Simultaneous Measurement of Thyroxine and Triiodothyronine Peripheral Turnover Kinetics in Man

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ABSTRACT Serum triiodothyronine (T_3) kinetics in man have been difficult to define presumably due to the interference of iodoproteins generated during the peripheral metabolism of T_3. The use, in the present study, of an anion-column chromatographic method for separation of serum T_3 as well as thyroxine (T_4) from these iodoproteins has overcome this technical handicap. Simultaneous measurement of serum ^{125}I-T_3 and ^{131}I-T_4 kinetics were performed in 31 subjects from the clinical categories of euthyroid, primary hypothyroid, thyrotoxic and posttreatment hypothyroid Graves' disease, factitial thyrotoxic, and idiopathically high and low thyroxine-binding globulin states. The normal mean T_3 fractional turnover rate (kT_3) was 0.68 (half-life = 1.0 days), increased in toxic Graves' disease patients to 1.10 (half-life = 0.63 days), and decreased in primary hypothyroid patients to 0.50 (half-life = 1.38 days). The mean T_3 equilibration time averaged 22 hr except in hypothyroid and high thyroxine-binding globulin (TBG) patients where the equilibration period was delayed by 10 hr. The mean T_3 distribution space in normal subjects was 38.4 liters. This was reduced in subjects with high TBG levels (26 liters) and increased in patients with low TBG and in all hyperthyroid states (53–55 liters). The normal serum T_3 concentration was estimated by radioimmunoassay to be 0.106 μg/100 ml. Combined with the mean T_3 clearance value of 26.1 liters/day, the calculated T_3 production rate was 27.6 μg/day. The mean T_3 production rate increased to 201 μg/day in thyrotoxic Graves' disease patients and was reduced to 7.6 μg/day in primary hypothyroid subjects. T_3 production rate was normal in subjects with altered TBG states. The ratio of T_3 to T_4 production rate in normal subjects was 0.31 and was unchanged in patients with altered TBG values. This ratio was increased in all Graves' disease patients with the highest value being 0.81 in the posttreatment hypothyroid Graves' disease group. This apparent preferential production of T_3 may have been responsible for the retention of rapid turnover kinetics for T_3 and T_4 observed in treated Graves' disease patients. The finding that factitial thyrotoxic patients also displayed similar rapid T_3 and T_4 turnover kinetics indicates that these alterations are not a unique feature of Graves' disease per se. When comparing the peripheral turnover values for T_3 and T_4 in man, it is apparent that alterations in metabolic status and serum TBG concentration influence both hormones in a parallel manner; however, changes in metabolic status seem to have a greater influence on T_3 kinetics while alterations in TBG concentrations have a greater effect on T_4. These observations probably relate to the differences in TBG binding affinity and peripheral tissue distribution of these two hormones.

INTRODUCTION

Since the introduction of radioactive iodine labeled thyroxine (T_4)[1] as a testing tool in clinical research, numerous studies of T_4 peripheral metabolism have been performed in man (1). By contrast, comparatively few

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[1] *Abbreviations used in this paper:* DS, distribution space; kT_3, triiodothyronine fractional turnover rate; kT_4, thyroxine fractional turnover rate; MCR, metabolic clearance rate; RIA, radioimmunoassay; T_3, triiodothyronine; T_4, thyroxine; TBG, thyroxine-binding globulin; U, urinary.

investigations have dealt with the metabolism of triiodothyronine (T_3), and the information available is variable and at times conflicting. Early estimates of the biological half-life of T_3 in euthyroid human subjects were reported to be greater than 2 days (2), while recently published values have varied between 1.30 and 1.6 days (3–5). This difficulty in accurately assessing T_3 kinetics probably relates to the generation of circulating iodoproteins appearing during T_3 degradation. Surks and Oppenheimer have found that these iodoproteins appear chemically and biologically similar to serum albumin and interfere with the conventional measurements of labeled T_3 in the serum (6). While comparing the peripheral deiodination rates of labeled T_3 and T_4 in man (7), we have observed that the rate of T_3 degradation, measured by assessing the rate of urinary excretion of radioactive label, is more rapid than the values previously cited in the literature. This observation, coupled with the iodoprotein studies of Surks and Oppenheimer (6), stimulated our interest in assessing labeled T_3 and T_4 kinetics in normal subjects and in patients with alterations in thyroid status.

METHODS

The subjects employed in this investigation were from the inpatient and outpatient services of the Los Angeles County–University of Southern California Medical Center. Subject classification was established by clinical examination and conventional thyroid testing (see Table 1). The eight euthyroid control subjects were either normal volunteers or patients with mild nonthyroidal illnesses such as duodenal ulcer or mild exogenous obesity. The six patients with primary hypothyroidism had spontaneous thyroid failure as adults. The thyrotoxic Graves' disease group was comprised of seven subjects all manifesting classic signs and symptoms of hyperthyroidism. Subjects were selected who displayed a variety of serum T_4 values including patients No. 6 and No. 7 who had normal serum total and free thyroxine determinations. None of the patients had been taking an antithyroid drug (methimazole) for more than 1 wk before the time of the study. The three patients with hypothyroid Graves' disease developed their hypothyroidism as a result of inadvertent overtreatment with methimazole; they had been hypothyroid for a period of 2–3 months before study and had developed gross myxedema. The three patients with factitial thyrotoxicosis had been ingesting thyroid hormone in an effort to control mild exogenous obesity and/or mental depression. Subject 1 in this group had been taking 0.9 mg L-thyroxine daily, while subjects 2 and 3 were each ingesting 9 gr of desiccated thyroid daily. In each instance, these doses of thyroid hormone had been maintained for periods in excess of 1 yr. The patients with idiopathically high and low TBG values were clinically euthyroid and in good health.

Pulse T_3 and T_4 tracer studies. The thyroid iodine uptake was blocked in all euthyroid and hypothyroid subjects by the administration twice daily of 5 drops of a saturated solution of potassium iodide. In addition to receiving potassium iodide, hyperthyroid subjects received 30–60 mg of methimazole in divided daily doses. Serum was drawn for stable T_3, T_4, and free T_4 determinations before the institutions of these drugs. After establishing a thyroid blockade, 30–50 μCi of ^{131}I-T_4 were given intravenously to initiate the study. Timed serum samples were collected twice daily for the next 7 days to measure T_4 disposal rates. 2–4 days after the administration of the T_4 tracer, a pulse dose of 40–100 μCi of ^{125}I-T_3 was administered intravenously. Beginning 16–20 hr later, serial serum samples were drawn at 1- to 2-hr intervals over a 24 to 36 hr period. In addition, serial timed urine samples were collected at approximately 2-hr intervals until the completion of the study. The ^{125}I-T_3 and ^{131}I-T_4 tracers were obtained from Industrial Nuclear Co., St. Louis, Mo.; specific activities were greater than 30 μCi/μg at time of injection. The purity of the radioactive tracers was verified before their administration employing a descending chromatographic paper system utilizing amyl alcohol, 2 N NH_3. The labeled tracers were more than 95% pure with the majority of the contaminants being labeled iodide. The contaminating iodide was subsequently removed during the processing of the serum samples and standards and therefore did not influence the final results.

Processing of serum samples. Serum ^{125}I-T_3 and ^{131}I-T_4 were separated from the nonthyronine labeled materials using a 23 × 0.8 cm glass column containing 26 mm of Dowex (Dow Chemical Co., Midland, Mich.) 1–2 X anion exchange resin, 100–200 mesh, acetate cycle (Curtis Nuclear Corporation, Los Angeles, Calif.). Any slow draining columns were replaced, as uniform draining time was essential to obtain reproducible results. Serum samples of 1 ml each were pipetted into three separate test tubes and 5 ml of 1.0 N NaOH were pipetted into each tube at 2-min intervals. After 5 min of incubation, each sample was poured into the anion exchange column; each tube was rinsed with approximately 1 ml distilled water which also was poured into the column. After the column had been allowed to drain, the second and third test tubes were poured into the same column in a similar manner. Thus, three successive serum samples were applied to each column. The columns were then washed successively with 1% acetic acid, three times with 15% acetic acid, and finally by 0.8 ml of glacial acetic acid and all eluates discarded. Then, 3 ml of 59% acetic acid were added to the column, the eluate collected in a counting tube, and the ^{131}I and ^{125}I activities were determined in an automatic well-type scintillation counter employing a dual channel spectrometer (Baird-Atomic, Inc., Cambridge, Mass.). Initial washings of the column with 1% and 15% acetic acid served to eliminate contaminating iodoproteins from the test samples. When a serum sample containing ^{131}I labeled albumin was passed through the same procedure, no ^{131}I activity was measured in the thyronine fraction. Additionally, when a serum sample containing only ^{131}I-iodide was used, less than 1% appeared in the thyronine fraction. Using this procedure, the average recovery for a single run was 58.1 ±1.6% (±SD) for ^{125}I-T_3 and 54.7 ±2.1% for ^{131}I-T_4. Appropriate ^{125}I-T_3 and ^{131}I-T_4 standards were prepared in pooled unlabeled serum to approximate the same level of activity as that of the test samples and were processed in a similar manner. All serum samples from study subjects were processed in one run in an effort to eliminate the interassay variability. The activities of the ^{131}I and ^{125}I were expressed in terms of per cent of the injected dose per liter and plotted against time on semilogarithmic coordinates. Calculations of the fractional turnover rates, distribution spaces, clearances, and production rates of T_4 and T_3 were performed as described by Sterling and Chodos (8).

Processing of urine sample. Each urine sample was col-

lected in a 250 ml polypropylene bottle containing 3 ml RAI 400 anion exchange resin, chloride cycle, 20–50 mesh (Mallinckrodt Chemical Works, St. Louis, Mo.). The urine was incubated in resin for 24 hr at room temperature to facilitate the uptake of labeled iodide on the resin. Each sample was decanted and the residual resin was transferred to a glass counting vial and counted in a well-type scintillation counter employing a dual channel spectrometer (Baird-Atomic, Inc., Cambridge, Mass.). Net counts for each isotope were expressed as a ratio of $^{125}I/^{131}I$ and plotted on semilogarithmic coordinates against time.

Metabolic clearance by constant infusion technique. In five subjects, after completion of the T_4 and T_3 pulse tracer studies, the metabolic clearance of T_3 was measured by techniques similar to those described by Tait and Burstein for steroids (9). A constant infusion consisting of 1 liter of 0.9% sterile saline solution, to which 25 μCi ^{125}I-T_3 and 10 μCi ^{131}I had been added, was administered through an indwelling polyethylene catheter or pediatric scalp vein needle into a peripheral arm vein. Human serum albumin was incorporated into the solution to a final concentration of 0.5% in order to prevent adsorption of the isotopes to the glassware and intravenous tubing. The infusion rate was approximately 2 ml/hr. A pulse loading dose of ^{125}I-T_3, equal in radioactivity to 48 hr of the infusion, and ^{131}I, equal in radioactivity to 8 hr of the infusion, was given to expedite tracer equilibration. The constant infusion system employed was a portable roller-type pump (Holter R.D. 044, Holter Company, Bridgeport, Pa.). Isotopic equilibrium was determined by measuring the ratio of ^{125}I to ^{131}I in sequential serum and urine samples; when the serum and urinary $^{125}I/^{131}I$ ratio values became constant in three consecutive hourly samples, isotopic equilibration was assumed to have occurred. Generally this was observed after 14–24 hr of infusion. The subjects remained supine except when voiding urine samples.

Other laboratory studies performed. Thyroxine iodine by column and "free" thyroxine determinations were performed by Bio-Science Laboratories, Van Nuys, Calif. The maximal binding capacity of TBG was measured by the paper electrophoretic technique described by Ingbar (10). Total stable serum T_3 concentrations were measured by a radioimmunoassay (RIA) method as described by Chopra, Solomon, and Beall (11). All serum T_3 determinations were performed without the knowledge of the patient source. Statistical analysis of the data was performed by a standard *t* test for nonpaired groups of unequal size.

RESULTS

Serum T_3 and T_4 kinetic data. Fig. 1 illustrates representative examples of serum ^{125}I-T_3 disappearance slopes which were observed in euthyroid subjects and in patients with primary hypothyroidism and thyrotoxic Graves' disease. When plotted on semilogarithmic coordinates, radioactivity data from unextracted serum samples produced a nonlinear and uniformly more shallow disappearance slope than was observed in the corresponding extracted samples. Some of the ^{125}I activity lost in the extraction procedure was ^{125}I-iodide. However, since iodides possess a shorter biological half-life than T_3, the nonparallelism between the slopes

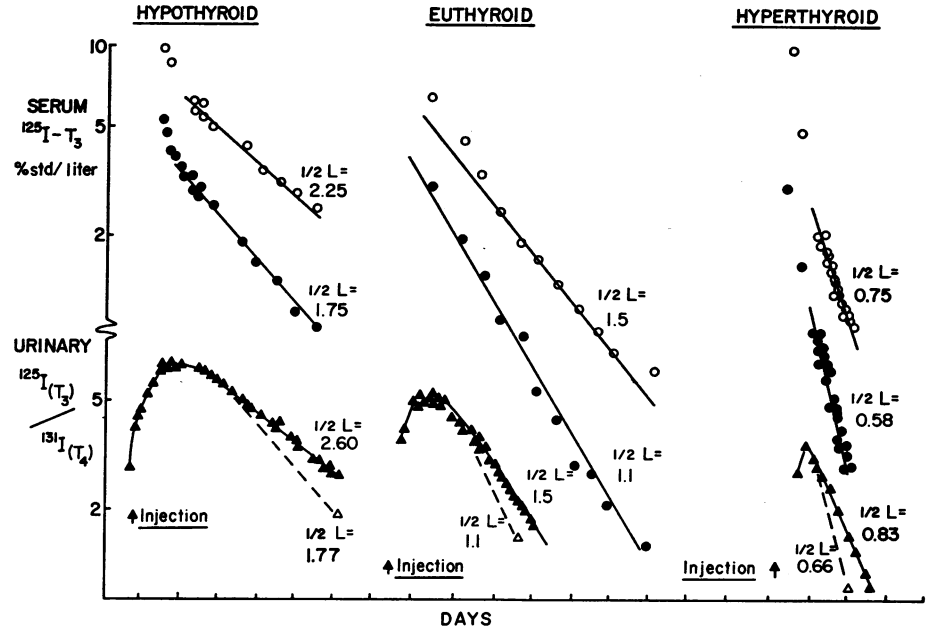

FIGURE 1 The whole serum (open circles) and extracted serum (closed circles) ^{125}I-T_3 activities are plotted for representative hypothyroid, euthyroid, and hyperthyroid subjects. The injection of the ^{125}I-T_3 tracer is denoted by the arrows. The closed triangles in the lower portion of the illustration represent the plot of urinary $^{125}I/^{131}I$ values and the dotted lines and open triangles represent the ^{125}I-T_3 disappearance slope based on urinary isotope calculation. ($\frac{1}{2}$ L = half-life in days.)

TABLE I

The Kinetics of Triiodothyronine and

Subject	Age	Height	Weight	Serum thyroxine		Thyroxine-binding globulin capacity	Serum triiodothyronine	Triiodothyronine equilibration time
				Total	Free			
	yr	*cm*	*kg*	*μg/100 ml*	*ng/100 ml*	*μg/100 ml*	*ng/100 ml*	*hr*
Normal								
1	58	178	52	4.4	1.4	14.3	55	24
2	32	178	70	4.5	1.4	21.3	122	22
3	25	179	112	3.5	1.2	—	—	17
4	35	179	76	4.2	1.5	20.4	120	23
5	51	163	75	2.9	1.1	19.8	188	27
6	63	148	68	6.8	1.8	33.3	85	23
7	54	168	57	5.0	1.1	28.8	105	16
8	54	173	77	3.8	1.7	20.8	67	24
Mean	46.5	171	73.3	4.4	1.4	22.7	106	22
±SE	4.9	3.9	6.4	0.4	0.1	2.4	16.7	1.3
Primary hypothyroid								
1	63	163	81	0.8	0.4	28.8	—	32
2	51	165	88	2.2	0.6	20.1	52	26
3	57	173	75	0.8	0.3	28.1	50	34
4	45	160	55	0.5	0.3	20.5	43	46
5	43	152	45	0.7	0.2	20.0	30	20
6	54	150	80	0.5	0.1	15.4	30	39
Mean	52.2	161	70.6	0.9	0.3	22.2	41	33
±SE	3.1	3.5	6.9	0.3	0.1	2.1	4.7	3.8
P‖	—	—	—	0.1	0.1	0.9	0.005	0.02
Graves' disease Thyrotoxic								
1	36	165	54	15.4	8.6	22	669	31
2	25	160	51	8.0	4.6	18	413	17
3	19	163	65	7.9	2.9	20	240	16
4	25	168	59	7.8	2.7	18	225	25
5	30	160	60	6.1	2.3	28	185	16
6	32	158	50	3.7	1.6	15	438	—
7	26	165	70	3.9	1.4	18	138	28
Mean	27.6	163	58	7.5	3.4	20	330	22
±SE	2.1	1.3	2.8	1.48	0.95	1.6	70.8	2.7
P‖				<0.1	<0.1	<0.4	<0.01	>0.9
Hypothyroid								
1	35	158	62	1.9	0.9	32	—	32
2	60	142	53	0.9	0.5	16	75	16
3	40	168	59	0.9	0.4	22	75	22
Mean	45	156	58	1.2	0.6	23	75	23
±SE	7.6	7.6	2.7	0.3	0.2	4.7	0	4.7
P‖				<0.01	<0.01	<0.9	<0.2	<0.9

* kT_3 and kT_4 equal the fractional turnover rate values for T_3 and T_4 measured in the serum. $^{Uk}T_3$ and $^{Uk}T_4$ represent these same values but measured as the urinary appearance rate of iodide derived from the deiodination of T_3 and T_4.

‡ T_3MCR, Metabolic clearance rate of T_3 determined by constant infusion.

§ Thyroxine iodine values multiplied by 1.53 to give total hormone when calculating thyroxine disposal rate.

‖ *P* value refers to the significance of the difference compared to normal group.

Fractional turnover rates*				Distribution space		Clearance rate			Production rate	
kT_4	kT_3	$_{UK}T_3$	$_{UK}T_3/T_4$	T_4	T_3	T_4	T_3	T_4‡ MCR	T_4§	T_3
%/24 hr				liters		liters/24 hr			μg/24 hr	
11.7	88	87	75	11.6	37.2	1.36	32.7	—	91.5	18.0
12.6	77	78	65	12.6	40.0	1.59	30.8	—	109.5	37.6
10.5	63	58	46	11.9	36.0	1.25	22.7	—	67.0	—
14.4	69	71	60	10.8	40.5	1.56	27.9	28.0	100.2	33.5
9.6	59	59	50	9.1	38.2	0.87	22.5	28.1	38.6	42.3
9.4	60	63	48	15.0	35.0	1.41	21.0	—	146.7	17.9
9.3	69	69	58	10.8	38.7	1.00	26.3	—	76.5	27.6
11.9	58	53	42	11.3	41.7	1.34	24.2	—	77.9	16.2
11.2	67.9	67.3	55.5	11.6	38.4	1.30	26.1	—	88.5	27.6
0.6	3.7	4.0	3.9	0.6	0.8	0.09	1.5	—	11.5	3.99
9.5	43	36	26	23.8	38.3	2.26	16.5	—	27.7	—
7.2	41	50	43	17.5	38.0	1.26	15.6	13.0	42.4	8.11
8.1	40	35	27	11.8	41.7	0.96	16.7	16.3	11.8	8.35
8.5	66	54	45	8.8	33.3	0.75	22.0	—	5.74	9.46
11.3	63	53	42	14.9	35.3	1.68	22.2	—	18.1	6.66
10.5	46	39	29	11.5	39.2	1.21	18.0	—	9.26	5.40
9.2	50	45	35	14.7	37.6	1.35	18.5	—	19.2	7.60
0.6	4.7	3.6	3.6	2.2	1.2	0.22	1.2	—	5.6	0.71
0.05	0.01	0.01	0.01	0.2	0.6	0.9	0.01	—	0.01	0.001
17.3	119	86	69	9.5	45.5	1.64	54	47	386	361
26.9	119	111	84	17.4	90.9	4.68	108	—	573	446
18.5	84	111	92	12.0	60.0	2.22	50	—	268	120
13.9	95	83	69	13.9	42.0	1.93	40	—	230	90
27.0	138	125	92	17.4	40.0	4.70	55	—	439	102
21.9	84	74	52	12.1	53.0	2.65	45	—	150	197
21.6	131	105	83	9.6	49.3	2.07	65	—	124	89.7
21.0	110	99.3	77.3	13.1	54.4	2.84	60	—	310	201
1.84	8.4	7.0	5.5	1.24	6.6	0.49	8.6	—	61.7	54.9
<0.01	<0.01	<0.01	<0.01	<0.3	<0.05	<0.01	<0.01	—	<0.01	<0.01
10.2	49	54	44	11.3	53.0	1.15	26	—	33.5	—
13.0	51	63	50	7.4	30.1	0.96	15	—	13.2	11.3
12.4	72	53	41	9.7	42.5	1.20	31	—	16.5	23.3
11.9	57.3	56.7	45	9.5	41.9	1.10	24	—	21.1	17.3
0.9	7.4	3.2	2.6	1.1	6.6	0.07	4.7	—	6.3	6.0
<0.6	<0.3	<0.1	<0.1	<0.2	<0.7	<0.2	<0.7	—	<0.01	<0.2

TABLE I—

Subject	Age	Height	Weight	Serum thyroxine		Thyroxine-binding globulin capacity	Serum triiodo thyroninr	Triiodothyronine equilibration time
				Total	Free			
	yr	*cm*	*kg*	*μg/100 ml*	*ng/100 ml*	*μg/100 ml*	*ng/100 ml*	hr
Factitial hyperthyroid								
1	25	178	77	10.3	4.8	27	—	24
2	45	163	65	8.7	3.6	—	128	19
3	41	173	77	8.0	3.6	29	285	25
Mean	37	171	73	9.0	4.0	28	207	23
±SE	6.1	4.4	4	0.7	0.4	—	78.5	1.9
$P\|\|$				<0.01	<0.01	—	<0.3	<0.8
Idiopathic elevated thyroxine-binding globulin								
1	67	162	91	8.4	1.7	41	288	29
2	46	168	125	6.8	1.7	37	150	31
3	43	152	45	8.4	2.0	57	110	26
4	38	173	59	5.5	1.5	43	120	43
Mean	49	164	80	7.3	1.7	44.5	167	32
±SE	6.4	4.5	18	0.7	0.1	4.4	41.2	3.5
$P\|\|$				0.01	0.05	<0.01	0.3	0.05
Idiopathic low thyroxine-binding globulin								
1	60	163	50	0.9	1.6	2	40	29
2	53	178	70	1.0	1.0	10	40	16

of the data plotted from nonextracted and extracted serum could not be explained solely on this basis. The generation of ^{125}I labeled iodoproteins from T_3 would more likely account for this flattening of the disappearance curve of the unextracted sera (6). The linearity observed in the extracted ^{125}I-T_3 serum slope suggests that such contamination had been effectively eliminated by column extraction.

In the euthyroid subjects, the daily fractional turnover rate for T_3 (kT_3) in the extracted serum was 67.9%. In the primary hypothyroid group, kT_3 decreased to 49.8%, while in the thyrotoxic Graves' and factitial hyperthyroid groups kT_3 increased to 110 and 98.3%, respectively. Insignificant changes in kT_3 were seen in the hypothyroid Graves' disease patients and subjects with idiopathic alterations in TBG. These findings are consistent with the conclusion that kT3 is affected by alterations in metabolic status, independent of changes in circulating TBG values. In these same subjects, kT_4 was affected similarly by alterations in metabolic status, but kT_4 was also altered by changes in serum TBG levels.

Analysis of urinary $^{125}I/^{131}I$ turnover kinetics. Representative samples of the urinary $^{125}I/^{131}I$ ratio plots are shown in Figs. 1 and 2. Since ^{125}I-T_3 and ^{131}I-T_4 normally are excluded from the urine, measurement of the urinary $^{125}I/^{131}I$ ratio reflects the deiodination of the precursor labeled hormones, namely, ^{125}I-T_3 and ^{131}I-T_4 (12).

The slope described by the urinary $^{125}I/^{131}I$ values after injection of ^{125}I-T_3 can be divided into three phases. The first phase describes the equilibration of ^{125}I-T_3 in the extrathyroidal T_3 pool. This phase was characterized by a rapid increase in the $^{125}I/^{131}I$ urinary values. The point at which the urinary $^{125}I/^{131}I$ values formed a linear exponential slope can be taken as the time when the T_3 tracer had achieved full equilibration; this time interval was observed to be 22 hr in euthyroid subjects. It was not significantly altered in any of the study groups except in those patients with high TBG levels and patients with primary hypothyroidism; in these groups T_3 equilibrium was prolonged for approximately 10 hr beyond the normal control values.

The second phase was marked by the $^{125}I/^{131}I$ urinary ratio values forming a linear slope on semilogarithmic coordinates (as illustrated in Figs. 1 and 2). Since this slope ($^{Ur}T_3/T_4$) represented the ratio of the fractional turnover rates of the labeled precursor hormones (i.e., ^{125}I-T_3 and ^{131}I-T_4), it was possible to mathematically derive the fractional turnover rate of serum ^{125}I-T_3 by the following analysis:

Assuming that the urinary $^{125}I/^{131}I$ slope was the result of two declining exponential functions, the mathemati-

(*Continued*)

Fractional turnover rates*				Distribution space		Clearance rate			Production rate	
kT_4	kT_3	$^{Uk}T_3$	$^{Uk}T_3/T_4$	T_4	T_3	T_4	T_3	T_3‡ MCR	T_4§	T_3
%/24 hr				liters		liters/24 hr			μg/24 hr	
15.1	93	75	60	11.8	51	1.78	47	—	280	—
15.2	83	84	69	12.0	59	1.82	49	—	242	62.7
16.1	119	106	90	16.4	50	2.64	60	—	323	171
15.5	98.3	88.3	73	13.4	53	2.08	52	—	282	117
0.3	10.7	9.2	8.9	1.5	2.8	0.28	4	—	23	54
<0.01	<0.05	<0.1	<0.2	<0.3	<0.01	<0.05	<0.01	—	<0.01	<0.2
7.1	64	62	55	7.9	19	0.56	12	—	72	34.6
8.0	50	49	41	7.9	30	0.63	15	—	66	22.5
6.8	69	66	59	8.6	27	0.58	19	—	75	20.9
9.2	59	55	46	7.8	28	0.72	17	—	61	20.4
7.8	60.5	58	50	8.1	26	0.62	16	—	68	24.6
0.5	4.1	3.8	4.1	0.2	2.4	0.04	1.5	—	3.1	3.4
<0.01	<0.3	<0.2	<0.4	<0.01	<0.01	<0.01	<0.1	—	<0.2	<0.6
28.9	82	66	37	13.1	55	3.79	45	—	52	18
23.7	75	64	40	21.9	74	5.19	56	—	64	22

cal expression for the ratio of two different equations can be written:

$$(1)\qquad \frac{^{125}I}{^{131}I} = \frac{A_1 e^{-^{Uk}T_3 t}}{A_2 e^{-^{Uk}T_4 t}} = A_3 e^{-^{Uk}T_3/T_4 t},$$

where ^{125}I and ^{131}I represent urinary ^{125}I and ^{131}I values at any time t; A_1, A_2, and A_3 are constants, $^{Uk}T_3$ and $^{Uk}T_4$ are the urinary fractional turnover rates for ^{125}I-T_3 and ^{131}I-T_4, respectively. Thus:

$$(2)\qquad -(^{Uk}T_3 - {}^{Uk}T_3) = {}^{Uk}T_3/T_4,$$

$$(3)\qquad {}^{Uk}T_4 + {}^{Uk}T_3/T_4 = {}^{Uk}T_3 = {}^{k}T_3,$$

where $^{Uk}T_3/T_4$ can be obtained directly from the urinary ratio slope and $^{Uk}T_4$ can be assumed to equal the fractional turnover rate of T_4 measured in serum (kT_4). As seen in Fig. 1 and Table I, $^{Uk}T_3$ values closely correlated with ($r = 0.91$, $P < 0.001$) the corresponding serum kT_3 measurements. This served to verify the accuracy of the direct serum kT_3 measurements.

Although kT_3 and $^{Uk}T_3$ were similar, it is of interest that $^{Uk}T_3$ values were generally less than the corresponding serum kT_3 determinations. This difference, which averaged 7.3% in all of the study groups, was found to be significant on paired t test ($P < 0.001$). It probably can be accounted for, in part, by the distorting effect of ^{125}I iodoproteins produced from the labeled T_3 (6). Assuming that the fraction degradation rate of the labeled iodoprotein is much less than that of labeled triiodothyronine, it would be expected that gross alterations in urinary $^{125}I/^{131}I$ ratio slope values would not be seen until the majority of the injected ^{125}I-T_3 tracer had disappeared. Indeed, a loss of linearity of the urinary slope values was not observed until 5–10 days after the injection of ^{125}I-T_3 tracer which denoted the beginning of the third phase.

The $^{Uk}T_3/T_4$ value in the second phase also provided an index of the relative fractional turnover rates of ^{125}I-T_3, as compared to ^{131}I-T_4. A marked increase in this ratio value was noted in the factitial hyperthyroid and toxic Graves' disease groups, while lower values were evident in the patients with primary hypothyroidism and those with idiopathically low TBG levels. A rise in the $^{Uk}T_3/T_4$ value would indicate that the change in fractional turnover rate for T_3 was greater than that for T_4. It is apparent from Table I and Fig. 2 that hyperthyroidism accelerates T_3 degradation to a greater degree than T_4 and that the reverse is true in hypothyroidism. An exception was the decrease in the $^{Uk}T_3/T_4$ values seen in the idiopathic low TBG group which resulted from an increase in T_4 degradation rather than a decrease in the T_3 degradation.

In the third phase, the urinary ratio values were observed to become fixed or to rise with time. This

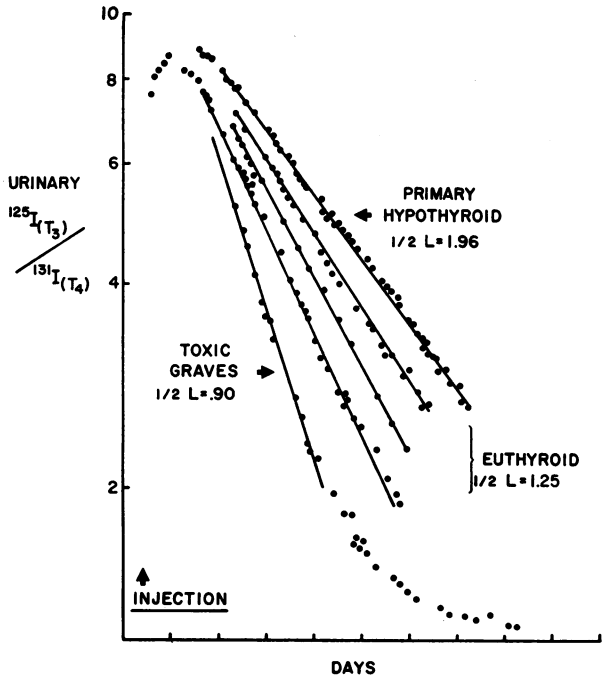

FIGURE 2 Urinary $^{125}I/^{131}I$ values are plotted after the injection of ^{125}I-T_3 in euthyroid subjects and in representative patients with thyrotoxic Graves' disease or primary hypothyroidism. ($\frac{1}{2}$ L = half-life in days.)

indicated that the precursor to the ^{125}I-iodide in the urine possessed a biological half-life greater than that of ^{131}I-T_4 or, in other words, greater than 7 days on the average. This would be consistent with the estimated biological half-life of 12–15 days for the albumin-like labeled material produced as a by-product of T_3 degradation (6).

Distribution space of T_3 (T_3 DS) and T_4 (T_4 DS). In euthyroid subjects T_3 DS was 38.4 ±0.8 liters and the T_4 DS was 11.6 ±0.6 liters (±SEM). The T_3 DS was increased to 64.5 ±9.5 liters in the low TBG group and to 54.4 ±6.6 liters in the thyrotoxic Graves' disease patients, while it was reduced to 26.0 ±2.4 liters in subjects with elevated TBG values. The T_3 DS was not significantly altered in the other study groups. It should be noted that the TBG levels in hypothyroid patients were not significantly different from those seen in the control group. The T_4 DS generally paralleled the alterations in T_3 DS in the various clinical states studied, but the changes were small and with the exception of subjects with elevated TBG levels, were not statistically significant.

The ratio of T_3 DS/T_4 DS was 3.31 in the euthyroid group and was not changed in subjects with altered TBG levels. There was a tendency in hyper- and hypothyroid states for respective increases and decreases in this ratio value to occur (4.15 in hyperthyroid Graves', 2.55 in primary hypothyroid subjects, $P < 0.05$). Thus, it appears that alterations in circulating TBG levels similarly affect the distribution spaces for T_3 and T_4, while changes in metabolic state alter T_3 DS to a greater extent than T_4 DS. Additionally, an increased T_3 DS/T_4 DS ratio of 4.41 was observed in hypothyroid Graves' disease subjects.

T_3 and T_4 clearances. In the euthyroid group, T_3 clearance was found to be 26.1 ±1.5 liters and T_4 clearance to be 1.3 ±0.09 liters/day. In thyrotoxicosis, T_3 and T_4 clearances were both significantly increased to 60 and 2.8 liters and the converse of 18.5 and 1.35 liters was present in the hypothyroid patients. In the group with elevated TBG values, T_3 and T_4 clearances were decreased to 0.62, while they were markedly increased in two subjects with low TBG levels.

Metabolic clearance rate determinations. In five study subjects (two controls, two hypothyroid, and one hyperthyroid patient), T_3 metabolic clearance rate was determined by employing a constant infusion of ^{125}I-T_3. Generally, there was excellent correlation ($r = 0.96$, $P < 0.01$) between the values as determined by the pulse tracer technique and the constant infusion method (Table I).

Hormonal production. In the euthyroid control group, daily blood production rates were 28 μg for T_3 and 88 μg for T_4. As might be expected, these values were not altered in euthyroid subjects with idiopathically high or low TBG values. In contrast, a 3½-fold increase in T_4 and over a 7-fold increase in T_3 production rate was found in the thyrotoxic Graves' disease patients which gave a ratio of T_3 to T_4 production of 0.64 ($P < 0.05$). This preferential T_3 production was seen most prominently in the hypometabolic Graves' disease patients where the T_3 to T_4 production ratio was increased to 0.81. In the primary hypothyroid group, there was a 4-fold decrease in both T_3 and T_4 production rates.

DISCUSSION

The method for measurement of serum T_3 kinetics described in this study appears to combine both technical simplicity and accuracy. Although solvent extraction methods (6, 12) could have been employed, the anion exchange column system proved to be less time consuming and more reproducible to cleanly separate labeled iodoproteins and iodothyronines. Substantiation that the column method achieved this goal was revealed by the following findings: (*a*) serum ^{125}I-T_3 disappearance curves were linear when plotted on semilogarithmic coordinates (Figs. 1 and 2); (*b*) the mathematical analysis of urinary $^{125}I/^{131}I$ values verified the accuracy of the serum T_3 turnover measurements; (*c*) studies of the metabolic clearance rate (MCR) of T_3 by con-

stant infusion closely approximated the results obtained by pulse T_3 kinetic studies.

The fractional turnover rates observed for T_3 in this study substantially differed from those reported by Woeber, Sobel, Ingbar, and Sterling (5) in hyperthyroidism and by Zaninovich, Volpe, and Ezrin in subjects with altered TBG states (4). Either the failure to appreciate (4), or adequately compensate for (5), the presence of iodoproteins formed from T_3 degradation may have been responsible for these differences. With the exception of the limited data reported by Surks and Oppenheimer (6), it is evident that all previously reported labeled T_3 disappearance curves, whether in serum (2–5, 13–15) or in the whole body studies (16), suffer from the same technical problem of failure to eliminate the influence of iodoproteins.

Estimates of T_3 distribution space (T_3 DS) may be in error since the single compartmental model system used in this study assumes that T_3 disposal during equilibration is the same as after equilibration. The observed rise in urinary $^{125}I/^{131}I$ ratio values during the equilibration phase (Figs. 1 and 2) indicated that T_3 deiodination was substantially less during than after equilibration. Since deiodination constitutes the major route of degradation for T_3, this would result in an underestimation of T_3 disposal during the equilibration and, in turn, would cause an underestimation of T_3 DS. On the other hand, the serum T_3 disappearance slope during the equilibration phase may reflect the clearance of the T_3 tracer, and this must be considered in calculating MCR. This error can be compensated for by using a two compartmental model (9). An apparent 20% overestimation of T_3 DS would result in normal subjects if a single rather than a two compartmental model system were used (15).

In spite of these potential shortcomings, the magnitude of error in calculating MCR using the single compartmental model method would not appear to be great. Similar MCR values were obtained in five of our subjects by the constant infusion method which does not suffer from these technical handicaps. Moreover, Cavalieri, Steinberg, and Searle (17) have recently presented values for T_3 MCR using the constant infusion method in normal and Graves' disease subjects which closely approximated the values seen in our patient population. Their T_3 MCR values were 26.0 liters/day in euthyroid and 52.3 liters/day in toxic Graves' disease subjects while our values were 26.1 liters/day and 60.0 liters/day, respectively. The reason that the single compartmental model model appears to satisfactorily approximate T_3 clearance is that the loss of the T_3 tracer during the equilibration phase appears to be relatively small until the tracer approaches its ultimate distribution volume. In other words, the rapid equilibrating compartments do not represent major sites for T_3 disposal.

Comparison of T_3 and T_4 kinetics revealed differences as well as similarities in peripheral metabolism. It was observed that kT_3 and kT_4 were altered in a parallel manner by changes in metabolic rate and TBG levels, but that alterations in metabolic status seemed to influence kT_3 to a greater extent than kT_4, while changes in TBG altered kT_4 to a greater degree than kT_3. Since T_3 and T_4 appear to be bound by TBG extracellularly, it is fair to assume that the extracellular distribution space for T_3 is equal to that of T_4, or about 5 liters (18). Thus, only about 15% of the entire extrathyroidal T_3 pool would appear to be extracellular. It is not surprising, therefore, that T_3 is affected by changes in metabolic status since it is predominantly an intracellular hormone. On the other hand, approximately 50% of the T_4 is in the extracellular fluid compartment bound to TBG (18), and it is equally logical that TBG alterations will influence kT_4 to a greater degree than kT_3. Therefore, one may conclude that the differences in the magnitude of change in kT_3 and kT_4 observed in the various study groups are best explained by the differences in the extrathyroidal distribution of these two hormones. Oppenheimer, Schwartz, Shapiro, Bernstein, and Surks have come to essentially the same conclusions from the study of T_3 and T_4 peripheral metabolism in four euthyroid subjects (19).

However, several other aspects of T_3 and T_4 peripheral metabolism are less clear. For instance, why is the T_3 distribution space 3½ times greater than that for T_4? Since the extracellular binding for T_3 and T_4 are predominantly to TBG and the intrahepatic distribution space for T_4 is estimated to be greater than that for T_3 (20), this difference is even more puzzling. Additionally, why was T_3 equilibration delayed as long as 22 hr in euthyroid subjects? Presumably this relates to the slow entrance of T_3 into the extrahepatic intracellular compartment. As has been observed by Cavalieri, Steinberg, and Searle (20), the egress of T_3 into this compartment is quite slow and, as we confirmed in the present study, is not altered by hypermetabolic states or by decreases in circulating TBG concentrations. Thus, it would appear that future investigation will be necessary to solve these puzzling observations.

The measurement of T_3 concentration in the serum has been technically difficult and still must be considered an area of controversial investigation (21–27). It would appear that the values previously reported by the method of Sterling, Bellabarba, Newman, and Brenner may be erroneously high (23). We have recently developed a double-column chromatographic method for measurement of serum T_3 concentration which allowed correction for some of the methodological artifacts, particu-

larly the monodeiodination of T_4 to T_3 (28). This has provided a more accurate assessment of serum T_3 concentration, but the correction factors are large and the results are, therefore, subject to some overcorrection, particularly at low serum T_3 levels. The recent development of a radioimmunoassay method for measurement of serum T_3 in unextracted serum would therefore appear to represent a substantial methodological improvement (11).

The apparently inappropriately high kT_3 and kT_4 values found in Graves' disease subjects with normal and subnormal T_4 values, requires some further clarification. A high kT_4 value relative to metabolic status in patients with treated Graves' disease was initially described by Ingbar and Freinkel (29). Subsequent investigations have substantiated this observation and have indicated that an augmentation in hepatic T_4 incorporation and degradation are probably responsible for the elevated kT_4 values (30, 31). Recently, Schussler and Vance (32) and Farmer, Smitherman, Beschi, and Pittman (33) have demonstrated that T_3 administration to euthyroid subjects, in replacement or subreplacement doses, is capable of increasing kT_4, implying that T_3 is capable of increasing the rate of T_4 degradation. Additionally, Sterling and coworkers have reported elevated serum T_3 values in treated Graves' disease subjects in whom serum T_4 values have returned to normal or hypothyroid levels (23). In the present study the following observations would appear to be relevant: (*a*) increases in the T_3 DS/T_4 DS and T_3/T_4 production ratios were found in the hypothyroid Graves' disease group; (*b*) a positive correlation between T_3 production rate and kT_4 was observed ($r = 0.72$, $P < 0.001$) when excluding altered TBG states; (*c*) two thyrotoxic Graves' disease patients, who displayed normal free and total T_4 values with elevated serum T_3 levels, had rapid T_3 and T_4 kinetics similar to those of the remainder of the patients with thyrotoxic Graves' disease; (*d*) patients with factitial thyrotoxicosis evidenced the same kinetic changes for T_3 and T_4 as were observed in the thyrotoxic Graves' disease group. Thus, it would appear that the presence of a large fractional turnover rate for T_4 in treated Graves' disease patients may not represent, as previously speculated, an expression of "an integral part of this disorder per se" (34), but rather it is probably a manifestation of a preferential T_3 secretion present in this condition.

It is evident from the foregoing discussion that T_3 production plays a major role in determining the pattern of T_3 and T_4 kinetics. Additionally, T_3 production would appear to have a considerable influence on peripheral hormone action. If one assumes that T_3 has 4 times the metabolic potency of T_4, then T_3 might account for more than half of all hormonal activity produced in euthyroid subjects and, in the case of Graves' disease patients, it could account for better than three fourths of total hormonal action. This preeminent role of T_3, both in normal and pathological states, would suggest the importance of this hormone in assessing thyroid status in man.

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