Simultaneous Measurement of Thyroxine and Triiodothyronine Peripheral Turnover Kinetics in Man

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ABSTRACT Serum triiodothyronine (T.) kinetics in man have been difficult to define presumably due to the interference of iodoproteins generated during the peripheral metabolism of Ts. The use, in the present study, of an anion-column chromatographic method for separation of serum T. as well as thyroxine (T.) from these iodoproteins has overcome this technical handicap. Simultaneous measurement of serum ¹²⁵I-T₈ and ¹²¹I-T₄ 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 thyroxinebinding globulin states. The normal mean T_s fractional turnover rate (kT_s) was 0.68 (half-life = 1.0 days), increased in toxic Graves' disease patients to 1.10 (halflife = 0.63 days), and decreased in primary hypothyroid patients to 0.50 (half-life = 1.38 days). The mean T_s 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_s 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_s concentration was estimated by radioimmunoassay to be 0.106 µg/100 ml. Combined with the mean T_s clearance value of 26.1 liters/day, the calculated T. production rate was 27.6 µg/day. The mean T: 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. production rate was normal in subjects with altered TBG states. The ratio of T_s to T_s 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 T₈ may have been responsible for the retention of rapid turnover kinetics for Ts and Ts observed in treated Graves' disease patients. The finding that factitial thyrotoxic patients also displayed similar rapid Ts and Ts turnover kinetics indicates that these alterations are not a unique feature of Graves' disease per se. When comparing the peripheral turnover values for T_s and T_s 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_s kinetics while alterations in TBG concentrations have a greater effect on T. 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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¹Abbreviations used in this paper: DS, distribution space; kT₃, triiodothyronine fractional turnover rate; kT₄, thyroxine fractional turnover rate; MCR, metabolic clearance rate; RIA, radioimmunoassay; T₂, triiodothyronine; T₄, thyroxine; TBG, thyroxine-binding globulin; U, urinary.

investigations have dealt with the metabolism of triiodothyronine (T_{*}), and the information available is variable and at times conflicting. Early estimates of the biological half-life of Ts 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_{*} kinetics probably relates to the generation of circulating iodoproteins appearing during Ts 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 Ts in the serum (6). While comparing the peripheral deiodination rates of labeled T₈ and T₄ in man (7), we have observed that the rate of T_s 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 Ts and Ts 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 I). 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 T4 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_4 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 in-

stitutions of these drugs. After establishing a thyroid blockade, 30-50 µCi of ¹⁸¹I-T₄ were given intravenously to initiate the study. Timed serum samples were collected twice daily for the next 7 days to measure T. disposal rates. 2-4 days after the administration of the T. tracer, a pulse dose of 40-100 µCi of 125I-T₈ 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 ¹²⁵I-T₈ and ¹³¹I-T₄ tracers were obtained from Industrial Nuclear Co., St. Louis, Mo.; specific activities were greater than 30 μ Ci/ μ g 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 NH2. 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 ¹²⁵I-T₈ and ¹⁸¹I-T₄ 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 181 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 ¹⁸¹I labeled albumin was passed through the same procedure, no ¹⁸¹I activity was measured in the thyronine fraction. Additionally, when a serum sample containing only ¹⁸¹I-iodide was used, less than 1% appeared in the thyronine fraction. Using this procedure, the average recovery for a single run was $58.1 \pm 1.6\%$ $(\pm s_D)$ for ¹²⁵I-T₈ and 54.7 $\pm 2.1\%$ for ¹⁸¹I-T₄. Appropriate ¹²⁵I-T₈ and ¹⁸¹I-T₄ 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 181 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. and T. 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 ¹²⁶I/¹⁸¹I and plotted on semilogarithmic coordinates against time.

Metabolic clearance by constant infusion technique. In five subjects, after completion of the T. and T. pulse tracer studies, the metabolic clearance of T₃ 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-Ts and 10 µCi 181 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 ¹²⁵I-T₃, equal in radioactivity to 48 hr of the infusion, and ¹⁸¹I, 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 ¹²⁵I to ¹⁸¹I in sequential serum and urine samples; when the serum and urinary 125 I/181 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_s concentrations were measured by a radioimmunoassay (RIA) method as described by Chopra, Solomon, and Beall (11). All serum T_s 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: and T, kinetic data. Fig. 1 illustrates representative examples of serum ¹²⁵I-T. 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 ¹²⁶I activity lost in the extraction procedure was ¹²⁶I-iodide. However, since iodides possess a shorter biological half-life than Ts, the nonparallelism between the slopes



FIGURE 1 The whole serum (open circles) and extracted serum (closed circles) $^{126}I-T_a$ activities are plotted for representative hypothyroid, euthyroid, and hyperthyroid subjects. The injection of the $^{126}I-T_a$ tracer is denoted by the arrows. The closed triangles in the lower portion of the illustration represent the plot of urinary $^{126}I/^{126}I$ values and the dotted lines and open triangles represent the $^{126}I-T_a$ disappearance slope based on urinary isotope calculation. ($\frac{1}{2}L = half$ -life in days.)

TABLE I The Kinetics of Triiodothyronine and

				Serum t	hyroxine	Thyroxine- binding	Serum	Triiodothyronine equilibration time	
Subject	Age	Height	Weight	Total	Free	capacity	thyronine		
	yr	cm	kg	µg/100 ml	ng/100 ml	µg/100 ml	ng/100 ml	hr	
Normai	50	179	50		1 4	14.2		24	
1	20	170	52 70	4.4	1.4	14.5	33	24	
2	32	170	10	4.5	1.4	21.5	122	17	
3	25	179	76	3.3	1.2	20.4	120	23	
5	51	163	75	7.2	1.5	10.8	188	23	
5	63	148	68	6.8	1.1	33 3	85	27	
7	54	168	57	5.0	1.0	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 h	ypothyroid								
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	
$P \parallel$				0.1	0.1	0.9	0.005	0.02	
Graves' di Thyroto	sease xic								
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	
$P \parallel$				<0.1	<0.1	<0.4	<0.01	>0.9	
Hypothyr	oid								
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	
$P \parallel$				<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. UkT_3 and UkT_4 represent these same values but measured as the urinary appearance rate of iodide derived from the deiodination of T_3 and T_4 . $\ddagger T_3MCR$, 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.

 $\parallel P$ value refers to the significance of the difference compared to normal group.

Thyroxine Peripheral Metabolism

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Production rate		te	learance ra	C	ution mace	Dietrib	es *	turnover rat	Fractional	
 T	 T4§	T₁‡ MCR	T:	T4	T:	T ₄	UkTs/T4	UET:	kT:	kT4
24 hr	µg/	·	liters/24 hr		ilers			/24 hr	%	
18	91.5		32.7	1.36	37.2	11.6	75	87	88	11.7
37.	109.5		30.8	1.59	40.0	12.6	65	78	77	12.6
_	67.0		22.7	1.25	36.0	11.9	46	58	63	10.5
33.	100.2	28.0	27.9	1.56	40.5	10.8	60	71	69	14.4
42.	38.6	28.1	22.5	0.87	38.2	9.1	50	59	59	9.6
17.	146.7		21.0	1.41	35.0	15.0	48	63	60	9.4
27	76.5		26.3	1.00	38.7	10.8	58	69	69	9.3
16.	77.9		24.2	1.34	41.7	11.3	42	53	58	11.9
27.	88.5		26.1	1.30	38.4	11.6	55.5	67.3	67.9	11.2
3.9	11.5		1.5	0.09	0.8	0.6	3.9	4.0	3.7	0.6
	27.7	_	16.5	2.26	38.3	23.8	26	36	43	9.5
8.	42.4	13.0	15.6	1.26	38.0	17.5	43	50	41	7.2
8.	11.8	16.3	16.7	0. 96	41.7	11.8	27	35	40	8.1
9.4	5.74		22.0	0.75	33.3	8.8	45	54	66	8.5
6.0	18.1		22.2	1.68	35.3	14.9	42	53	63	11.3
5.4	9.26		18.0	1.21	39.2	11.5	29	39	46	10.5
7.0	19.2		18.5	1.35	37.6	14.7	35	45	50	9.2
0.7	5.6		1.2	0.22	1.2	2.2	3.6	3.6	4.7	0.6
0.0	0.01		0.01	0.9	0.6	0.2	0.01	0.01	0.01	0.05
361	386	47	54	1.64	45.5	9.5	69	86	119	17.3
446	573		108	4.68	90.9	17.4	84	111	119	26.9
120	268	<u> </u>	50	2.22	60.0	12.0	92	111	84	18.5
90	230		40	1.93	42.0	13.9	69	83	95	l 3.9
102	439		55	4.70	40.0	17.4	92	125	138	27.0
197	150	_	45	2.65	53.0	12.1	52	74	84	21.9
89.7	124		65	2.07	49.3	9.6	83	105	131	1.6
201	310	_	60	2.84	54.4	13.1	77.3	99.3	110	1.0
54.9	61.7		8.6	0.49	6.6	1.24	5.5	7.0	8.4	1.84
<0.0	<0.01		<0.01	<0.01	<0.05	<0.3	<0.01	<0.01	<0.01	0.01
	33.5		26	1.15	53.0	11.3	44	54	49	0.2
11.3	13.2		15	0.96	30.1	7.4	50	63	51	3.0
23.3	16.5		31	1.20	42.5	9.7	41	53	72	2.4
17.3	21.1	-	24	1.10	41.9	9.5	45	56.7	57.3	1.9
6.0	6.3	-	4.7	0.07	6.6	1.1	2.0	3.2	7.4	0.9
<0.2	<0.01	_	<0.7	<0.2	<0.7	<0.2	<0.1	<0.1	<0.3	0.0

TABLE I-

				Serum t	hyroxine	Thyroxine- binding	Serum	Triiodothyronine equilibration time	
Subject	Age	Height	Weight	Total	Free	globulin capacity	triiodo thyroninr		
	yr	cm	kg	µg/100 ml	ng/100 ml	µg/100 ml	ng/100 ml	hr	
Factitial h	yperthyroid	1							
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	
$P \parallel$				< 0.01	<0.01		<0.3	<0.8	
Idiopathic	elevated th	yroxine-bi	nding glob	ulin					
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	
$P \parallel$				0.01	0.05	< 0.01	0.3	0.05	
Idiopathic	low thyrox	ine-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 ¹²⁵I labeled iodoproteins from T₈ would more likely account for this flattening of the disappearance curve of the unextracted sera (6). The linearity observed in the extracted ¹²⁵I-T₈ serum slope suggests that such contamination had been effectively eliminated by column extraction.

In the euthyroid subjects, the daily fractional turnover rate for T_{*} (kT_{*}) in the extracted serum was 67.9%. In the primary hypothyroid group, kT_{*} decreased to 49.8%, while in the thyrotoxic Graves' and factitial hyperthyroid groups kT_{*} increased to 110 and 98.3%, respectively. Insignificant changes in kT_{*} 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_{*} was affected similarly by alterations in metabolic status, but kT_{*} was also altered by changes in serum TBG levels.

Analysis of urinary ¹⁸⁵I/¹⁸¹I turnover kinetics. Representative samples of the urinary ¹²⁵I/¹⁸¹I ratio plots are shown in Figs. 1 and 2. Since ¹⁸⁵I-T₈ and ¹⁸¹I-T₄ normally are excluded from the urine, measurement of the urinary ¹²⁵I/¹⁸¹I ratio reflects the deiodination of

the precursor labeled hormones, namely, $^{126}\mathrm{I}\text{-}\mathrm{T}_{\$}$ and $^{131}\mathrm{I}\text{-}\mathrm{T}_{\$}$ (12).

The slope described by the urinary ¹²⁵I/¹⁵¹I values after injection of ¹²⁶I-T₈ can be divided into three phases. The first phase describes the equilibration of ¹²⁶I-T₈ in the extrathyroidal T₈ pool. This phase was characterized by a rapid increase in the ¹²⁶I/¹⁵¹I urinary values. The point at which the urinary ¹²⁶I/¹⁵¹I values formed a linear exponential slope can be taken as the time when the T₈ 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 T₈ equilibrium was prolonged for approximately 10 hr beyond the normal control values.

The second phase was marked by the $^{125}I/^{181}I$ urinary ratio values forming a linear slope on semilogarithmic coordinates (as illustrated in Figs. 1 and 2). Since this slope ($^{116}T_8/T_4$) represented the ratio of the fractional turnover rates of the labeled precursor hormones (i.e., $^{126}I-T_8$ and $^{126}I-T_4$), it was possible to mathematically derive the fractional turnover rate of serum $^{126}I-T_8$ by the following analysis:

Assuming that the urinary ¹²⁸I/¹²⁸I slope was the result of two declining exponential functions, the mathemati-

	Duadu	Clearance rate			Distribution appear		Fractional turnovar rates*			
uction rate	Produc	T ₂ ‡								
T:	T₄§	MCR	T:	T₄	T3	T.	ULT2/T4	ULT 3	kT3	kT₄
g/24 hr	µg/		liters/24 hr			lit		24 hr	%/	
	280		47	1.78	51	11.8	60	75	93	15.1
62.7	242		49	1.82	59	12.0	69	84	83	15.2
171	323	—	60	2.64	50	16.4	90	106	119	16.1
117	282	_	52	2.08	53	13.4	73	88.3	98.3	15.5
54	23		4	0.28	2.8	1.5	8.9	9.2	10.7	0.3
<0.2	<0.01		<0.01	< 0.05	<0.01	<0.3	<0.2	<0.1	< 0.05	<0.01
34.6	72		12	0.56	19	7.9	55	62	64	7.1
22.5	66		15	0.63	30	7.9	41	49	50	8.0
20.9	75		19	0.58	27	8.6	59	66	69	6.8
20.4	61		17	0.72	28	7.8	46	55	59	9.2
24.6	68		16	0.62	26	8.1	50	58	60.5	7.8
3.4	3.1		1.5	0.04	2.4	0.2	4.1	3.8	4.1	0.5
<0.6	<0.2		<0.1	<0.01	< 0.01	< 0.01	<0.4	<0.2	<0.3	<0.01
18	52		45	3.79	55	13.1	37	66	82	28.9
22	64		56	5.19	74	21.9	40	64	75	23.7

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

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

where ¹²⁵I and ¹³¹I represent urinary ¹²⁵I and ¹³¹I values at any time t; A₁, A₂, and A₃ are constants, ^{Uk}T₃ and ^{Uk}T₄ are the urinary fractional turnover rates for ¹²⁵I-T₃ and ¹³¹I-T₄, respectively. Thus:

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

(3)
$$U^{k}T_{4} + U^{k}T_{3}/T_{4} = U^{k}T_{3} = {}^{k}T_{3}$$

where ${}^{Uk}T_s/T_4$ can be obtained directly from the urinary ratio slope and ${}^{Uk}T_4$ can be assumed to equal the fractional turnover rate of T₄ measured in serum (kT₄). As seen in Fig. 1 and Table I, ${}^{Uk}T_s$ values closely correlated with (r = 0.91, P < 0.001) the corresponding serum kT₈ measurements. This served to verify the accuracy of the direct serum kT₈ measurements.

Although kT_s and ^{va}T_s were similar, it is of interest that ^{va}T_s values were generally less than the corresponding serum kT_s 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 ^{vas}I iodoproteins produced from the labeled T_s (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 ¹²⁶I/⁵⁸¹I ratio slope values would not be seen until the majority of the injected ¹²⁶I-T₈ tracer had disappeared. Indeed, a loss of linearity of the urinary slope values was not observed until 5–10 days after the injection of ¹²⁶I-T₈ tracer which denoted the beginning of the third phase.

The UkT₃/T₄ value in the second phase also provided an index of the relative fractional turnover rates of ¹²⁵I-T₃, as compared to ¹³¹I-T₄. 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 "Ts/Ts value would indicate that the change in fractional turnover rate for T_s was greater than that for T₄. It is apparent from Table I and Fig. 2 that hyperthyroidism accelerates T₈ degradation to a greater degree than T. and that the reverse is true in hypothyroidism. An exception was the decrease in the ^{Uk}T₃/T₄ values seen in the idiopathic low TBG group which resulted from an increase in T₄ degradation rather than a decrease in the Ts degradation.

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



FIGURE 2 Urinary ¹³⁶I/¹⁸¹I values are plotted after the injection of ¹³⁵I-T₈ 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 ¹²⁶I-iodide in the urine possessed a biological half-life greater than that of ¹²⁶I-T₄ 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 T₃ degradation (6).

Distribution space of T_* (T_* DS) and T_* (T_* DS). In euthyroid subjects T_* DS was 38.4 ± 0.8 liters and the T_* DS was 11.6 ± 0.6 liters ($\pm sem$). The T_* 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_* 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_* DS generally paralleled the alterations in T_* 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_{*} DS/T_{*} 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_{*} and T_{*}, while changes in metabolic state alter T_{*} DS to a greater extent than T_{*} DS. Additionally, an increased T_{*} DS/T_{*} DS ratio of 4.41 was observed in hypothyroid Graves' disease subjects.

T: and T: clearances. In the euthyroid group, T: clearance was found to be 26.1 ± 1.5 liters and T. clearance to be 1.3 ± 0.09 liters/day. In thyrotoxicosis, T: and T. 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: and T. 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), T₈ metabolic clearance rate was determined by employing a constant infusion of ¹⁹⁸I-T₈. 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 μ g for T_s and 88 μ g for T₄. As might be expected, these values were not altered in euthyroid subjects with idiopathically high or low TBG values. In contrast, a $3\frac{1}{2}$ -fold increase in T₄ and over a 7-fold increase in T₈ production rate was found in the thyrotoxic Graves' disease patients which gave a ratio of T₈ to T₄ production was seen most prominently in the hypometabolic Graves' disease to 0.81. In the primary hypothyroid group, there was a 4-fold decrease in both T₈ and T₄ production rates.

DISCUSSION

The method for measurement of serum T_s 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 ¹³⁵I-T_s disappearance curves were linear when plotted on semilogarithmic coordinates (Figs. 1 and 2); (b) the mathematical analysis of urinary ¹³⁵I/⁴⁵¹I values verified the accuracy of the serum T_s turnover measurements; (c) studies of the metabolic clearance rate (MCR) of T_s by con-

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

The fractional turnover rates observed for T_* 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_* 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_* 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_s distribution space (T_s DS) may be in error since the single compartmental model system used in this study assumes that T_s disposal during equilibration is the same as after equilibration. The observed rise in urinary 125 I/121 ratio values during the equilibration phase (Figs. 1 and 2) indicated that Ts deiodination was substantially less during than after equilibration. Since deiodination constitutes the major route of degradation for T_s, this would result in an underestimation of T₂ disposal during the equilibration and, in turn, would cause an underestimation of T_s DS. On the other hand, the serum T_s disappearance slope during the equilibration phase may reflect the clearance of the Ts 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_s 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 T₃ MCR using the constant infusion method in normal and Graves' disease subjects which closely approximated the values seen in our patient population. Their T₃ 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 T₈ clearance is that the loss of the T_s 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_s disposal.

Comparison of T. and T. kinetics revealed differences as well as similarities in peripheral metabolism. It was observed that kTs and kTs were altered in a parallel manner by changes in metabolic rate and TBG levels, but that alterations in metabolic status seemed to influence kT₃ to a greater extent than kT₄, while changes in TBG altered kT₄ to a greater degree than kT₈. Since Ts and Ts appear to be bound by TBG extracellularly, it is fair to assume that the extracellular distribution space for T₂ is equal to that of T₄, or about 5 liters (18). Thus, only about 15% of the entire extrathyroidal Ts pool would appear to be extracellular. It is not surprising, therefore, that T₈ is affected by changes in metabolic status since it is predominantly an intracellular hormone. On the other hand, approximately 50% of the T₄ is in the extracellular fluid compartment bound to TBG (18), and it is equally logical that TBG alterations will influence kT₄ to a greater degree than kT₃. Therefore, one may conclude that the differences in the magnitude of change in kTs and kTs 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 Ts and Ts peripheral metabolism in four euthyroid subjects (19).

However, several other aspects of Ts and Ts peripheral metabolism are less clear. For instance, why is the T_s distribution space $3\frac{1}{2}$ times greater than that for T_s ? Since the extracellular binding for Ts and Ts are predominantly to TBG and the intrahepatic distribution space for T₄ is estimated to be greater than that for T_s (20), this difference is even more puzzling. Additionally, why was T₂ equilibration delayed as long as 22 hr in euthyroid subjects? Presumably this relates to the slow entrance of T₃ into the extrahepatic intracellular compartment. As has been observed by Cavalieri, Steinberg, and Searle (20), the egress of Ts 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_{\bullet} 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_{\bullet} concentration which allowed correction for some of the methodological artifacts, particu-

larly the monodeiodination of T₄ to T₈ (28). This has provided a more accurate assessment of serum T₈ concentration, but the correction factors are large and the results are, therefore, subject to some overcorrection, particularly at low serum T₈ levels. The recent development of a radioimmunoassay method for measurement of serum T₈ in unextracted serum would therefore appear to represent a substantial methodological improvement (11).

The apparently inappropriately high kT₃ and kT₄ values found in Graves' disease subjects with normal and subnormal T4 values, requires some further clarification. A high kT4 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 T₄ incorporation and degradation are probably responsible for the elevated kT₄ values (30, 31). Recently, Schussler and Vance (32) and Farmer, Smitherman, Beschi, and Pittman (33) have demonstrated that Ts administration to euthyroid subjects, in replacement or subreplacement doses, is capable of increasing kT₄, implying that T₃ is capable of increasing the rate of T₄ degradation. Additionally, Sterling and coworkers have reported elevated serum T₃ values in treated Graves' disease subjects in whom serum T₄ 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 T₃ DS/T₄ DS and T₃/T₄ production ratios were found in the hypothyroid Graves' disease group; (b) a positive correlation between T₃ production rate and kT₄ 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 T4 values with elevated serum T8 levels, had rapid T₃ and T₄ 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 T₃ and T₄ as were observed in the thyrotoxic Graves' disease group. Thus, it would appear that the presence of a large fractional turnover rate for T4 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 T₃ secretion present in this condition.

It is evident from the foregoing discussion that T_s production plays a major role in determining the pattern of T_s and T_4 kinetics. Additionally, T_s production would appear to have a considerable influence on peripheral hormone action. If one assumes that T_s has 4 times the metabolic potency of T_4 , then T_s might account for more than half of all hormonal activity pro-

duced 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_s , both in normal and pathological states, would suggest the importance of this hormone in assessing thyroid status in man.

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