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Research Article

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The Extrathyroidal Conversion Rate of Thyroxine to Triiodothyronine in Normal Man

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ABSTRACT Eight normal subjects were administered tracer amounts of a ^{14}C-labeled thyroxine, L-[tyrosyl-^{14}C] T_4, by multiple injections. Then serial blood samples were collected for isolation of the thyroxine, triiodothyronine, and tetraiodothyroacetic acid fractions by a combination of column and paper chromatographies. The chromatographic artifacts were corrected by adding to the sera a purified ^{3}H-labeled thyroxine, D,L-[α,β-^{3}H] T_4 immediately after the separation of sera from blood. 1–2% of the serum ^{14}C radioactivity was observed in the triiodothyronine fraction and 2–4% of the serum ^{14}C radioactivity was observed in the tetraiodothyroacetic acid fraction. Complete kinetic studies of thyroxine and triiodothyronine were compared in the same individual in four of the subjects. The extrathyroidal conversion rates of thyroxine to triiodothyronine were calculated from data obtained during both the injection and the postinjection periods as functions of the ^{14}C-labeled thyroxine and triiodothyronine remaining in the body at time t and their fractional turnover rates. The average daily rate of the extrathyroidal conversion of thyroxine to triiodothyronine was 4% of the extrathyroidal thyroxine pool or 33% of the total thyroxine production. The amount of triiodothyronine generated by this pathway (22 μg/day) was found to contribute 31% of the extrathyroidal triiodothyronine pool or 41% of the daily triiodothyronine production. This pathway is a major source of triiodothyronine production. The extrathyroidal conversions of thyroxine to triiodothyronine and tetraiodothyroacetic acid are major metabolic pathways of thyroxine in normal man.

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INTRODUCTION

Since the presence of triiodothyronine (T_3)[1] in human plasma was first reported in 1952, the source of this circulating hormone has never been clearly defined (1). It is known that some triiodothyronine is released by the thyroid gland into the blood. At least in the lower animals, the concentration of triiodothyronine is higher in the venous effluent than the arterial blood of the thyroid (2, 3). The possibility of extrathyroidal deiodination of thyroxine (T_4) to triiodothyronine had also been entertained by many investigators. However, in the early human studies, the attempts to identify ^{131}I-labeled T_3 following a single injection of ^{131}I-labeled T_4 only yielded equivocal results (4, 5). Volpert, Greenberg, and Werner found triiodothyronine in both normal pituitary and transplanted pituitary tumor of mice after the injection of ^{131}I-labeled thyroxine (6). Albright, Larson, and Tust detected triiodothyronine after incubating ^{131}I-labeled thyroxine with rat kidney tissue (7). While there were such exceptions, most animal studies by either in vivo or in vitro techniques also failed to substantiate deiodination of T_4 as a significant pathway that gives rise to circulating T_3 (6–8). More recently Braverman, Ingbar, and Sterling analyzed the total serum T_4 and T_3 in hypothyroid patients maintained on pharmacological doses of L-thyroxine (9). The T_3:T_4 ratios in their sera were found to be higher than the ratio in the ingested L-thyroxine preparation. These same investigators also administered multiple injections of ^{125}I-labeled thyroxine to two athyreotic patients. Approximately 1–2% of the serum radioactivity was found to be in the triiodothyronine fraction and twice that in the tetraiodothyroacetic acid (Tetrac) fraction. This study strongly suggested the presence of extrathyroidal conversion of T_4 to T_3 in man. In a study by Pittman, Nakafuji, and Read in normal men, ^{14}C-

[1] *Abbreviations used in this paper:* K, fractional turnover-rate; T_3, triiodothyronine; T_4, thyroxine; Tetrac, tetraiodothyroacetic acid; V_d, volume of distribution.

labeled T_3 and Tetrac were also regularly observed after repeated intravenous injections of ^{14}C-labeled T_4 (10). The present report contains our measurement of the rate of this extrathyroidal conversion of T_4 to T_3 in similarly prepared normal subjests. T_4 and T_3 kinetic studies were compared in the same individuals in order to define the biological significance of the pathway of extrathyroidal conversion of T_4 to T_3 in man.

METHODS

Materials. Two types of radiothyroxines were used in the studies. One radiothyroxine was labeled with ^{14}C in the nonphenolic ring and the alanine side chain, L-[tyrosyl-^{14}C] T_4. It was synthesized by the method of Shiba and Cahnmann by Amersham-Searle Corp., Arlington Heights, Ill. and it had a specific activity of 135 mCi/mmole (11). The L-[tyrosyl-^{14}C] T_4 was used to calculate the T_4 to T_3 conversion rates. The other radiothyroxine was labeled with ^3H in the alpha and beta carbons of the side chain, D,L-[α,β-^3H] T_4, with a specific activity of 125 mCi/mmole. The D,L-[α,β-^3H] T_4 was synthesized by Dr. J. Nunez with the method of Nunez, Jacquemin, and Roche (12). The sample contained primarily L-thyroxine but it was not free of some D-thyroxine. The D,L-[α,β-^3H] T_4 was used only as a methodological control in the correction for artifactual T_4 deiodination and for the calculation of T_4 recoveries during our chromatographic manipulations. In addition, a ^{131}I-labeled triiodothyronine, L-[3′-^{131}I] T_3 was purchased from the Abbott Laboratories, North Chicago, Ill., specific activity 1.79×10^4 mCi/mmole. The purity of radioactive T_4 and T_3 was 98% or greater before use. They were prepared for injection in a sterile solution of 1% ethanol, 0.9% scdium chloride, and 1% human albumin as described previously (13). Every thyroxine dose was chromatographed in at least two solvent systems and the presence of triiodothyronine was assayed directly in a liquid scintillation counter as described below. Two D,L-[α,β-^3H] T_4 standards were prepared and they contained 0.52 and 0.50% of ^3H-labeled triiodothyronine. Five injection doses of D,L-[α,β-^3H] T_4 were prepared. The range of ^3H-labeled triiodothyronine contained in these preparations was 0.46–0.81%. Seven injection doses of L-[tyrosyl-^{14}C] T_4 were prepared. The range of ^{14}C-labeled triiodothyronine detected in these doses was 0.59–0.82%.

Subjects. Studies were carried out in eight young, healthy male subjects free of any past history or family history of thyroid disorder. The details of their clinical information are listed in Table I. Throughout the experiment the subjects were housed in the Clinical Research Center of the University of Alabama Medical Center. Each subject was given daily intravenous injections of L-[tyrosyl-^{14}C] T_4 for 10 days. The total T_4 dose was 8–11 μg per day. Throughout the experiment serial blood samples were taken. In addition, 24-hr urines and feces were collected for radioisotope recoveries. On the 20th day of experiment, an intravenous injection of D,L-[α,β-^3H] T_4 was given to four of the subjects (97.4 μg). The D,L-[α,β-^3H] T_4 experiment was carried out to determine the rate at which the extrathyroidal conversion of T_4 to T_3 reaches an equilibrium. During the 24th–27th days of experiment these same four subjects were given Lugol's solution orally, five drops, t.i.d. (three times a day). They were given 200 μCi of L-[3′-^{131}I] T_3 intravenously in one injection for the kinetic studies of T_3. During the studies with D,L-[α,β-^3H] T_4 and L-[3′-^{131}I] T_3, the collections of blood, urine, and feces were made every 12 hr.

Laboratory procedures. Sera were separated immediately after blood collection. Into those samples which did not contain previously injected D,L-[α,β-^3H] T_4, a small amount of purified D,L-[α,β-^3H] T_4 was added as a control for T_4 recovery and for artifactual T_3 production. The amounts of ^3H-labeled T_3 found at the end of all chromatographic manipulations were used to correct for the in vitro T_4 to T_3 conversion. For samples which contained previously injected D,L-[α,β-^3H] T_4, the ^3H-labeled T_3 found in the blood 10 min after the injection of D,L-[α,β-^3H] T_4 was used for similar correction.

The T_4, T_3, and Tetrac fractions were isolated from serum by a combination of column and filter-paper chromatographies (14). The serum samples were deproteinized on a resin column, Dowex AG, W-X2 (Dow Chemical Co., Midland, Mich.) (H^+ form). After washing the column with water and 0.15 M ammonium acetate, the T_4, T_3, and Tetrac fractions were eluted with 7.4 N ammonium hydroxide. The eluent was condensed by lyophilization and applied on a No. 3 Whatman paper. The two-dimensional chromatogram was developed in a solvent of hexane, tertiary amyl alcohol, and 2 N ammonia (1:5:6) in a descending system for the first direction and then in an ascending system in the second direction. A small amount of carrier T_3 (5 μg/μl) was added into the condensed eluent to facilitate visualization of the T_3 area on the paper chromatogram under ultraviolet light. T_4 was present in adequate amount to be detected by ultraviolet light without the use of carrier T_4. The T_4, T_3, and Tetrac areas were cut out in 2×0.5 cm strips. The mean T_4 recovery was 54.8% with a range of 45.8–62.8%. For each run of serum, a tracer amount of a purified L-[3′-^{131}I] T_3 was added to a portion of the same sample and the paired portions were carried through the entire

TABLE I

Clinical Information of the Subjects

Patients, age, and sex			Thyroidal uptake of ^{131}I	Total serum T_4	Total serum T_3	T_4:T_3 ratios	L-[tyrosyl-^{14}C] T_4 inj.
yr			*% 24 hr^{-1}*	*μg/100 ml*	*ng/100 ml*		*dpm $\times 10^6$ day^{-1}*
J. McD.	22	M	17.8	8.0	230	35	3.090
D. D.	21	M	7.8	4.5	140	32	7.713
D. V.	21	M	32.7	6.5	165	39	4.480
B. H.	23	M	36.1	7.0	160	44	4.480
J. Mu.	22	M	20.2	6.5	210	31	4.452
D. G.	22	M	16.7	5.5	190	29	4.279
J. S.	22	M	17.5	4.2			
J. Ma.	23	M	22.8	4.9			

chromatographic procedure simultaneously in a parallel fashion. The T_3 recovery was calculated from the recovered L-[3′-^{131}I] T_3. The over-all T_3 recovery by this method was 17.2–31.8%. In the present study some serum samples were controlled by D,L-[α,β-^{3}H] T_4. Both the trace amounts of ^{3}H-T_4 contaminant and the trace amounts of ^{3}H-labeled T_3 which was produced by the in vitro deiodination of D,L-[α,β-^{3}H] T_4, contributed ^{3}H activity to the T_3 zone on paper chromatograms. The per cent of ^{3}H radioactivity detected in the T_3 zone was assumed to be the same as the percent of ^{14}C radioactivity contributed by the same artifacts which were corrected for in our calculation of ^{14}C-T_3. The over-all recovery of thyroxine calculated from the D,L-[α,β-^{3}H] T_4 standard was 7.9–19.0%.

The ^{14}C and ^{3}H activities were assayed in a dioxane scintillator (1% PPO, 0.05% POPOP, and 5% naphthalene) with a liquid scintillation counter (Nuclear-Chicago Corporation, Des Plaines, Ill.). The gamma radioactivity of ^{131}I was assayed in a well scintillation counter (Nuclear-Chicago Corporation, Des Plaines, Ill.).

The half-times of T_4 were obtained from the ^{14}C data 24 hr after the last injection of L-[tyrosyl-^{14}C] T_4. The disappearance of ^{14}C radioactivity in blood was followed for 9–12 days during which time five to seven samples were collected from each subject for the calculation of half-times.

In the study of T_3 kinetics, the ^{131}I activities in both the native serum and the protein precipitate were assayed. The latter was used in our calculation. The serum was precipitated by the following procedure. To 1.0 ml of serum was first added 25 μg/ml of carrier sodium iodide and enough propylthiouracil to make a 10^{-5} M solution, followed by 1.0 ml of cold 20% trichloroacetic acid. The resulting precipitate was washed three times with cold 5% trichloroacetic acid.

The measurements of the total serum T_4 and T_3 were carried out by the Boston Medical Laboratory, Waltham, Mass. The serum T_4 was measured by a modification of the method of Murphy and Pattee (15). The serum T_3 was measured according to a modified method of Sterling, Bellabarba, Newman, and Brenner (14).

Calculation. The serum radioactivity was plotted as a function of time. The half-time ($t_{\frac{1}{2}}$) of T_4 or T_3 was obtained from the respective linear regression. The fractional turnover-rate (K) and the zero time volume of distribution (V_d) were calculated by a method similar to that described by Ingbar and Freinkel (16). The total body pool was derived from the product of the serum concentration and the volume of distribution. The daily production rate (or disposal rate) was calculated from the product of the serum concentration, the volume of distribution, and the fractional turnover-rate of T_4 or T_3. In the study of T_3 kinetics, the problem of iodoproteins that arises as a product of T_3 degradation was avoided by using only the data collected between 24–72 hr after an injection of L-[3′-^{131}I] T_3 (17).

The kinetics in the extrathyroidal conversion of T_4 to T_3 are calculated by the following:

A = the daily injection dose of radiothyroxin in dpm.
$[T_4]$ = the amount of injected L-[tyrosyl-^{14}C] T_4 in the body in dpm at time t.
$[T_3]$ = the amount of L-[tyrosyl-^{14}C] T_3 in the body in dpm at time t.
K_4 = the fractional turnover-rate of thyroxine, per day.
K_3 = the fractional turnover-rate of triiodothyronine, per day.
λ = the extrathyroidal conversion rate of thyroxine to triiodothyronine per day.
t^* = the time of last injection of radiothyroxin in days.
t = the time of blood sample collection in days.

In this calculation the following assumptions are made:

(*a*) K_3, K_4, and λ are independent of both time and the amount of T_3 and T_4 present.
(*b*) λ/K_4 is close to zero.
(*c*) A units of labeled T_4 are administered daily for t^* days.

$$\frac{d[T_4]}{dt} = A - K_4[T_4]. \tag{1}$$

For $A \neq 0$, the solution of (1) is

$$[T_4] = \frac{A}{K_4}(1 - e^{-K_4 t}). \tag{2}$$

For $A = 0$, i.e. for $t > t^*$, the solution to (1) is

$$[T_4] = Ce^{-K_4 t} \tag{3}$$

where C must be evaluated from the initial conditions. In this case, the initial conditions are that at $t = t^*$ $[T_4]$ is given by, from (2), $A(1 - e^{-K_4 t^*})/K_4$. Using this, equation (3) becomes

$$[T_4] = \frac{A}{K_4}(1 - e^{-K_4 t^*})e^{K_4(t^* - t)}. \tag{4}$$

The extrathyroidal conversion of T_4 to T_3 can be described by

$$\frac{d[T_3]}{dt} = \lambda[T_4] - K_3[T_3]. \tag{5}$$

As was the case for $[T_4]$, the solution of (5) will have one of two forms, depending on whether or not $A = 0$. For $A \neq 0$, the initial condition is that $[T_3] = 0$ for $t = 0$, and the solution to (5) is

$$[T_3] = \frac{\lambda A}{K_3 K_4 (K_3 - K_4)} \times [K_3(1 - e^{-K_4 t}) - K_4(1 - e^{-K_3 t})]. \tag{6}$$

For $A = 0$, the initial condition is that, at $t = t^*$, $[T_3]$ is given by (6), with t^* replacing t. Using this, the solution to (5) becomes

$$[T_3] = \frac{\lambda A}{K_3 K_4 (K_3 - K_4)}[K_3(1 - e^{-K_4 t^*})e^{K_4(t - t^*)} - K_4(1 - e^{-K_3 t^*})e^{-K_3(t - t^*)}]. \tag{7}$$

For $t \leq t^*$, the extrathyroidal conversion rate, λ, can be found, after dividing (6) by (2), as

$$\lambda = \frac{K_3(K_3 - K_4)}{K_3 - K_4\dfrac{1 - e^{-K_3 t}}{1 - e^{-K_4 t}}}\frac{[T_3]}{[T_4]} \tag{8}$$

For $t > t^*$, λ can be found by dividing (7) by (4).

$$\lambda = \frac{K_3(K_3 - K_4)}{K_3 - K_4\left(\dfrac{e^{K_3 t^*} - 1}{e^{K_4 t^*} - 1}\right)e^{-(K_3 - K_4)t}}\frac{[T_3]}{[T_4]} \tag{9}$$

RESULTS

The clinical information of our subjects are summarized in Table I. Our subjects were given 10 daily injections of L-[tyrosyl-^{14}C] T_4. During the entire 20 days of study the average ^{14}C recovery from urine was 37.6% of the injected dose with a range of 27.7–50.3%. The fecal collections from five of the eight subjects were complete. The average recovery of ^{14}C from feces was 13.0% of the injected dose with a range of 9.2–14.4%. Fig. 1 shows the cumulative and daily recoveries of ^{14}C

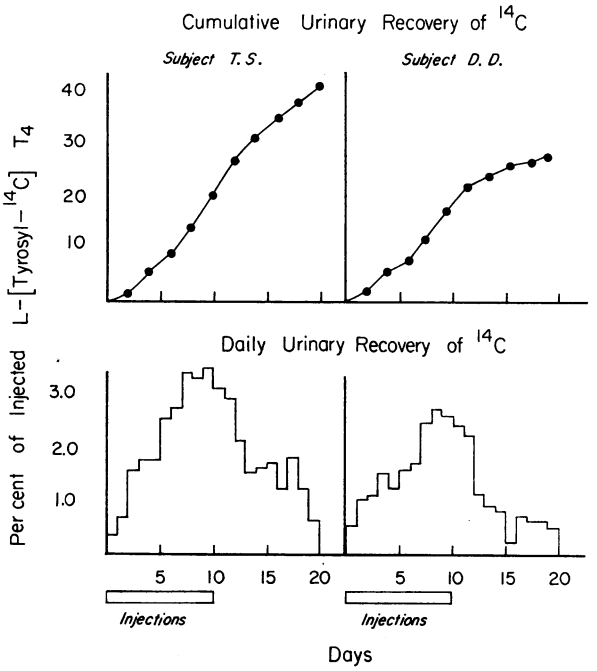
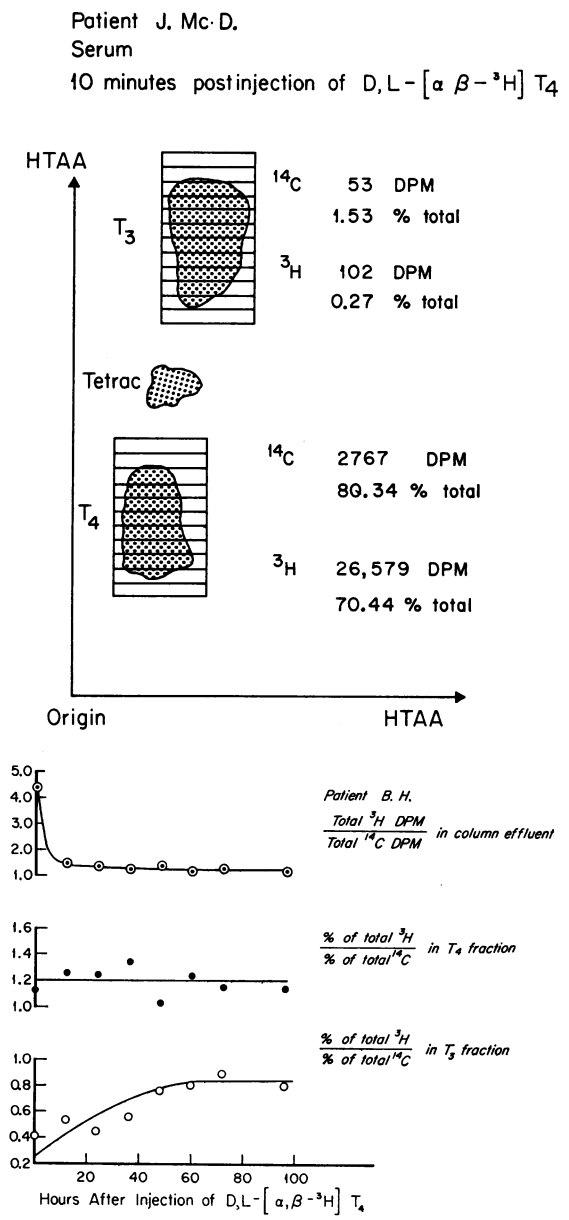


FIGURE 1 ^{14}C recoveries from urine. Each subject was given L-[tyrosyl-^{14}C] T_4 8–11 μg/day intravenously for 10 days.

from urine of subjects J. S. and D. D. This pattern of urinary recovery was typical of the remaining subjects of the group.

In all serum samples studied, ^{14}C radioactivity was consistently found in the T_4, T_3, and Tetrac fractions. The ^{14}C-labeled Tetrac was always found in equal or slightly larger amounts than the ^{14}C-labeled T_3. The ^{14}C radioactivity in the T_3 fraction represented 1.56 ±0.50% of the total ^{14}C radioactivity in serum. The recovery of Tetrac was not measured. Of the total radioactivity applied to the chromatogram paper, 0.4–4.3% was found in the Tetrac fraction.

In all the subjects studied trace amounts of D,L-[α,β-^3H] T_4 were added to sera either in vitro or in vivo as a control. The need of such a control is shown in Fig. 2. Subject J. McD. had previously been equilibrated with L-[tyrosyl-^{14}C] T_4. Then he was given an injection of D,L-[α,β-^3H] T_4. A serum sample drawn 10 min after the injection of D,L-[α,β-^3H] T_4 showed 0.27% ^3H activity in the T_3 fraction which was likely due to the D,L-[α,β-^3H] T_3 in the injection dose, the D,L-[α,β-^3H] T_4 remaining in the T_3 fraction and some D,L-[α,β-^3H] T_3 formed artifactually in vitro. The serum study of a similarly prepared subject, B. H., is shown in Fig. 3 in which the $^3H/^{14}C$ ratios of the deproteinized serum, the T_4 and T_3 fractions are plotted as functions of time. The $^3H/^{14}C$ ratios of the deproteinized serum and of the T_4 fraction remained nearly constant after the initial equilibration of D,L-[α,β-^3H] T_4 with the body pool of T_4. The $^3H/^{14}C$ ratio in the T_3 fraction rose and did not reach a plateau until 48 hr after the injection of D,L-[α,β-^3H] T_4. If the ^3H activity in the T_3 fraction was due to T_3 contamination in the D,L-[α,β-^3H] T_4 dose and chromatographic artifacts, the $^3H/^{14}C$ ratio in the T_3 fraction should have fallen or remained con-



FIGURES 2 and 3 The subjects had been equilibrated with L-[tyrosyl-^{14}C] T_4 previously and were then given one injection of D,L-[α,β-^3H] T_4, intravenously, 97.4 μg. Serial blood samples were collected. The two-dimensional paper chromatograms of the deproteinized sera were developed in a solvent system of hexane:tertiary amyl alcohol:2 N ammonium hydroxide in both directions. The T_4 and T_3 areas were cut out and assayed for ^{14}C and ^3H radioactivities.

stant. Therefore, the radioactivity found in the T_3 fraction was contributed primarily by the in vivo conversion of radioactive T_4 to T_3 in addition to the artifacts mentioned before.

It is assumed that during chromatographic manipulations the ^3H and ^{14}C-labeled thyroxines contributed similar fractions of their respective radioactivities to the T_3 area on paper chromatograms due to T_4 remaining in the T_3 fraction and due to T_4 to T_3 conversion in vitro. Therefore, the fraction of ^3H activity was used to correct for the contribution of ^{14}C due to these artifacts in the T_3 area. When this value was equal to or less than the labeled T_3 contaminant in the original T_4 injection dose, no net correction was made. In actuality, no net correction was necessary in most of our calculations of $[T_3]$. For example: to 10 ml serum of J. Mu. trace amounts of ^{131}I-labeled T_3 standard were added. To another 10 ml serum of J. Mu. trace amounts of a ^3H-labeled T_4 standard were added. After all the chromatographic manipulations, 62 dpm of ^3H was recovered from the T_3 area of the final paper chromatogram or 0.48% of the total ^3H activity applied to the paper. From the same T_3 area, 55 dpm of ^{14}C activity was recovered. The over-all T_3 recovery calculated from the ^{131}I T_3 standard was 17.7%. Since the per cent of ^3H-labeled T_3 contaminant in the original injection dose of ^3H-T_4 was approximately the same (0.50%) as that recovered in the T_3 area, no correction for artifacts was made. Therefore, the serum concentration of ^{14}C-labeled $T_3 = 5.5 \text{ dpm} \div 17.7 \times 100 = 31 \text{ dpm ml}^{-1}$. The chromatographic data are summarized in Table II.

From sera collected during L-[tyrosyl-^{14}C] T_4 injections the $[T_4]$ value or the amount of L-[tyrosyl-^{14}C] T_4 remaining in the body at time t was calculated from equation (2), $[T_4] = A/K_4(1 - e^{-K_4t})$. The extrathyroidal conversion rate, λ, was calculated from equation (8), $\lambda = (K_3[K_3 - K_4])/(K_3 - K_4[1 - e^{-K_3t}]/[1 - e^{-K_4t}])[T_3]/[T_4]$. From sera collected after all 10 injections of L-[tyrosyl-^{14}C] T_4, $[T_4]$ was calculated from equation (4), $[T_4] = A/K_4(1 - e^{-K_4t^*})e^{K_4(t^*-t)}$, and λ from equation (9),

$$\lambda = \frac{K_3(K_3 - K_4)}{K_3 - K_4\left(\frac{e^{K_3t^*} - 1}{e^{K_4t^*} - 1}\right)e^{-(K_3-K_4)t}} \frac{[T_3]}{[T_4]}.$$

However, for interest of comparison, $[T_4]$ values calculated from two other methods are listed in Table III along with those calculated from equations (2) and (4). In one of the methods the serum concentration of L-[tyrosyl-^{14}C] T_4 was obtained from the ^{14}C activity found in the T_4 area of the final paper chromatogram and the over-all T_4 recovery. In turn, $[T_4]$ was calculated from the serum L-[tyrosyl-^{14}C] T_4 concentration and the known volume of distribution of T_4. As shown in Table III, the $[T_4]$ values derived by this method

TABLE II

T_3 Radioactivities on the Paper Chromatograms in the Sera and in the Body, $[T_3]$, at Time t

Subject	Experiment	Time	T_3 radioactivities: Chromatograms	T_3 radioactivities: Sera	Total pool $[T_3]$
		day	*dpm T_3 area^{-1}*	*dpm ml^{-1}*	*dpm × 10^5*
J. McD.	1	16.0	31.8	14.4	6.404
	2	20.0	52.8	12.3	5.487
	3	20.5	30.5	7.1	3.168
	4	21.0	49.7	11.6	5.162
	5	21.5	27.3	6.4	2.835
	6	22.0	28.0	6.5	2.910
B. H.	1	8.0	31.3	13.2	5.027
	2	16.0	84.4	19.3	7.334
	3	16.5	64.2	14.7	5.586
	4	17.0	125.4	28.7	10.906
	5	17.5	50.4	11.5	4.370
	6	18.0	111.2	25.4	9.652
	7	18.5	105.6	24.1	9.158
	8	19.0	101.6	23.2	8.816
	9	20.0	84.0	19.2	7.296
D. V.	1	8.0	53.9	34.8	12.013
	2	16.0	40.5	9.3	3.195
	3	16.5	50.8	11.6	4.005
	4	17.0	33.9	7.8	2.674
	5	17.5	32.5	7.4	2.563
	6	18.0	42.9	9.8	3.384
	7	19.0	21.1	4.8	1.663
	8	20.0	15.6	3.6	1.232
D. D.	1	12.0	108.4	40.3	18.735
	2	17.0	54.7	19.2	8.947
	3	17.5	69.7	23.2	10.807
	4	18.0	104.1	34.7	16.145
	5	18.5	73.9	24.6	11.458
	6	19.0	95.8	32.0	14.857
	7	20.0	79.2	26.4	12.281
D. G.	1	20.0	27.3	8.6	3.694
J. Mu.	1	10.0	55.4	31.2	13.429

9–20 ml of the serum samples were applied to the column for deproteinization. The over-all recovery of T_3 after chromatographies was 17.2–31.8%. The T_3 radioactivity in serum (dpm ml^{-1}) = T_3 radioactivity on paper chromatogram (dpm) ÷ total serum volume used (ml) ÷ per cent of T_3 recovery × 100. T_3 radioactivity in the body (dpm) = T_3 radioactivity in serum (dpm ml^{-1}) × T_3 volume of distribution (1) × 1,000.

agree with those calculated from equations (2) and (4) in most instances. The other method estimated $[T_4]$ by serially subtracting the urinary and fecal recoveries of ^{14}C from the total L-[tyrosyl-^{14}C] T_4 injected. As seen in Table III, because of the unavoidable loss of specimen during any long term collection, this last method vastly overestimates the amount of L-[tyrosyl-^{14}C] T_4 remaining in the body.

In four of the eight subjects, kinetic studies of both T_4 and T_3 were carried out. The results are shown in Table IV. The results from the T_4 studies showed a mean half-time ($t_{\frac{1}{2}}$) of the disappearance of ^{14}C from serum, 5.62 ±1.21 days (mean ±SD). The mean fractional turnover-rate (K) was 12.93 ±2.62% per day.

TABLE III

Estimated L-[Tyrosyl-^{14}C] T_4 in the Body, [T_4], DPM × 10^5

Subject	Experiment	Method 1	Method 2	Method 3
J. McD.	1	120.8	111.3	188.0
	2	84.9	121.6	177.1
	3	81.2	105.5	177.1
	4	77.7	104.3	173.8
	5	74.3	66.9	167.0
	6	71.1	77.6	163.8
B. H.	1	220.2	275.4	221.7
	2	111.0	186.6	294.5
	3	103.8	154.4	287.9
	4	97.1	231.3	281.4
	5	90.9	142.0	275.1
	6	85.0	175.7	271.1
	7	79.5	155.6	266.1
	8	74.4	140.9	266.1
	9	65.1	132.8	260.7
D. V.	1	193.8	233.6	202.6
	2	75.1	66.2	255.6
	3	68.2	61.5	250.3
	4	61.1	52.9	238.7
	5	57.9	50.5	231.6
	6	53.1	43.1	220.4
	7	44.6	41.5	217.8
	8	37.5	32.8	206.7
D. D.	1	316.3	413.5	535.1
	2	158.2	484.6	429.2
	3	147.6	158.7	428.4
	4	137.7	164.1	423.2
	5	128.5	153.2	419.8
	6	119.9	156.9	415.2
	7	104.4	191.2	407.3
D. G.	1	86.4	183.0	71.5
J. Mu.	1	250.0	266.7	216.2

Estimation of the amount of injected L-[tyrosyl-^{14}C] T_4 remaining in the body at time t, [T_4], by three different methods. Method 1 used the equations (2) and (4). Method 2 used the serum concentration of L-[tyrosyl-^{14}C] T_4 and the volume of distribution of T_4. Method 3 used subtraction of the ^{14}C recovery from excreta from the total amount of L-[tyrosyl-^{14}C] T_4 injected.

The mean zero time volume of distribution (V_d) was 10.1 ±1.8 liters. The average extrathyroidal pool of T_4 was 635.0 ±130.0 μg (total T_4) which resulted in a mean T_4 production rate of 82.4 ±25.7 μg per day.

In the same four subjects the results of the T_3 kinetic study showed a mean half-time of 0.92 ±0.11 days (mean ±SD). The average fractional turnover-rate of T_3 was 76.12 ±7.16% per day. The average zero time volume of distribution of T_3 was 40.9 ±4.8 liters. The mean extrathyroidal pool of T_3 was 74.8 ±15.8 μg. Lastly, the mean daily production rate of T_3 was 57.4 ±14.7 μg.

Sufficient data were available to calculate the extrathyroidal conversion rate of T_4 to T_3, λ, in six of the subjects. Analyses were carried out in six to nine samples of serum from each subject in the majority of instances. The results of λ calculated by equations (2) and (8) or equations (4) and (9) gave essentially similar results. They were grouped together in Table V. The mean conversion rate from the entire group of six subjects was 4.16 ±1.44% of extrathyroidal T_4 pool per day (mean ±SD), or 33.4 ±11.0% of T_4 production per day. In four of the six subjects the amount of T_3 generated by the pathway of extrathyroidal conversion was calculated with each subject's own kinetics of T_4 and T_3. For the remaining two subjects, the amount of T_3 converted from T_4 was calculated with the mean values obtained from the other four subjects. The results showed that 26.1 ±9.5 μg of thyroxine was metabolized each day by the pathway of extrathyroidal conversion which gave rise to an average of 21.9 ±7.9 μg of T_3. The amount of T_3 thus derived constituted an average of 31.0 ±14.7% of the total extrathyroidal pool of T_3. More important, it constituted an average of 41.2 ±20.9% of the daily production rate of T_3.

DISCUSSION

In the past, in vivo evidence of extrathyroidal conversion of T_4 to T_3 were sought by many studies in animals and in man. Most of these studies employed the technique of administering radiothyroxine in one injection, and then the presence or absence of radioactive T_3 was demonstrated by chromatography. The conclusions of most of these studies were equivocal and controversial (6). This controversy can be explained partly by the kinetics of T_3 itself. As shown by the results of our present study and that reported by other investigators, this hormone has a fractional turnover-rate of 70% per day and a half-time of approximately 1 day (17–20). After multiple injections of L-[tyrosyl-^{14}C] T_4, the ^{14}C labeled T_3 constituted only 1.6% of the total serum radioactivity. Therefore, demonstration of the extrathyroidal conversion of T_4 to T_3 amidst the multiple artifacts inherent of chromatographic techniques is fraught with pitfalls. (*a*) Thyroidal contribution of T_3, (*b*) contamination of the T_4 dose by T_3, (*c*) incomplete separation of the T_4 and T_3 fractions, and (*d*) artifactual production of T_3 during chromatography are some of the pitfalls.

The procedures followed in our present study were adopted to overcome these very pitfalls. The radiothyroxines used in the study were labeled with ^{14}C or ^{3}H rather than with a radioiodine, therefore the thyroidal contribution of T_3 in blood was excluded from our calculation. The L-[tyrosyl-^{14}C] T_4 doses were chromato-

TABLE IV

Kinetic Studies of T_4 and T_3 in the Same Subjects

Patients		$t_{\frac{1}{2}}$	K	V_d	Extra-thyroidal pool	Production rate
		day	*% day^{-1}*	*liter*	*μg*	*μg day^{-1}*
Thyroxine	J. McD.	7.85	8.83	7.5	600.0	53.0
	B. H.	5.19	13.35	12.5	875.0	116.8
	D. V.	3.99	17.35	10.2	663.0	115.0
	D. D.	5.00	13.86	10.0	450.0	62.4
	D. G.	6.30	11.00	10.1*	556.0	61.2
	J. Mu.	5.37	12.91	10.1*	665.0	85.9
	Mean	5.62	12.93	10.1	635.0	82.4
	±SD	1.21	2.62	1.8	130.0	25.7
Triiodo-thyronine	J. McD.	0.86	80.35	44.5	102.4	82.3
	B. H.	0.98	70.50	38.0	60.8	42.9
	D. V.	1.06	65.48	34.5	56.9	37.3
	D. D.	0.79	88.13	46.5	65.1	57.4
	D. G.	0.92*	76.12*	40.9*	77.7	59.2
	J. Mu.	0.92*	76.12*	40.9*	85.9	65.4
	Mean	0.92	76.12	40.9	74.8	57.4
	±SD	0.11	7.16	4.8	15.8	14.7

$t_{\frac{1}{2}}$, the half-time of the radioactivity disappearance from serum. K, the fractional turnover-rate. V_d, zero time volume of distribution. Each mean of the group is shown with its standard deviation.

* No study was carried out for this subject and the value was not included in the mean.

graphed at the beginning and the end of injection period to determine the exact amounts of contamination by ^{14}C-labeled T_3 which was a very small correction in the present study since most of the sera were collected 5 days after the last ^{14}C-T_4 injection. As shown in Fig. 3, when serial blood samples were collected after an injection of D,L-[α,β-^{3}H] T_4, the ^{3}H activity in the T_3 fraction of blood rose with time suggesting that thc formation of ^{3}H-T_3 was primarily due to metabolie events. If the ^{3}H radioactivity in the T_3 fraction was solely due to contamination of the T_4 dose by T_3, the ^{3}H activity should have cleared at the same rate as

TABLE V

The Extrathyroidal Conversion Rates of T_4 to T_3 (λ)

Subjects	Experiments	% T_4 metabolized per day		% T_3 generated per day	
		Extrathyroidal pool	Production rate	Extrathyroidal pool	Production rate
J. McD.	6	3.6 ±0.8	40.8	17.7	22.0
B. H.	9	4.9 ±1.9	36.9	59.3	84.0
D. V.	8	2.5 ±1.0	14.5	24.5	37.4
D. D.	7	6.8 ±1.9	48.8	39.1	44.4
D. G.	1	2.8	25.3	16.6	21.8
J. Mu.	1	4.4	34.4	28.8	37.8
Mean ±SD		4.2 ±1.4	33.4 ±11.0	31.0 ±14.7	41.2 ±20.9

λ, the extrathyroidal conversion rate. The results of the first four subjects were calculated from their own kinetic data (Table IV). The results of the last two subjects were calculated from the mean values of the first four subjects listed in Table IV.

^{14}C-T_3 and the $^3H/^{14}C$ ratios in the T_3 fraction should not have risen with time. The problems of incomplete separation of T_4 and T_3 fractions and the in vitro conversion of T_4 to T_3 are inherent of column and paper chromatographies. The contributions of these artifacts to our ^{14}C-T_3 measurements were partly corrected for by the addition of D,L-[α,β-^3H] T_4 to serum samples in our study. At least part of the ^3H radioactivity in the T_3 fraction was the combined result from the D,L-[α,β-^3H] T_4 that remained in the T_3 fraction and the D,L-[α,β-^3H] T_3 that was formed artifactually. Therefore, the ^3H activity in the T_3 fraction was used to correct for the ^{14}C radioactivity in the T_3 fraction which was due to the contaminating L-[tyrosyl-^{14}C] T_4 and the in vitro production of L-[tyrosyl-^{14}C] T_3. When these corrections were applied, the net ^{14}C radioactivity in the T_3 fraction was never negative in any sample drawn 48 hr after an injection of radiothyroxin. Our results convincingly showed that the pathway of extrathyroidal conversion of T_4 to T_3 is operative in normal man and they support the recent report of Braverman et al (9).

All our serum samples were analyzed by two-dimensional chromatography at the final stage of study. A solvent of hexane: tertiary amyl alcohol: ammonia was used in developing the chromatograms in both directions because this solvent system is particularly suitable in the separation of T_4, T_3, Tetrac, and triiodothyroacetic acid (21). The metabolism of T_4 to Tetrac was observed in animals by many observers before (22–24) and was also observed by Braverman et al. in man (9). Our results agreed with the last report and showed that in a state of near isotopic equilibrium, ^{14}C-labeled Tetrac was found in equal or slightly larger amounts than ^{14}C-labeled T_3. Since the clearance rate of Tetrac was reported to be slower than that of T_3 by some early studies, the higher serum concentrations of ^{14}C-labeled Tetrac could not be interpreted to mean a higher production rate for Tetrac than for T_3 in man (25). Recently, Pittman, Read, Chambers, and Nakafuji (13) administered a mixture of D,L-[α,β-^3H] T_4 and another T_4-labeled with ^{14}C in the phenolic ring to normal subjects. Approximately 50–60% of the radioactivity was eventually recovered from urine after 3 wk. The $^3H/^{14}C$ ratios of the urine were the same as the $^3H/^{14}C$ ratios of the T_4 dose suggesting that the deiodinated metabolites of T_4 retained an intact diphenyl ether structure. Therefore, most likely the T_3 and Tetrac formed from T_4 metabolism are in turn deiodinated before urinary excretion without undergoing cleavage of the diphenyl ether.

Detailed study of the T_4 and T_3 kinetics were carried out in the same individual in four of our subjects. Our study of T_4 showed a mean $t\frac{1}{2}$ of 5.62 days, mean turnover-rate of 12.9% per day, mean zero time volume of distribution of 10.1 liter, mean extrathyroidal thyroxine pool 635 μg (total T_4) and mean daily production 82.4 μg. Despite the fact that our results were obtained from the mean of only four subjects, these values were essentially in agreement with the results published by Berson and Yalow; Ingbar and Freinkel; and Sterling and Chodos (26, 16, 27). Our mean turnover-rate and production rate of T_4 were slightly higher than the values given in literature which can be explained partly by the fact that our subjects were young and healthy and their values were obtained after approximately 70% of the extrathyroidal T_4 pool was equilibrated with the injected ^{14}C-T_4.

Our study of T_3 kinetics showed a mean $t\frac{1}{2}$ of 0.92 per day, a mean fractional turnover-rate of 76.1% per day, a mean zero time volume of distribution 40.9 liter, a mean extrathyroidal T_3 pool of 74.8 μg (total T_3) and a mean production rate of 57.4 μg. The formation of a ^{131}I-labeled protein as a degradation product of injected L-[3′-^{131}I] T_3 was reported only recently (17). Comparison of our results with the earlier reports is difficult but our results are in general agreement with the more recent reports of T_3 kinetics by Surks, Woeber, Nicoloff, and Cavalieri and their respective coworkers (17–20). Again, our values of the turnover-rate are slightly higher. Until recently measurements of stable T_3 were not made widely. The absolute production rate or disposal rate of T_3 were not reported by most investigators. Our value of T_3 production rate agreed with that given by Woeber, Sobel, Ingbar, and Sterling 60 μg per day (18). There are several reported methods for the measurement of stable T_3 in serum employing column and paper chromatographies as well as gas-liquid chromatography (14, 28, 29). The value of T_3 in normal serum by these three methods is well above 200 ng per 100 ml. However, there appeared to be no agreement on the value of stable T_3 in normal serum (30). Apparently no satisfactory correction for chromatographic artifacts as discussed earlier in this paper has been found. The stable serum T_3 in our study was measured by a modified method of Sterling et al. (14) in the Boston Medical Laboratory, Waltham, Mass. Measured by this method the T_3 concentration in normal serum is 150–250 ng per 100 ml which must be taken as an approximation. However, for clinical purposes and in normal subjects this method has been found very reproducible. In the presence of elevated serum T_4, the measurement of T_3 becomes too high partly due to incomplete separation of the T_4 and T_3 fractions. Braverman et al. (9) reported that their athyreotic patients, whose serum T_4 measurements were elevated to 2–3 times the normal value, were eumetabolic despite serum T_3 measurements in the range of 269–680 ng per 100 ml (9). This dis-

crepancy can be explained partly by the fact that in the presence of excessive T_4, the T_4 contamination of the T_3 fraction becomes significant in displacement analysis and falsely elevates the T_3 measurements.

The sera studied were collected from our subjects both during and after the injection period of L-[tyrosyl-^{14}C] T_4. While these different conditions required different methods of calculation, they yielded similar results. Our calculation yielded a mean extrathyroidal conversion rate of T_4 to T_3, 4.16% of the extrathyroidal T_4 per day. More significantly this pathway was shown to metabolize approximately 33% of the T_4 produced daily. Therefore, approximately 22 μg of T_3 was generated from the extrathyroidal conversion of T_4. However the contribution of this 22 μg of T_3 to the over-all production of T_3 is more difficult to assess because of our reservation over the stable T_3 determinations. If these determinations were valid, then extrathyroidal conversion of T_4 to T_3 contributed 41% of the total daily production of T_3.

In conclusion, our study conclusively shows that the pathways of extrathyroidal conversion of T_4 to T_3 and T_4 to Tetrac are operative in normal men. The amount of T_4 metabolized by this pathway accounts for 33% of the total T_4 production and contributes 41% of the total T_3 production each day. Therefore, the extrathyroidal conversion of T_4 to T_3 is a physiologically important pathway in normal man.

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