K_{12}) of the extrathyroidal conversion of T_4 to T_3 was also estimated by the compartment analysis. The T_3 distribution volume (V_3) of pool 3, in which T_3 is utilized and degraded, was about 60% of total distribution volume ($V = V_1 + V_2 + V_3$) in normal subjects, whereas only about 25% of the extrathyroidal T_4 pool was in the intracellular compartment, indicating that T_3 is predominantly an intracellular hormone. The V_3/V ratios of T_3 were almost constant in a normal ratio of about 60% in hypothyroid patients before and after treatment. On the other hand, the V_3/V ratios of T_4 were elevated in patients with untreated hypothyroidism, and they returned almost to the normal range after administration of T_4. Thus, the V_3/V ratio of T_4 bore a significant inverse relation to the free T_4 concentration in all subjects ($r = -0.68$, $P < 0.05$). The findings suggested a redistribution of T_4 into the cellular compartment in patients with untreated hypothyroidism. In normal subjects values for K_{12} averaged 3.00±0.68% per day of the extrathyroidal T_4 pool, and the amount of T_3 generated by the conversion of T_4 (mean±SD, 17±5 μg/day) was found to contribute approximately 70% of the daily T_3 production (mean ±SD, 24±5 μg/day). The patients with untreated hypothyroidism had elevated K_{12} (mean±SD, 4.16±0.72% per day). Because of diminution of extrathyroidal T_4 pool, the amount of T_3 converted from T_4 was markedly diminished in the untreated patients (mean±SD, 4±3 μg/day), and it agreed closely with T_3 production rate (mean±SD, 3±2 μg/day). In the patients treated with T_4 K_{12} returned almost to the normal range (mean±SD, 2.59±0.47% per day), and the amount of T_3 arising from deiodination of T_4 (mean±SD, 24±6 μg/day) corresponded closely to T_3 production rate (mean±SD, 21±5 μg/day). Furthermore, a highly significant correlation was evident in the plots of serum T_3 concentrations against the amounts of T_3 generated by the conversion of T_4 in all subjects ($r = +0.85$, $P < 0.001$). The results indicated that the amount of T_3 formed by the extrathyroidal conversion of T_4 is a major determinant of serum T_3 concentration in normal subjects and in patients with hypothyroidism before and after treatment.

INTRODUCTION

Extrathyroidal conversion of thyroxine (T_4)[1] to triiodothyronine (T_3) was demonstrated by recent studies

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[1] *Abbreviations used in this paper:* BMR, basal metabolic rate; $(CR)_{in\ vivo}$, in vivo conversion ratio for T_4 to T_3; [^{125}I]T_4, ^{125}I-labeled L-T_4; [^{131}I]T_3, ^{131}I-labeled L-T_3; MCR, metabolic clearance rate of thyroid hormones; T_3, triiodothyronine; T_4, thyroxine; TCA, trichloracetic acid.

TABLE I

Clinical and Laboratory Findings

Subject	Sex	Age	Body wt	BMR	Thyroid hormones concentration			
					T_4	Free T_4 %	Free T_4	T_3
		yr	*kg*	%	*μg/100 ml*	%	*ng/100 ml*	*ng/100 ml*
Normal								
Y. O.	M	21	52		7.2	0.023	1.66	130
M. U.	M	22	67		7.8	0.020	1.56	132
M. E.	M	23	64		10.4	0.027	2.81	180
I. N.	M	25	62		6.4	0.023	1.47	121
S. Y.	M	28	61		8.0	0.023	1.84	146
Mean±SD					8.0	0.023	1.87	142
					±1.5	±0.002	±0.04	±23
Untreated hypothyroidism								
Y. U.	F	24	39	−13.8	1.7	0.016	0.27	40
K. T.	F	45	66	−11.0	1.2	0.016	0.19	30
M. Y. (1)*	F	52	49	−16.2	0.8	0.021	0.17	25
M. K. (1)*	F	60	55	−15.5	0.3	0.015	0.05	12
Mean±SD					1.0	0.017	0.17	27
					±0.6	±0.003	±0.09	±12
Hypothyroid patients treated with T_4								
M. Y. (2)	F	52	47	0	9.4	0.025	2.35	142
D. K.	M	53	44	+13.2	8.6	0.026	2.24	92
M. K. (2)	F	60	48	+11.0	10.4	0.030	3.12	120
Mean±SD					9.5	0.027	2.57	118
					±0.9	±0.003	±0.48	±25
Normal range (25 volunteers)					6.1–11.2	0.020–0.030	1.47–3.15	85–180

* The numbers in parentheses indicate the first and second studies in patients M. Y. and M. K.

in both athyreotic patients (1) and normal man (2–4). Moreover, the findings have indicated that the conversion of T_4 to T_3 was a physiologically important pathway in man (3–5).

In the present paper, the rate of this conversion was determined in normal subjects and in patients with primary hypothyroidism before and after treatment. In addition, the intracellular distribution of T_3 and T_4 was estimated by the three-compartment analysis of serum disappearance curves of radioactive T_3 and T_4. The study revealed that T_3 derived from the extrathyroidal conversion of T_4 is the main source of the extrathyroidal T_3 pool in normal subjects and in patients with hypothyroidism before and after treatment.

METHODS

Clinical materials

The subjects of this study were five normal volunteers, four patients with untreated primary hypothyroidism, and three hypothyroid patients made euthyroid by the daily oral administration of 150 μg of T_4. T_4 was administered in two doses given at 12-h intervals. One patient (D. K.) was studied after about 7 wk of the treatment. Two patients (M. Y. and M. K.) with untreated hypothyroidism were restudied after about 10 wk of the treatment. All the patients were hospitalized in the endocrine section of Tenri Hospital. Diagnosis was established on the basis of the clinical picture and determinations of basal metabolic rate (BMR), thyroid ^{131}I uptake, and free T_4 concentration in serum. The untreated patients had low BMR and free T_4 concentrations and presented the typical clinical pictures of hypothyroidism. Patients with clinically detected liver or renal diseases were not included in this study. Table I lists the clinical and laboratory data obtained in all subjects.

^{125}I-labeled L-T_4 ($[^{125}I]T_4$) and ^{131}I-labeled L-T_3 ($[^{131}I]T_3$) turnover studies

Both $[^{125}I]T_4$ and $[^{131}I]T_3$ were obtained from Abbott Laboratories, North Chicago, Ill., and utilized without further purification. All subjects received 5 drops of Lugol's solution orally three times a day for 3 days before and throughout the study. The two tracer materials diluted in 0.125 g/100 ml albumin in normal saline solution were injected into an antecubital vein, and venous blood was taken from the opposite arm 1 and 5 min and 1, 3, 5, 7, 9, 12, and 24 h after the injection. Thereafter, blood samples were obtained twice a day for 3 days and subsequently once daily until 2 wk after the injection. Serum samples were subjected to trichloracetic acid (TCA) precipitation to remove inorganic iodide and were extracted four times with ethanol in order to measure nonextractable iodine (6), according to the method of Oppenheimer, Schwartz, Shapiro, Bernstein, and Surks (7). The difference between the TCA-precipi-

table iodine and nonextractable iodine was considered to be iodothyronine. The radioactivities of ^{131}I and ^{125}I were measured in a well type scintillation counter equipped with a pulse height analyzer to a precision of at least 2% and were converted into radioactivity per liter of serum as percent of the administered dose.

Method of analysis

In the present study, the extrapolation method and the three-compartmental method were employed for analyzing the kinetic data.

Extrapolation method. Semilogarithmic plots were made of the disappearance curves of radioactive thyroid hormones in serum. The final slope was taken as the fractional turnover rate, the time-zero intercept of this slope was used to estimate the total distribution volume, and the products of the fractional turnover rate and the total distribution volume were the metabolic clearance rates of thyroid hormones (MCR) (8–12).

Three-compartmental method. The disappearance curves of radioactive thyroid hormones were resolved into three components by the method of peeling (13). The mathematical equation is given by

$$Y = h_1e^{-g_1t} + h_2e^{-g_2t} + h_3e^{-g_3t}$$

where g_i and h_i are the slope and the intercept, respectively, of the i^{th} component and i is either the index 1, 2, or 3.

A three-pool model was then formulated, which is illustrated in Fig. 1. The first pool (pool 1) in this model is serum. The second pool (pool 2), exchanging with serum, is the interstitial fluid compartment, and the third pool (pool 3) is the compartment in which thyroid hormones are utilized and degraded and from which it is irreversibly removed. Pool 3 is clearly not homogenous and represents, at least, muscle, kidney, and liver. The parameters λ_{ij} are fractional turnover rates, and the subscripts denote passage from compartment j to compartment i. λ_{03} is the fractional irreversible loss rate of T_3 or T_4 leaving the site of degradation. It was assumed that the thyroid hormone degradation system does not distinguish between the isotopic tracer and the native unlabeled compound. Thus, refined g_i and h_i were used to compute the parameter by the method described by Skinner, Clark, Baker, and Shipley (14). Moreover, the volume of each pool was obtained by the following formulae:

$$V_1 = \frac{100}{h_1 + h_2 + h_3}$$

$$V_2/V_1 = \frac{\lambda_{21}(\lambda_{23} + \lambda_{03})}{(\lambda_{12} + \lambda_{32})(\lambda_{23} + \lambda_{03}) - \lambda_{23}\lambda_{32}}$$

$$V_3/V_1 = \frac{\lambda_{21}\lambda_{32}}{(\lambda_{12} + \lambda_{32})(\lambda_{23} + \lambda_{03}) - \lambda_{23}\lambda_{32}}$$

These equations were based upon the assumption of a steady and uniform concentration of thyroid hormones throughout the three compartment. The calculation was made by a Hewlett-Packard calculator (model 9100A, Hewlett-Packard Co., Palo Alto, Calif.).

The sum of the volumes of pools 1, 2, and 3, $V_1 + V_2 + V_3$, represented the total distribution volume of thyroid hormones, and the MCR was the products of volume of the pool 3 (V_3), from which T_3 or T_4 is irreversibly removed,

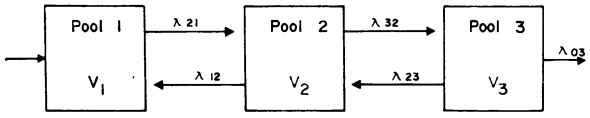

FIGURE 1 A three-pool model describing the serum disappearance of radioactive thyroid hormones. V_1, V_2, and V_3 indicate the distribution volumes of pools 1, 2, and 3, respectively. The parameters λ_{ij} are fractional turnover rates, and the subscripts denote passage from compartment j to compartment i. λ_{03} is the fractional irreversible loss rate of thyroid hormones leaving the site of degradation. Pool 1: serum. Pool 2: interstitial fluid. Pool 3: sites of utilization and degradation.

and the fractional irreversible loss rate (λ_{03}) of T_3 or T_4 leaving the site of degradation (MCR = $V_3 \times \lambda_{03}$).

The disappearance curves of radioactive thyroid hormones were also resolved into two components. The mean sum of squares of residuals for this set was compared with that obtained for the resolution of the curves into three components. The resolution of the curve into more than four components was not possible, since a fourth component is not discernible by eye, nor can it be obtained by the peeling procedure.

Since it has previously been demonstrated (15) that thyroxine distribution volume was dependent only on body weight and independent of height, in all subjects the distribution volume and the MCR of thyroid hormones were adjusted to a 60 kg body wt.

Paper chromatographic study

In addition to the turnover studies, T_4 and T_3 were isolated from serum obtained 5 min and 4, 7, and 10 days after the injection by a combination of column and paper chromatography. Since the radioactivity of injected [^{131}I]T_3 was negligible in serum obtained 4, 7, and 10 days after the injection, a small amount of previously purified [^{131}I]T_3 was added to serum as control for T_3 recovery. The serum samples were deproteinized, and T_4 and T_3 were eluted by the method described by Sterling, Bellabarba, Newman, and Brenner (16). The two-dimensional chromatogram was developed in a solvent of butanol, ethanol, and ammonia in an ascending system for the first direction and then in hexane, tertiary amyl alcohol, and ammonia (17) in a descending system for the second direction. Satisfactory separation of T_4 and T_3 was obtained by this chromatography. To correct for the in vitro T_4 to T_3 conversion, during analyses of four sera of each subject small amounts of previously purified [^{125}I]T_4 and [^{131}I]T_3 were added to a pooled serum, and T_4 and T_3 were separated by the same chromatographic procedure simultaneously in a parallel fashion. The T_3 area on the paper chromatogram was identified by autoradiography. The T_3 area was cut out, and the radioactivity of ^{131}I and of ^{125}I was counted in a well type scintillation counter equipped with a pulse height analyser to a precision of at least 2%.

Approximately half of previously purified [^{131}I]T_3, which was added to serum as a control for T_3 recovery, was localized in the two-dimensionally separated T_3 spot (mean ±SD, 52.9±8.3% in all subjects). A serum sample drawn 5 min after the injection of [^{125}I]T_4 showed about 2% ^{125}I activity in the T_3 area, which was likely due to the [^{125}I]T_3 in the injection dose and some [^{125}I]T_3 formed artifactually in vitro. [^{125}I]T_3 contaminated in the dose was cleared

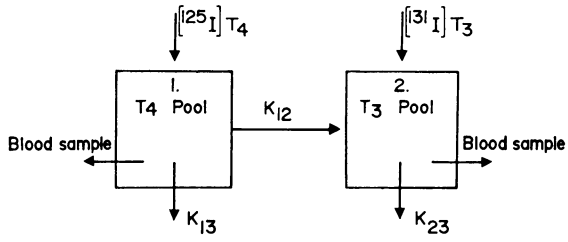

FIGURE 2 A two-pool model describing the kinetics of $[^{125}I]T_4$ and $[^{131}I]T_3$. K_{12} is the rate of conversion of T_4 to T_3. The sum of K_{12} and K_{13} is the fractional turnover rate of $[^{125}I]T_4$. K_{23} is the fractional turnover rate of $[^{131}I]T_3$.

rapidly, as anticipated from the results of the $[^{131}I]T_3$ turnover study, and it was nearly negligible 4, 7, and 10 days after the injection.

The ratio of ^{125}I activity found in the T_3 spot of paper chromatogram to the total ^{125}I activity in serum was approximately 1.8% with a range of 1.18–2.76% in all subjects. The result of the two-dimensional analysis of pooled serum, to which previously purified $[^{125}I]T_4$ was added, revealed that ^{125}I activity found in the T_3 spot on paper was about 0.4% of total ^{125}I activity in serum (mean±SD, 0.39 ±0.02%); values averaged 24.2% of the ratio of $[^{125}I]T_3$/total ^{125}I in serum from the subjects (mean±SD, 24.2± 7.1%). Thus, the radioactivity found in the T_3 area was contributed primarily by the in vivo conversion of radioactive T_4 to T_3 in addition to the artifacts mentioned previously (18, 19).

The in vivo conversion ratio for T_4 to T_3 $[(CR)_{\text{in vivo}}]$ was calculated as follows:

$$(CR)_{\text{in vivo}} = \left[\frac{^{125}I \text{ in } T_3 \text{ area of chromatogram of serum from subject}/R}{\text{Total } ^{125}I \text{ in serum of subject}} - \frac{^{125}I \text{ in } T_3 \text{ area of chromatogram of pooled serum}/R'}{\text{Total } ^{125}I \text{ in pooled serum}}\right] \times 2 \quad (1)$$

where R and R' were recoveries of ^{131}I in T_3 spots of paper chromatograms of serum from subject and of pooled serum, respectively. Since it was assumed that in commercial preparations of $[^{125}I]T_4$ the 3′ and 5′ positions are randomly labeled with iodine, monodeiodination of T_4 would yield only one molecule of T_3 containing ^{125}I for each two molecules formed (1), and this has been proven (20). Thus, values for $(CR)_{\text{in vivo}}$ were corrected by multiplying the measured values by 2. $(CR)_{\text{in vivo}}$ was almost constant during days 4–10, suggesting that the conversion reaction reached equilibrium 4 days after the injection of $[^{125}I]T_4$.

Estimation of the rate of extrathyroidal conversion of T_4 to T_3

To estimate the conversion rate of T_4 to T_3, a two-pool model was formulated to describe the kinetics of $[^{125}I]T_4$ and $[^{131}I]T_3$ in serum. As shown in Fig. 2, pool 1 is the extrathyroidal T_4 pool, and pool 2 is the extrathyroidal T_3 pool. K_{12} is the rate of conversion of T_4 to T_3. Metabolism of $[^{125}I]T_4$ injected into pool 1 may be represented by Eq. 2.

$$\frac{dQ[^{125}I]T_4(t)}{dt} = -Q[^{125}I]T_4(t) \times (K_{12} + K_{13}) \quad (2)$$

where Q is the total radioactive thyroid hormones in the body and $K_{12}+K_{13}$ is a fractional turnover rate of $[^{125}I]T_4$, which is the slope of the third component of the $[^{125}I]T_4$ disappearance curve from serum. The change in $[^{125}I]T_3$ in pool 2 derived from $[^{125}I]T_4$ is dependent on the rate of conversion of T_4 to T_3 and T_3 clearance.

$$\frac{dQ[^{125}I]T_3(t)}{dt} = Q[^{125}I]T_4(t) \times K_{12} - Q[^{125}I]T_3(t) \times K_{23} \quad (3)$$

where K_{23} is a fractional turnover rate of $[^{131}I]T_3$, which is the slope of the last component of the disappearance curve of $[^{131}I]T_3$. From the solution of these two equations, the desired rate of conversion of T_4 to T_3 was obtained, as represented by Eq. 4.

$$K_{12} = \frac{(V)T_3}{(V)T_4} \times \frac{q[^{125}I]T_3(t)}{q[^{125}I]T_4(t)} \times [K_{23} - (K_{12} + K_{13})] \quad (4)$$

where $(V)T_3$ and $(V)T_4$ were the T_3 and T_4 distribution volumes, which were obtained by the three-compartment analysis as mentioned above, and q is the concentration of radioactive thyroid hormone in serum. $q[^{125}I]T_3(t)/q[^{125}I]T_4(t)$ was the ratio for the in vivo T_4 to T_3 conversion estimated by paper chromatographic study. The conversion ratio was the mean value of those obtained 4, 7, and 10 days after the injection of $[^{125}I]T_4$ in each subject.

Determination of T_4 concentration and of T_3 concentration in serum

Free T_4 fractions in serum were determined by the magnesium precipitation method as described by Sterling and Brenner (21). The free T_4 concentrations were the products of free T_4 percentages and T_4 concentrations in serum. The determinations of serum T_4 concentrations were carried out according to the method of Murphy and Pattee (22). The concentration of T_3 in serum was determined by radioimmunoassay. Antibodies to T_3 were raised in rabbits by injection of T_3-bovine serum albumin conjugates which were prepared by the method of Gharib, Ryan, Mayberry, and Hockert (23). T_3 binding of serum protein was blocked by the addition of 8-anilino-1-naphthalene-sulfonic acid.

The lowest T_3 concentration detectable in the present assay was 25 ng/100 ml or less when a 0.1 ml serum sample was assayed. Sera containing less than 25 ng/100 ml of T_3 were assayed by using 0.2-ml serum samples. The T_3 concentrations determined by using 0.2-ml serum samples were approximately twice those determined by using 0.1-ml serum samples in the present assay. Serum T_3 concentrations ranged from 85 to 180 ng/100 ml in 25 normal volunteers (mean±SD, 128±25 ng/100 ml). The coefficients of variation for triplicate determinations within an assay were 4.0% in a normal subject, 1.7% in a thyrotoxic patient, and 7.0% in a hypothyroid patient. There was no significant difference in T_3 concentrations of the same serum determined in two consecutive assays in three normal subjects, three thyrotoxic patients, and three hypothyroid patients by the paired t test $(0.1 < P\{|t| \geqq 1.634\} < 0.2)$.

All free T_4 fractions and the concentrations of T_4 and T_3 were determined in serum drawn before injection of radioactive thyroid hormones. Moreover, additional values were obtained on sera taken during the course of the study in hypothyroid patients during treatment with T_4. In no case was there any evidence of a systemic change suggesting alteration of the "steady state" during the course of the turnover studies.

RESULTS

The principal results obtained are presented in Tables I–III.

[^{131}I]T_3 turnover study. The mean sum of squares obtained from the data of all subjects for the two-pool model (mean±SE, 1.3241±0.2619) was approximately five times the value obtained for the three-pool model (mean±SE, 0.2745±0.0538). The findings indicated a better fit with the latter model. Moreover, the values for the ratios of the volume of pool 1 to that of pool 2 (V_2/V_1) for all subjects averaged 2.59±0.51 (mean±SD) in the case of the three-pool model and corresponded closely to previous estimates obtained by employing a different methodology (13). The results provide further support for the three-pool model.

The total distribution volume of T_3, representing the sum of the volumes of pools 1, 2, and 3, and the MCR of T_3, representing the product of the irreversible loss rate and the volume of pool 3 ($\lambda_{03} \times V_3$), were appreciably lower than those obtained by the extrapolation method in all subjects. The differences were greater, the larger volume and the higher the clearance rate (Table II).

Values for total distribution volume of T_3 obtained by the three-compartmental method averaged 46.6±12.8 liters/60 kg body wt (mean±SD) in normal subjects. The T_3 distribution volume of pool 3, in which T_3 is utilized and degraded, ranged from 15.1 to 41.0 liters/60 kg body wt in normal subjects (mean±SD, 26.6±10.4 liters/60 kg body wt); values averaged 56% of the total distribution volume of T_3 ($V_3/V = 56 \pm 9\%$, Fig. 3). The result indicates that approximately 60% of the entire extrathyroidal T_3 was in the intracellular compartment in normal subjects.

The patients with untreated hypothyroidism had markedly diminished T_4 and T_3 concentrations in serum and they showed reduced total distribution volume of T_3. The T_3 distribution volume of pool 3 in these patients (mean±SD, 16.6±2.4 liters/60 kg body wt) was also appreciably diminished, as compared with 26.6±10.4 liters/60 kg body wt of normal subjects, although these differences were not statistically significant (Table II). The value of the ratio V_3/V (mean±SD, 52±5%) was almost the same as that of the normal subjects (mean±SD, 56±9%, Fig. 3). In the patients after treatment with T_4, the T_3 and T_4 concentrations returned to almost normal range, and their total distribution volume

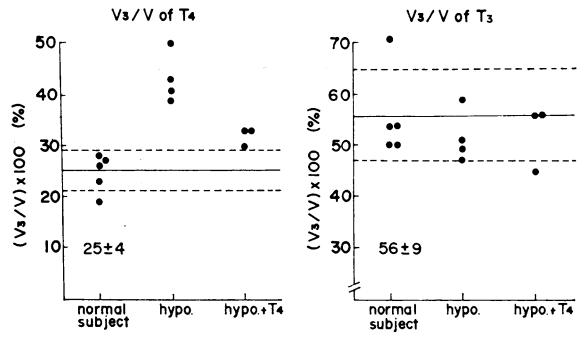

FIGURE 3 The intracellular distribution of thyroid hormones. V_3, the distribution volume of pool 3, in which thyroid hormones are utilized and degraded; V, the total distribution volume of thyroid hormones; hypo., patients with untreated hypothyroidism; hypo. + T_4, patients treated with T_4. Mean values and standard deviations in normal subjects were indicated by the solid horizontal lines and the broken horizontal lines, respectively.

and the volume of pool 3 were entirely normal. Therefore, the ratio V_3/V was within the normal range in the patients treated with T_4 (Fig. 3). These findings illustrated maintenance of the normal V_3/V ratio, approximately 60%, despite alterations of the T_3 distribution volume and of T_3 and T_4 concentrations in serum in hypothyroid patients before and after treatment.

The fractional turnover rate of [^{131}I]T_3, which was obtained by the extrapolation method, ranged from 0.280 to 0.594/day (mean±SD, 0.429±0.119/day) in normal subjects, values slightly lower than those of the euthyroid subjects recently reported (4, 24–28). The turnover rates in patients with hypothyroidism before and after treatment were almost the same as those of the normal subjects (Table II).

Values for the fractional irreversible loss rate of T_3 leaving the site of degradation, which was estimated by the three-compartmental analysis, averaged 0.706±0.240/day in normal subjects. There was no significant difference between the irreversible loss rate in normal subjects and that in hypothyroid patients before and after treatment. Moreover, the fractional irreversible loss rate was greater than the fractional turnover rate obtained by the extrapolation method (Table II).

The distinct diminution of the MCR's, estimated by the three-compartmental analysis, was found in three of four patients with untreated hypothyroidism, and the mean MCR (mean±SD, 12.0±2.4 liters/day per 60 kg body wt) in this group was significantly lower than in normal subjects ($P < 0.05$, Table II). The patients with maintenance of a euthyroid state by the administration of T_4 had almost normal MCR's (Table II).

The daily production rate of T_3, representing the product of the MCR by the three-compartmental analysis and T_3 concentration in serum, ranged from 17 to 28 μg/

TABLE II
Results of

Subject	Sex	Age	T_3 kinetic data: Extrapolation method V^*	K	MCR*	Three-compartment analysis V^*	V_3^*	λ_{03}	MCR*	T_3DR^*
		yr	*liter*	*per day*	*liter/day*	*liter*	*liter*	*per day*	*liter/day*	*μg/day*
Normal										
Y. O.	M	21	49.1	0.594	29.2	42.9	21.4	1.008	21.6	28
M. U.	M	22	34.4	0.481	16.6	30.5	15.1	0.864	13.1	17
M. E.	M	23	44.9	0.428	19.3	40.5	21.9	0.720	15.8	28
I. N.	M	25	69.3	0.280	19.4	61.6	33.4	0.480	16.1	19
S. Y.	M	28	73.1	0.361	26.4	57.4	41.0	0.456	18.7	27
Mean±SD			54.2	0.429	22.2	46.6	26.6	0.706	17.0	24
			±16.5	±0.119	±5.3	±12.8	±10.4	±0.240	±3.2	±5
Untreated hypothyroidism										
Y. U.	F	24	38.3	0.505	19.4	33.6	16.9	0.912	15.4	6
K. T.	F	45	38.2	0.324	12.4	33.3	19.7	0.504	9.9	3
M. Y. (1)	F	52	37.0	0.387	14.3	33.7	15.7	0.744	11.7	3
M. K. (1)	F	60	33.8	0.434	14.7	28.7	14.1	0.768	10.8	1
Mean±SD			36.8	0.413	15.2	32.3	16.6	0.732	12.0	3
			±2.1	±0.076	±3.0	±2.4	±2.4	±0.169	±2.4	±2
P			NS	NS	NS	NS	NS	NS	<0.05	<0.001
Hypothyroid patients treated with T_4										
M. Y. (2)	F	52	39.7	0.463	18.4	34.8	19.5	0.912	17.8	25
D. K.	M	53	58.9	0.407	23.9	48.7	27.1	0.624	16.9	16
M. K. (2)	F	60	47.0	0.465	21.9	43.5	18.9	0.936	17.6	21
Mean±SD			48.5	0.445	21.4	42.3	21.8	0.824	17.4	21
			±9.7	±0.033	±2.8	±7.0	±4.6	±0.174	±0.5	±5
P			NS	NS	NS	NS	NS	NS	NS	NS

V, total distribution volume of thyroid hormones; K, fractional turnover rate of radioactive thyroid hormones; V_3, distribution volume of pool 3, in which thyroid hormones are utilized and degraded; λ_{03}, fractional turnover rate of thyroid hormones irreversibly leaving the site of degradation; T_3DR, T_3 production rate; ETT_4, extrathyroidal T_4 pool; T_4DR, T_4 production rate; P, probability that the values in hypothyroid patients are identical to the corresponding values in normal subjects; NS, not significant.

* The value was adjusted to 60 kg body wt.

day per 60 kg body wt in normal subjects (mean±SD, 24±5 μg/day per 60 kg body wt). A markedly diminished production rate of T_3 was found in all the patients with untreated hypothyroidism (mean±SD, 3±2 μg/day per 60 kg body wt), and it returned to almost normal range after treatment with T_4 (Table II).

[^{125}I]T_4 turnover study. In all subjects in the present study, the total distribution volume and the MCR of T_4, estimated by the three-compartmental method, agreed closely with those as determined by the extrapolation method (Table II). The value for the ratio of volume of pool 3 to total distribution volume of T_4 (V_3/V) averaged 25±4% in normal subjects, indicating that approximately one-fourth of extrathyroidal T_4 was in the intracellular compartment (Fig. 3). The patients with untreated hypothyroidism had markedly elevated T_4 distribution volumes of pool 3, in which T_4 is utilized and degraded (mean±SD, 4.8±0.3 liters/60 kg body wt vs. 2.1±0.4 liters/60 kg body wt in the normals, $P < 0.001$, Table II). Moreover, it was of interest that the mean value of the V_3/V ratios in these patients was almost twice the normal mean (mean±SD, 43±5% vs. 25±4% in the normals, $P < 0.001$, Fig. 3).

The T_4 distribution volume of pool 3 in patients N. Y. and M. K., who were studied before and after treatment, returned to high normal values after treatment with T_4 (Table II). The V_3/V ratios in the two patients were also reduced after the treatment (Fig. 3). Thus, the mean values of T_4 distribution volume of pool 3 (mean±SD, 3.7±0.2 liters/60 kg body wt) and of the V_3/V ratios (mean±SD, 32±2%) in the patients after treatment with T_4 were significantly lower than those in the untreated patients ($P < 0.01$), although they were still elevated when compared with those of normal sub-

T_4 kinetic data								
Extrapolation method			Three-compartment analysis					
V*	K	MCR*	V*	ETT_4*	V_3*	λ_{03}	MCR*	T_4DR*
liter	*per day*	*liter/day*	*liter*	*μg*	*liter*	*per day*	*liter/day*	*μg/day*
8.7	0.115	1.00	9.0	648	2.1	0.480	0.99	71
5.4	0.169	0.91	5.8	452	1.6	0.528	0.86	67
10.4	0.108	1.13	10.6	1,102	2.8	0.408	1.13	118
9.6	0.130	1.25	10.0	640	1.9	0.648	1.23	79
8.1	0.122	0.99	8.1	648	2.2	0.456	0.99	79
8.4	0.129	1.06	8.7	698	2.1	0.504	1.04	83
±1.9	±0.024	±0.13	±1.9	±241	±0.4	±0.091	±0.14	±20
12.2	0.071	0.86	11.9	202	4.6	0.192	0.88	15
10.8	0.074	0.80	11.0	132	4.8	0.168	0.80	10
10.7	0.084	0.90	10.9	87	4.5	0.193	0.87	7
10.4	0.091	0.94	10.2	31	5.1	0.168	0.85	3
11.0	0.080	0.88	11.0	113	4.8	0.180	0.85	9
±0.8	±0.009	±0.06	±0.7	±72	±0.3	±0.014	±0.04	±5
<0.05	<0.01	<0.05	NS	<0.01	<0.001	<0.001	<0.05	<0.001
11.3	0.095	1.08	11.5	1,081	3.7	0.288	1.09	95
12.2	0.087	1.09	12.1	1,041	3.9	0.264	1.05	90
11.9	0.091	1.08	11.9	1,238	3.5	0.288	1.03	107
11.8	0.091	1.08	11.8	1,120	3.7	0.280	1.06	97
±0.5	±0.004	±0.01	±0.3	±104	±0.2	±0.014	±0.03	±9
<0.05	<0.05	NS	<0.05	<0.05	<0.01	<0.01	NS	NS

jects (Table II and Fig. 3). The V_3/V ratio of T_4 bore a statistically significant inverse relation to the free T_4 concentration in sera of all subjects studied in the present paper ($r = -0.68$, $P < 0.05$, Fig. 4).

Values for the irreversible loss rate of T_4 leaving the site of degradation averaged 0.504±0.091/day in normal subjects. This was approximately four times the T_4 fractional turnover rate (mean±SD, 0.129±0.024/day) estimated by the extrapolation method. The irreversible loss rate of T_3 was less than two times the T_3 fractional turnover rate as mentioned above (Table II). These findings suggested that both T_4 and T_3 must return from the intracellular compartment without having undergone effective metabolic transformation, as demonstrated previously (29, 30). Moreover, the return of T_4 to the systemic circulation was more evident than in the case of T_3.

The patients with untreated hypothyroidism had diminished turnover rates of T_4 (mean±SD, 0.080±0.009/day). The turnover rates were still lower (mean±SD, 0.091±0.004/day) in the patients treated with T_4, although still somewhat elevated, as compared with 0.080 ±0.009/day in the untreated patients.

The irreversible loss rate of T_4, estimated by the three-compartment analysis, was markedly diminished in the patients with untreated hypothyroidism (mean± SD, 0.180±0.014 vs. 0.504±0.091/day in the normals): values were only about two times the fractional turnover rates of T_4. The patients treated with T_4 showed an elevated irreversible loss rate of T_4, as compared with that of untreated patients. However, the values were appreciably lower than those in normal subjects (Table II).

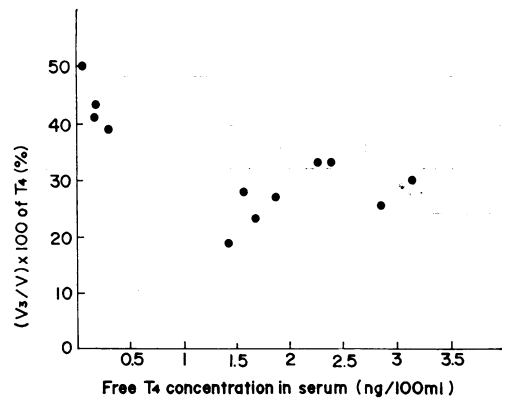

FIGURE 4 Relation between free T_4 concentration in serum and the ratio V_3/V of T_4. V_3, the T_4 distribution volume of pool 3, in which T_4 is utilized and degraded; V, the total distribution volume of T_4. The correlation coefficient is -0.68 in all subjects ($P < 0.05$).

The MCR obtained by the three-compartmental method and the T_4 production rate were 1.04±0.14 liters/day per 60 kg body wt and 83±20 μg/day per 60 kg body wt in normal subjects, respectively (Table II), values almost the same as those of normal subjects previously reported (8–12). The patients with untreated hypothyroidism had diminished clearance rates of T_4, and their T_4 production rates were also markedly diminished (Table II). The MCR's of T_4 in the patients after treatment of T_4 were within normal range (mean±SD, 1.06± 0.03 liters/day per 60 kg body wt, Table II), and, therefore, their production rates of T_4 were almost normal (mean±SD, 97±9 μg/day per 60 kg body wt, Table II).

The rate of the extrathyroidal conversion of T_4 to T_3. Values for the conversion ratio of T_4 to T_3 averaged 0.0199±0.0024 in normal subjects. Moreover, the average daily rate of the extrathyroidal conversion of T_4 to T_3 was 3% of the extrathyroidal T_4 pool (mean±SD, 3.00± 0.68% per day, Table III). The conversion ratios for T_4 to T_3 were elevated in patients with untreated hypothyroidism (mean±SD, 0.0432±0.0023 vs 0.0199± 0.0024 in the normals $P < 0.001$), and markedly elevated rates of the conversion occurred in three of four patients (Table III). The mean value of the conversion rate in these patients was significantly higher than in normal subjects (mean±SD, 4.16±0.72 vs. 3.00±0.68% per day in the normals, $P < 0.05$, Table III). The conversion ratio returned almost to the normal range after treatment with T_4, and the conversion rates also decreased during T_4 administration (Table III).

The products of the extrathyroidal T_4 pool and the conversion rate of T_4 to T_3 represented the micrograms

TABLE III

Extrathyroidal conversion of T_4 to T_3

Subject	Sex	Age	$(CR)_{in\ vivo}$*	K_{12}	ETT_4‡ × K_{12}/T_4DR‡	ETT_4‡ × K_{12} × 0.84	ETT_4‡ × K_{12} × 0.84/T_3DR‡
		yr		*per day*	%	*μg/day*	%
Normal							
Y. O.	M	21	0.0172	0.0393	35.2	21	75.0
M. U.	M	22	0.0199	0.0326	22.4	13	76.5
M. E.	M	23	0.0209	0.0256	23.7	24	85.7
I. N.	M	25	0.0233	0.0215	17.7	12	63.1
S. Y.	M	28	0.0182	0.0308	25.3	17	63.0
Mean±SD			0.0199	0.0300	24.9	17	72.7
			±0.0024	±0.0068	±6.4	±5	±9.7
Untreated hypothyroidism							
Y. U.	F	24	0.0408	0.0488	66.7	8	133.3
K. T.	F	45	0.0418	0.0316	40.0	3	100
M. Y. (1)	F	52	0.0459	0.0430	57.1	3	100
M. K. (1)	F	60	0.0444	0.0428	33.3	1	100
Mean±SD			0.0432	0.0416	49.3	4	108.3
			±0.0023	±0.0072	±15.3	±3	±16.7
P			<0.001	<0.05			
Hypothyroid patients treated with T_4							
M. Y. (2)	F	52	0.0276	0.0305	34.7	28	112
D. K.	M	53	0.0164	0.0211	24.4	18	112.5
M. K. (2)	F	60	0.0191	0.0261	30.0	27	130
Mean±SD			0.0210	0.0259	29.7	24	118
			±0.0058	±0.0047	±5.2	±6	±10
P			NS	NS			

ETT_4, extrathyroidal T_4 pool; T_4DR, T_4 production rate; T_3DR, T_3 production rate; K_{12}, rate of extrathyroidal conversion of T_4 to T_3; $ETT_4 \times K_{12} \times 0.84$, amount of T_3 generated by conversion of T_4 to T_3; *P*, probability that the values in hypothyroid patients are identical to the corresponding values in normal subjects; NS, not significant.

* Average of the values obtained 4, 7, and 10 days after the injection of [^{125}I]T_4.

‡ The value was adjusted to 60 kg body wt.

of T_4 estimated to undergo monodeiodination daily, and this was about 25% of the daily T_4 production (mean±SD, 24.9±6.4%) in normal subjects (Table III). The amount of T_3 generated by the conversion of T_4 to T_3 was obtained by multiplying the amount of T_4 converted to T_3 by 0.84, a factor used to ascertain the molar equivalent quantity of T_3. The values ranged from 12 to 24 μg/day per 60 kg body wt, averaging 17±5 μg/day per 60 kg body wt in normal subjects, and these were found to contribute approximately 70% of the daily T_3 production (mean±SD, 72.7±9.7%, Table III).

Despite elevations in daily rate of conversion for T_4 to T_3, the amount of T_3 generated from T_4 daily was markedly diminished in the patients with untreated hypothyroidism (mean±SD, 4±3 μg/day per 60 kg body wt), and it agreed closely with T_3 production rate (mean ±SD, 3±2 μg/day per 60 kg body wt). The amount of T_3 arising from deiodination of T_4 was elevated during administration of T_4 in patients with hypothyroidism (mean±SD, 24±6 μg/day per 60 kg body wt, Table III), and it was in close agreement with the daily production rates of T_3 in these patients (mean±SD, 21±5 μg/day per 60 kg body wt) and in normal subjects (mean±SD, 24±5 μg/day per 60 kg body wt). Surks, Schadlow, Stock, and Oppenheimer (5) calculated the ratio of T_4 disposal to T_3 disposal in hypothyroid patients during T_4 replacement therapy, and it was the proportion of T_4 converted to T_3. In the present paper values for this ratio were 31.6% (M. Y.), 21.1% (D. K.), and 23.4% (M. K.) in three patients treated with T_4 (mean±SD, 25.4±5.5%); values corresponded closely to those estimated by the chromatographic technique in the same patients (mean±SD, 29.7±5.2%, Table III). Finally, the amount of T_3 generated from T_4 daily bore a highly significant relationship to the serum T_3 concentration in all subjects studied in the present paper. The correlation coefficient was +0.85 ($P < 0.001$, Fig. 5).

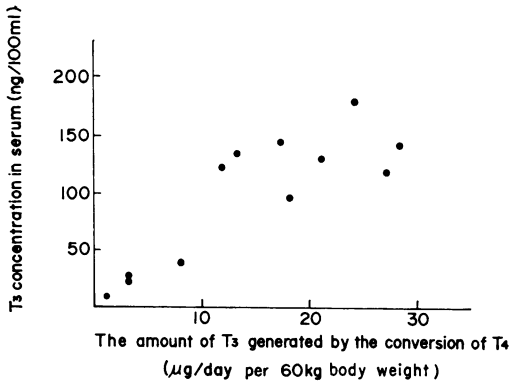

FIGURE 5 Relation between the amount of T_3 generated by the conversion of T_4 and the T_3 concentration in serum. The correlation coefficient is +0.85 in all subjects ($P < 0.001$).

DISCUSSION

Turnover studies of radioactive T_3 have been performed by Sterling, Lashof, and Man (8) and subsequently by several investigators (4, 5, 24–28, 31, 32). In a [^{131}I]T_4 turnover study the radioactivity in the serum consisted mainly of radioactive T_4, whereas nonextractable iodine was recently observed in the serum during radioactive T_3 metabolism (6). The nonextractable iodine interfered with the conventional measurement of radioactive T_3 in the serum (6). Thus, to separate serum T_3 from the iodoproteins, a solvent extraction method was described by Oppenheimer and his co-workers (7), and an anion-column chromatographic method was recently developed by Nicoloff, Low, Dussault, and Fisher (28). In the present paper, the solvent extraction method was employed, although it was time consuming. No data on the differences between the extraction method and the column chromatographic method were available in the present study.

There are several possible methods of analyzing kinetic data to estimate the MCR of T_3. Nearly all of the published estimates of the clearance rate for T_3 in normal subjects are based on the conventional, single-compartment approach. Serum disappearance curves of radioactive thyroid hormones are curvilinear when plotted semilogarithmically, indicating that a multicompartmental system exists for removal of thyroid hormone from serum in man. Moreover, it has been demonstrated previously that a significant portion of thyroid hormones are removed by the liver and the kidney (29). The findings obtained by utilizing single-compartmental kinetics do not provide direct insight into the cellular clearance of thyroid hormones. The parameters of cellular clearance could be obtained by study of models of thyroid hormone metabolism with techniques of multicompartmental analysis. In the present paper a three-pool model was proposed as the minimal model necessary to explain the observed disappearance curves of radioactive thyroid hormones from the serum. The mean sum of squares derived from the data of all subjects was extremely low for the three-pool model. Moreover, the V_2/V_1 ratio, calculated on the basis of the three-pool model and the experimental data, corresponded closely to the previous estimates obtained by a different technique (13). These results provide validation of the proposed model for thyroid hormone kinetics.

The MCR and total distribution volume of T_3 obtained by the three-compartmental analysis, which attempted to fit T_3 kinetics data to a three-compartment model, were significantly lower than those by the extrapolation method in all subjects studied in the present paper. Koutras and his co-workers (33), who employed a two-compartment model, obtained in seven euthyroid

subjects living in an endemic goiter area a mean clearance rate of 19.53 liters/day, values appreciably lower than those reported previously (24–28). However, our estimate of the clearance rate of T_3, based on the three-compartment model, was still lower than the value obtained by Koutras and his co-workers (33). In contrast, the MCR and total distribution volume of T_4 obtained by the extrapolation method agreed closely with those obtained by the three-compartmental analysis in all subjects studied in the present paper. The results suggested that the loss of the T_3 tracer during equilibration phase was much greater than in the case of T_4 turnover study, and it provided validation of the MCR obtained by the three-compartmental analysis.

The irreversible loss rate from the cellular compartment was greater than the fractional turnover rate, either in T_3 turnover or in T_4 turnover. This difference was especially evident in the T_4 turnover study. The findings suggested that the return of T_4 to systemic circulation was more significant than in the case of T_3. It has been previously postulated that about 15% of the entire extrathyroidal T_3 pool would appear to be extracellular (28, 34). In the present paper, in which both intracellular distribution and total distribution volume of T_3 were estimated by the three-compartmental analysis, approximately 60% of the extrathyroidal T_3 pool was in the intracellular compartment. On the other hand, only one-fourth of the extrathyroidal T_4 pool was in the intracellular compartment. Therefore, these results were consistent with the suggestion by previous studies that T_3 is predominantly an intracellular hormone (7, 28), although the cellular distribution of T_3 was relatively smaller than anticipated in previous studies (28, 34).

In the present study, it was remarkable that the V_3/V ratios of T_3 were almost constant, with normal values of approximately 60%, in patients with primary hypothyroidism before and after treatment, irrespective of alterations of T_3 and T_4 concentrations in serum. In contrast, marked elevation of the V_3/V ratio of T_4 was found in patients with untreated hypothyroidism, who had markedly diminished total T_4 concentrations and diminished free T_4 concentrations in serum. Moreover, the V_3/V ratio of T_4 returned almost to the normal range in the patients who showed normal T_4 concentrations in serum after T_4 administration. Thus, the V_3/V ratio of T_4 was found to be inversely related to the concentrations of free T_4. Previous reports suggested that in several states T_4 is shifted to cellular sites, so that the cellular pool of hormone comprises a greater than normal fraction of the total extrathyroidal pool (12, 35). The present findings also suggested a redistribution of T_4 into the cellular compartment in patients with untreated hypothyroidism.

Recently the extrathyroidal conversion of T_4 to T_3 was demonstrated by several investigators (1–5, 36–38). In the present paper, the average daily rate of the extrathyroidal conversion of T_4 to T_3 was approximately 3% of the extrathyroidal T_4 pool in normal subjects. The value was slightly lower than that obtained by Pittman, Chambers, and Read (3) based on studies with ^{14}C-labeled T_4. Moreover, the amount of T_3 generated by the conversion (extrathyroidal T_4 pool $\times$ the conversion rate of T_4 to T_3 $\times$ 0.84) was found to contribute approximately 70% of the daily T_3 production in normal subjects. It has been previously reported that approximately 40% of daily T_3 disposal was derived from the monodeiodination of T_4 (2, 3, 39). A recent report gives a mean value of 59±8% (4). The estimate is similar to the mean value obtained in the normal subjects of the present study, 73±10%. The discrepancy between our estimate and results of previous reports (2, 3, 39) can be explained partly by the difference in stable T_3 concentration in serum (3, 4).

In the present paper marked elevations of the conversion ratios were found in patients with untreated hypothyroidism, and their conversion rates were also elevated. It has been shown that spontaneous deiodination of [^{131}I]T_4 during paper chromatography could be inhibited by carrier T_4 (40). Hence, the ratio for T_4 to T_3 conversion, corrected for the in vitro T_4 to T_3 conversion in pooled serum, might be overestimated in patients with untreated hypothyroidism, who had markedly diminished T_4 concentration in serum. Despite elevation of the conversion rate, the amount of T_3 converted from T_4 was markedly diminished (4±3 μg/day), and it agreed closely with the T_3 production rate (3±2 μg/day) in these patients.

In hypothyroid patients treated with T_4 the amount of T_3 generated by the conversion of T_4 to T_3 (mean±SD, 24±6 μg/day) was in close agreement with the daily T_3 production in these patients (mean±SD, 21±5 μg/day) and the normal T_3 production rate (mean±SD, 24±5 μg/day). This finding was consistent with the assumption that the only source of T_3 was the monodeiodination of T_4 in patients after treatment with T_4, since thyroidal secretion of T_3 was negligible (5). Values for T_4 and T_3 disposal during administration of T_4 were employed to calculate the proportion of T_4 converted to T_3 (T_3 disposal/T_4 disposal) by Surks et al. (5), and they reported a proportion of 42.6% in hypothyroid patients during T_4 replacement therapy. Subsequently, a slightly lower proportion of T_4 converted to T_3 (mean±SD, 35.7±7.2%) was reported by Braverman et al. (4), who estimated the T_3 disposal/T_4 disposal ratios in normal female volunteers during administration of T_4. Moreover, several investigators (2, 3), who estimated the extent of conversion by the chro-

matographic technique, reported that approximately one-third of the T_4 produced was metabolized by interconversion to T_3. In the present paper the amount of T_4 metabolized by the conversion of T_4 to T_3 accounted for 25% of the T_4 production per day in normal subjects, according to the chromatographic technique. Furthermore, it is of interest that the proportion of T_4 converted to T_3, as obtained by the chromatographic method, corresponded closely to that obtained from the ratio of T_3 disposal/T_4 disposal in hypothyroid patients during administration of T_4. Although our estimate that 25% of T_4 is converted to circulating T_3 was slightly lower than results of previous reports (2–5), the finding supported the validity of our estimates of the conversion rate and of daily T_3 production.

In all subjects studied in the present paper the computation of the correlation coefficient of serum T_3 concentrations against the amount of T_3 generated by the conversion of T_4 showed a highly significant correlation. Previous reports (4, 5) have suggested that under normal physiologic circumstances a major fraction of T_3 in serum is derived from the peripheral deiodination of T_4 and that only a lesser fraction is derived from direct secretion by the thyroid gland. The findings of the present study illustrated that the amount of T_3 converted from T_4 was a major determinant of serum T_3 concentration in normal subjects and in patients with hypothyroidism before and after treatment.

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