

# Determination of Iodothyronine Absorption and Conversion of L-Thyroxine ( $T_4$ ) to L-Triiodothyronine ( $T_3$ ) using Turnover Rate Techniques

MARTIN I. SURKS, ALAN R. SCHADLOW, JERROLD M. STOCK, and  
JACK H. OPPENHEIMER

*From the Endocrine Research Laboratory, Division of Endocrinology,  
Department of Medicine, Montefiore Hospital and Medical Center and the  
Albert Einstein College of Medicine, Bronx, New York 10467*

**ABSTRACT** The absorption of L-thyroxine ( $T_4$ ) and L-triiodothyronine ( $T_3$ ) and the fractional rate of conversion of  $T_4$  to  $T_3$  were determined from the turnover rates of  $T_4$  and  $T_3$  in seven patients without endogenous thyroid function during separate treatment periods with these iodothyronines. Serum  $T_3$  concentration was measured by a radioimmunoassay procedure in which the iodothyronines are separated from the plasma proteins before incubation with anti- $T_3$  antibody. Metabolic clearance rates were calculated by an integral (noncompartmental) approach since the use of single compartment kinetics led to a 40% overestimation of the metabolic clearance rate of  $T_3$ . Based on the amount of hormone ingested and the observed hormonal turnover rates, the absorption of  $T_4$  and  $T_3$  (iodothyronine turnover/iodothyronine ingested) in man could be estimated. Absorption of  $T_3$  was complete in three subjects but decreased to 43% in a fourth who was suffering from mild congestive heart failure. Mean  $T_4$  absorption was  $48.0 \pm 2.6\%$  (SEM) for seven subjects. The mean fractional rate of  $T_4$  to  $T_3$  conversion determined during  $T_4$  replacement therapy ( $T_3$  turnover/ $T_4$  turnover) was 42.6% (range 30.7–50.8%). Thus, approximately one-half of the  $T_4$  which was deiodinated was converted to  $T_3$  suggesting that monodeiodination is an obligatory step in the peripheral metabolism of  $T_4$ . Calculations based on these results together with other available data suggest that under normal physiologic circumstances the major portion of the  $T_3$  pool is derived from monodeiodination of  $T_4$ .

## INTRODUCTION

The recent development of accurate methods for the determination of plasma L-triiodothyronine ( $T_3$ )<sup>1</sup> concentration (1–5) have for the first time allowed precise measurement of  $T_3$  turnover in man. In the following report we describe studies in which both  $T_3$  and L-thyroxine ( $T_4$ ) turnover rates were measured in patients without endogenous thyroid function but maintained in the euthyroid state by the administration of synthetic  $T_3$  or  $T_4$ . Since the amount of  $T_4$  or  $T_3$  administered was known and the turnover of these iodothyronines could be calculated it was possible to estimate both the absorption of  $T_4$  and  $T_3$  and the fractional conversion of  $T_4$  to  $T_3$  in man. Turnover was calculated from the product of the mean plasma iodothyronine concentration and the metabolic clearance rate. In the case of  $T_3$ , a newly developed radioimmunoassay technique was used for measurement of plasma concentration (6). Metabolic clearance rates were assessed by the application of non-compartmental assumptions to the analysis of the isotopic data (7).

The results of these studies, taken in conjunction with other available data suggest that (a) under normal conditions the human thyroid gland secretes largely  $T_4$ , (b) the source of circulating  $T_3$  in normal man is largely the monodeiodination of  $T_4$  in the peripheral tissues, and (c) monodeiodination in man, as in the rat (8), appears to be an obligatory intermediate step in the deiodination of  $T_4$  by tissues. Moreover, these studies indicate that application of single compartment kinetics leads

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<sup>1</sup> Abbreviations used in this paper: CR, conversion ratio; MCR, metabolic clearance rate;  $T_3$ , triiodothyronine;  $T_4$ , thyroxine; TCA, trichloroacetic acid; TSH, thyrotropin.

to a systematic overestimation of the metabolic clearance rate of  $T_3$  and underscores the desirability of using multicompartmental or noncompartmental approaches to the analysis of metabolic data obtained with isotopic  $T_3$  in man.

## METHODS

The turnover rate of  $T_4$  and  $T_3$  was measured in seven hypothyroid patients during hormonal replacement treatment with synthetic  $T_4$  (Synthroid, Flint Laboratories, Morton Grove, Ill.). Four were known to be athyreotic after surgical and radioiodine thyroidectomy for papillary-follicular thyroid carcinoma. They were without metastatic disease as assessed by total body scans and urinary excretion of radioiodine as well as routine roentgenography. The diagnosis in the remaining three patients was severe primary hypothyroidism. All subjects were clinically euthyroid at the time of study. Their serum concentrations of thyrotrophin (TSH) were less than 3  $\mu$ U/ml based on Research Standard A (obtained through the courtesy of R. Bangham, National Institute for Medical Research, Mill Hill, London) (9). The human TSH and the rabbit anti-human TSH antiserum were gifts of the National Pituitary Agency.

Turnover rates were calculated as the product of the metabolic clearance rates and mean plasma concentrations of each hormone. In the four athyreotic patients (R. W., A. F., V. DiG., and V. P.) the metabolic clearance rate of  $T_3$  was determined during treatment with synthetic  $T_3$  (Cytomel; Smith, Kline & French Laboratories, Philadelphia, Pa.) 4 wk before the metabolic clearance rate of  $T_4$  was measured. The metabolic clearance rates of  $T_3$  and  $T_4$  were measured simultaneously in the other patients. The plasma concentration of  $T_4$  was assessed both by competitive protein binding (10) and  $T_4$ -I by column (11) methods (Bioscience Laboratories, Van Nuys, Calif.). Plasma  $T_3$  was measured by radioimmunoassay as previously described (6). The average value of plasma hormone concentrations from three to eight different plasma samples was used in the calculation of turnover rates. Plasma samples for determination of hormone concentration were obtained between 8 and 9 a.m. just prior to the administration of the daily dose of  $T_4$ . They were stored at  $-20^\circ$  until the assays were performed.

L-thyroxine labeled with  $^{125}$ I (Tetramet- $^{125}$ I), specific activity = 40–60  $\mu$ Ci/ $\mu$ g, and L-triiodothyronine, labeled with either  $^{125}$ I (Triomet- $^{125}$ I), specific activity = 70–90  $\mu$ Ci/ $\mu$ g, or  $^{131}$ I (Triomet- $^{131}$ I), specific activity = 30–50  $\mu$ Ci/ $\mu$ g, were obtained from Abbott Laboratories, North Chicago, Ill. For the determination of metabolic clearance rates 20  $\mu$ Ci of either [ $^{125}$ I] $T_4$  or [ $^{125}$ I] $T_3$ , or a combined dose of 20  $\mu$ Ci [ $^{125}$ I] $T_4$  and 40  $\mu$ Ci [ $^{131}$ I] $T_3$ , were injected intravenously. Plasma samples were generally obtained every 2 h for the first 12 h after injection and at 24–48 h intervals thereafter. Plasma was obtained for 5 days for the  $T_3$  studies and for 12 days for the  $T_4$  studies. Five drops of Lugol's solution were administered twice a day throughout the study. All plasma samples were treated with trichloroacetic acid (TCA) to remove inorganic iodide (7). In the  $T_3$  metabolic clearance rate determinations, the plasma non-extractable iodine was measured by extraction with ethanol as previously described (7). Plasma radioiodothyronine concentration was calculated as the difference between the TCA-precipitable radioactivity and the nonextractable radioactivity. Radioactivity in all samples was measured in a two-channel Packard Autogamma Spectrometer to a statis-

tical precision of  $\pm 2\%$ . Metabolic clearance rates were calculated by both single compartment kinetics and the integral approach first described by Tait (12) for the steroid hormones and more recently applied to the iodothyronines (7).

## RESULTS

Plasma  $T_4$  concentration was within the normal range for all patients treated with 150–200  $\mu$ g  $T_4$ /day (Table I). The range of values for individual samples collected during a 12 day period was  $\pm 10\%$  of the mean value for each patient. Moreover, determinations of  $T_4$  concentration by competitive protein binding and by iodine analysis were in close agreement. Plasma  $T_3$  concentrations on different days during the study were within  $\pm 15\%$  of the mean  $T_3$  concentration for each patient (Table I). The mean plasma  $T_3$  concentration for all of the subjects,  $172 \pm 9.3$  (SE) ng/100 ml, was somewhat higher than the mean of untreated euthyroid individuals in our laboratory,  $146 \pm 24$  ng/100 ml.

Data for the metabolic clearance rates of  $T_3$  and  $T_4$  are shown in Table I and Fig. 1. Calculation by single compartment kinetics resulted in a larger estimate of metabolic clearance rate of  $T_3$  and  $T_4$  than by the integral (noncompartmental) approach. The difference was small (4.5%) in measurements of  $T_4$  metabolic clearance rate, but still significant statistically ( $P < 0.01$ , paired  $t$  test). For  $T_3$ , however, the mean metabolic clearance rate calculated by single compartment kinetics was 40.6% greater than by integral calculations (range 16.6–61.9%). The metabolic clearance rate derived from the integral (noncompartmental) calculation was therefore used in all calculations (Table I).

The absorption of the iodothyronines was estimated from the turnover rates of  $T_3$  or  $T_4$  during separate treatment periods with these preparations. Since, in these patients, the only source of iodothyronine is that absorbed from the enteric tract it follows that:

$$\begin{aligned} \text{iodothyronine turnover, } \mu\text{g/day} \\ = \text{iodothyronine absorbed, } \mu\text{g/day,} \\ \text{and} \end{aligned}$$

$$\begin{aligned} \text{absorption (\%)} \\ = 100 \times \frac{\text{iodothyronine turnover, } \mu\text{g/day}}{\text{iodothyronine ingested, } \mu\text{g/day}} \end{aligned}$$

During treatment with  $T_4$  from 39.8 to 54.6% of the ingested  $T_4$  was absorbed in these patients (mean  $48.0 \pm 2.6\%$ ) (Table I). Similar calculations of  $T_3$  absorption were made in four patients during a separate treatment period with 50–75  $\mu$ g  $T_3$ /day (Table II). In these subjects, plasma  $T_3$  concentration increased 300–500% after  $T_3$  ingestion, falling thereafter to pre-dose values.<sup>2</sup> The

<sup>2</sup> These data have been reported previously (6).

TABLE I  
Turnover of L-thyroxine ( $T_4$ ) and L-triiodothyronine ( $T_3$ ), Absorption and Conversion of  $T_4$  in Hypothyroid Patients Treated with Synthetic  $T_4$ \*

Hypothyroid Patients Treated with Thyroxine												
Patient	Body weight	T <sub>4</sub>					T <sub>3</sub>					Conversion** rate
		Plasma† T <sub>4</sub>	Metabolic‡ clearance rate	Turnover		Absorption‖	Plasma† T <sub>3</sub>	Metabolic‡ clearance rate	Turnover¶			
	kg	µg/100 ml	liter/day	µg/day	µmol/day	%	ng/100 ml	liter/day	µg/day	µmol/day	%	
R. W.	74.5	10.2(4)	1.03	105.8	0.136	52.9	149(6)	23.9	31.1	0.048	34.9	
A. F.	60.9	8.3(4)	0.96	79.5	0.102	39.8	162(5)	23.0	32.8	0.050	49.1	
V. DiG.	68.2	8.0(4)	0.92	73.7	0.095	49.2	146(6)	23.9	31.0	0.048	50.8	
V. P.	60.9	6.6(2)	1.14	75.0	0.097	37.6	209(6)	11.5	19.4	0.030	30.7	
J. B.	59.1	8.1(3)	1.20	96.6	0.124	48.3	202(4)	20.7	37.4	0.057	46.1	
R. A.	76.4	10.5(3)	1.04	109.1	0.140	54.6	162(4)	23.8	34.0	0.052	37.1	
A. S.	97.7	7.0(3)	1.54	107.5	0.138	53.8	172(4)	28.6	44.7	0.069	49.5	
Mean	71.1	8.4	1.12	92.5	0.119	48.0	172	22.2	32.9	0.051	42.6	
SEM	5.1	0.6	0.08	6.0	0.008	2.6	9.3	2.0	2.9	0.004	3.1	

\* The daily dose of  $T_4$  was 200 µg for all subjects except V. DiG. who received 150 µg.

† The number of plasma samples analyzed is shown in parenthesis. The average value is presented. For  $T_4$ , at least one sample was analyzed by  $T_4$ -I-by-column method (see Methods).

§ The metabolic clearance rates (MCR) of  $T_3$  and  $T_4$  were calculated by the integral (noncompartmental) method except for subject V. P. in whom the  $T_4$  MCR was calculated by the single compartment kinetic approach. The MCR of  $T_3$  and  $T_4$  were measured simultaneously in subjects J. B., R. A., and A. S. In the other four subjects, the MCR of  $T_3$  was determined 1 month earlier at a time when they were being treated with synthetic  $T_3$ , 50–75 µg/day.

||  $T_4$  absorption =  $100 \times T_4$  turnover, µg day<sup>-1</sup>/ $T_4$  ingested, µg day<sup>-1</sup>.

¶ The average  $T_3$  content of the  $T_4$  dose was 2.25% as determined by radioimmunoassay of  $T_3$  in solutions of tablets from different batches of synthetic  $T_4$ . Assuming 100% absorption of  $T_3$ , the absorbed  $T_3$  constituted a mean of 12.3% of the total  $T_3$  turnover. The values for  $T_3$  turnover were corrected for this source of  $T_3$  so that they represent  $T_3$  converted from  $T_4$  only.

\*\* Conversion rate =  $T_3$  turnover, µmol day<sup>-1</sup>/ $T_4$  turnover, µmol day<sup>-1</sup>.

mean plasma  $T_3$  concentration was calculated by integrating the area under a curve describing  $T_3$  concentration vs. time. The average absorption of  $T_3$  was 102.8% in three of the four subjects studied (range 92.9–113.3%) (Table II). In V. P., who was in mild congestive heart failure at the time of study,  $T_3$  absorption was 43.2%.

Since, during treatment with  $T_4$ , the only source of  $T_3$  is from the metabolism of  $T_4$ , a minimal estimate<sup>3</sup> of  $T_4$  to  $T_3$  conversion can be calculated from the  $T_3$  and  $T_4$  turnover rates. Thus, the  $T_4$  to  $T_3$  conversion ratio (CR), representing the percentage of the  $T_4$  turnover

converted to  $T_3$  is

$$100 \times \frac{T_3 \text{ turnover, } \mu\text{mol/day}}{T_4 \text{ turnover, } \mu\text{mol/day}}$$

The mean CR for the seven patients studied was  $42.6 \pm 3.1\%$  (range 30.7–50.8%) (Table I).

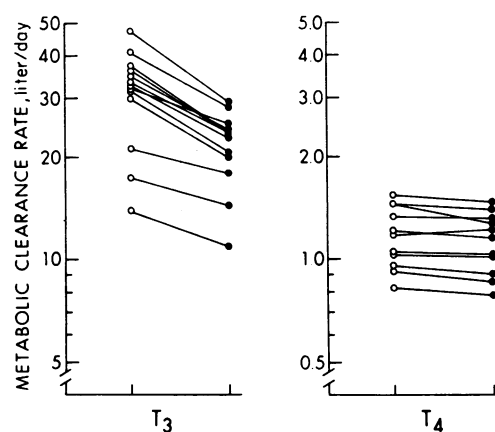


FIGURE 1 Comparison of  $T_3$  and  $T_4$  metabolic clearance rate calculations by single compartment kinetics (open circles) and by an integral (noncompartmental) approach (filled circles). In addition to the seven subjects described in this report, data from eight euthyroid subjects who were either normal volunteers or convalescing from nonthyroidal illness are included also.

<sup>3</sup> Strictly speaking, the values for the conversion ratio provided in these calculations should be considered to be only *minimal* estimates. It is theoretically possible that some of the  $T_3$  generated from  $T_4$  in a cell is irreversibly metabolized before it has the opportunity to enter the plasma sampling compartment. Nevertheless, this appears unlikely to be a source of major error. As pointed out in the Discussion, there is considerable evidence that  $T_3$  arises through the random deiodination of  $T_4$  in tissues, a process which would yield a theoretical maximum rate of  $T_3$  formation equal to one-half of the rate of total  $T_4$  deiodination. If the conversion ratio obtained in these studies were a significant underestimate of the actual rate of  $T_4$  to  $T_3$  conversion, the theoretical 50% limit of conversion would be exceeded. Under such circumstances, the calculated molar potency ratio of  $T_3$  to  $T_4$  would have to be substantially less than the 2–3:1 values which are generally accepted (13).

TABLE II  
Turnover and Absorption of L-Triiodothyronine ( $T_3$ ) in  
Hypothyroid Patients during Treatment with  $T_3$

Patient	Dose of $T_3$	Mean plasma* $T_3$	Metabolic clearance rate	Turnover†	Absorption‡
	$\mu\text{g/day}$	$\text{ng}/100\text{ ml}$	$\text{liter/day}$	$\mu\text{g/day}$	%
R. W.	75	322 (8)§	23.9	76.6	102.1
A. F.	75	303 (8)	23.0	69.7	92.9
V. DiG.	50	237 (8)	23.9	56.7	113.3
V. P.	75	282 (7)	11.5	32.4	43.2
Mean		286	20.6	58.9	87.9
SEM		18.3	3.0	10.9	15.5

\* Calculated by integration of the curve described by plotting plasma  $T_3$  concentration against time.

† See footnote to Table I for calculations.

§ Numbers in parentheses indicate the number of plasma samples analyzed.

## DISCUSSION

The accuracy of the rates of iodothyronine absorption and  $T_4$  to  $T_3$  conversion determined by turnover rate techniques as described herein depends on the precision of measurement of the mean plasma concentration and metabolic clearance rates of  $T_3$  and  $T_4$ . Whereas the methods employed for  $T_4$  determination are well established, only a limited experience is available with published methods for the determination of plasma  $T_3$ . There is general agreement, however, that radioimmunoassay procedures with specific anti- $T_3$  antibodies provide the most reliable measurements. Moreover, available evidence suggests that the presence of the plasma binding proteins in the assay mixture results in high  $T_3$  values (14). In the radioimmunoassay used for measurement of  $T_3$  in this study,  $T_3$  is separated from the plasma binding proteins before incubation with antibody (6). The mean plasma  $T_3$  concentration of euthyroid individuals by this method,  $146 \pm 24$  ng/100 ml is in good agreement with that of some published methods in which the binding of  $T_3$  to plasma proteins in the assay mixture is blocked by addition of other agents (2-5) but somewhat greater than that of other reports in which the mean plasma  $T_3$  of euthyroid individuals is in the range of 100-110 ng/100 ml (4, 5). The effect of a possible overestimation of plasma  $T_3$  by our method on the conclusions of the present study is discussed below.

Since the mean plasma concentration is required for the calculation of turnover rates, the relationship of the observed plasma  $T_3$  and  $T_4$  concentrations to the mean iodothyronine concentration must be considered. We have previously shown that the plasma  $T_4$  concentration may increase transiently after  $T_4$  ingestion in some patients (6). Since the increase is relatively small in mag-

nitude (20-40%) and short in duration (2-4 h), it does not influence significantly the mean plasma  $T_4$  concentration. In contrast to  $T_4$ , plasma  $T_3$  concentration remains relatively constant after a dose of  $T_4$  is ingested (6). Thus, during treatment with  $T_4$  the plasma concentration of  $T_3$  and  $T_4$  in samples obtained prior to administration of the daily  $T_4$  dose adequately represents the mean plasma iodothyronine concentration. It is notable that the mean plasma  $T_3$  concentration during  $T_4$  replacement therapy,  $172 \pm 9.3$  ng/100 ml, is higher than that of euthyroid subjects in our laboratory (6). Similar data have been reported by Lieblisch and Utiger (3). The possibility that the dose of  $T_4$  administered to these patients may be somewhat greater than necessary to produce euthyroidism is currently under investigation. During  $T_3$  treatment, the large and sustained (8-12 h) increase in  $T_3$  concentration which occurs after the hormone is ingested necessitates repeated sampling of plasma throughout the day to assess the mean plasma concentration (6).

An important component in the determination of the turnover rate is the measurement of the clearance rate by isotopic techniques. Conventionally, estimates of the clearance rates of iodothyronines have been made from the product of the apparent distribution volume as determined from the reciprocal of the zero-time extrapolation of the terminal plasma disappearance curve and the terminal fractional plasma removal rate (15). This procedure makes the tacit assumption of the existence of a single rapidly mixing compartment. The results of our studies clearly indicate that whereas no major error is introduced in estimating the  $T_4$  clearance rate in this fashion, a systematic 40% overestimation of the clearance rate is introduced when such analytic techniques are applied to  $T_3$ . The reason why  $T_4$  clearance rate can be measured by single compartmental methods may be related to the fact that the fractional rate of distribution of  $T_4$  is relatively rapid in comparison to the fractional rate of metabolism. In the case of  $T_3$ , the relatively more rapid rate of fractional hormone metabolism in relationship to its rate of distribution appears to invalidate the assumptions of single compartment kinetics. Our observations are at variance with those of Nicoloff, Low, Dussault, and Fisher (16) and Cavalieri, Steinberg, and Searle (17) who reported no difference in the clearance rates of  $T_3$  determined by single compartmental kinetics and by a noncompartmental approach in constant infusion experiments employing euthyroid subjects. However, an overestimation of the  $T_3$  clearance rate by single compartmental methods was observed by Cavalieri et al. (17) in thyrotoxic subjects. The basis of these discrepancies may perhaps be related to an inadequate duration of the  $T_3$  infusion in the euthyroid subjects. Under any circumstances, the integral ap-

proach first used by Tait for steroid clearance measurements (12) and later applied by us to the iodothyronines (7) appears to be both useful and a convenient technique for measuring the clearance rate of  $T_3$  by isotopic techniques. Although the  $T_3$  clearance measurements in some of the subjects was determined prior to measurement of  $T_4$  clearance and during treatment with  $T_3$ , mean  $T_3$  clearance and conversion ratios in this group were not significantly different from the values of the remaining three subjects in whom  $T_3$  and  $T_4$  clearance rates were measured simultaneously during  $T_4$  treatment.

Previous estimates of iodothyronine absorption in man have been based on measurements in the plasma or whole body of  $T_4$  or  $T_3$  radioactivity from isotopically labeled iodothyronine preparations which were administered orally (18–20). As pointed out by Hays (19), differences in the composition of the solution in which  $T_4$  is ingested may result in different values for absorption. The measurement of absorption by turnover rate techniques described in this report allows for the first time measurement of the absorption of hormone in the actual pharmaceutical preparations which patients ingest for replacement therapy. In agreement with previous reports,  $T_3$  absorption was essentially complete in patients without gastrointestinal disease (20, 21). The observation that  $T_3$  absorption was reduced to 50% in one patient suffering from mild congestive heart failure suggests that a relatively minimal degree of intestinal dysfunction may reduce  $T_3$  absorption, whereas published reports suggest that  $T_4$  absorption is reduced only in severe malabsorption (19). The average  $T_4$  absorption (50%) was somewhat less than that observed by Oddie, Fisher, and Epperson (18) (63.4%) who administered the dose in a capsule and by Hays (19) (74.4%) who gave the dose in a liquid form, but greater than observed by Hays for doses in capsules (41.7%) (19). Some of these differences may be due to the relatively small groups of subjects studied or to geographical factors.

The extrathyroidal conversion of  $T_4$  to  $T_3$  in man was first clearly demonstrated by Braverman, Ingbar, and Sterling (22). Subsequently, Sterling, Brenner, and Newman (23) and Pittman, Chambers, and Read (24) confirmed their observations and estimated the extent of conversion by measuring the concentration of radioactive  $T_3$  in plasma after injection of radioactive  $T_4$ . Both groups reported that as much as 33% of the  $T_4$  production was converted to  $T_3$ . Technical problems, however, may offer serious obstacles to this approach. Since only a small portion of the  $T_3$  pool is in the plasma (7) isotopically labeled  $T_3$  constitutes only 1–3% of the plasma radioactivity after injection of isotopically labeled  $T_4$ . The accurate measurement of such small amounts of labeled  $T_3$  in the presence of a large excess of labeled  $T_4$  is a formidable problem with inherent diffi-

culties in the estimation of chromatographic paper background radioactivity and in the assessment of overlap of a small fraction of  $T_4$  or tetraiodothyroacetic acid radioactivity into the  $T_3$  region of chromatograms or conversion of isotopic  $T_4$  to  $T_3$  during sample processing. These factors thus necessitated numerous adjustments (24). The conversion rate of 42% determined by turnover techniques which obviate these technical difficulties would therefore appear to be a more reliable estimate of this metabolic pathway. It is theoretically possible that the conversion rates in the three subjects with primary hypothyroidism were somewhat overestimated due to residual thyroidal secretion of  $T_3$ . This is unlikely since serum TSH concentration was undetectable on hormonal replacement therapy and since the mean conversion rate in this group did not differ significantly from that of the four athyreotic subjects.

Based on measurements of the conversion rate and the known biological activity of  $T_3$  in the rat, we have previously indicated that essentially all of the biological activity of  $T_4$  can be attributed to the  $T_3$  which is generated and suggested that  $T_4$  should be considered a prohormone (25). The more recent observation that propylthiouracil treatment causes a decrease in  $T_4$  to  $T_3$  conversion which fully accounts for the anti- $T_4$  effect of this agent strengthens this conclusion (8). The conversion rate of 42% observed in the current experiments in conjunction with a two to three fold greater biological activity of  $T_3$  compared with  $T_4$  (13) suggests that  $T_3$  effects all thyroidal activity in man as well as in the rat. The conclusion that  $T_3$  is the biologically active thyroid hormone is supported further by the recent demonstration of stereospecific low capacity, high affinity binding sites for  $T_3$  only in the rat anterior pituitary (26) and in the nuclei of liver and kidney (27).

Since peripheral  $T_4$  to  $T_3$  conversion results from monodeiodination of  $T_4$  the relationship of the amount of  $T_4$  converted to the amount of  $T_4$  deiodinated may help define the pathways in  $T_4$  metabolism which result in  $T_3$  formation. Approximately 85% of the  $T_4$  turnover is metabolized by deiodination (28–31). In the current studies the ratio:  $T_4$  converted/ $T_4$  deiodinated was 0.5 (range 0.35–0.6). Using a different technique to measure the  $T_4$  to  $T_3$  conversion rate we recently reported a similar relationship between  $T_4$  converted and  $T_4$  deiodinated both in normal rats and in animals in which the conversion rate was reduced by treatment with propylthiouracil (8). Thus, in man as well as in the rat, approximately one-half of the  $T_4$  deiodinated is converted to  $T_3$ . In earlier studies we have shown that during  $T_4$  metabolism there is no significant difference between the appearance in urine of iodide from the phenolic ring and tyrosyl ring and have suggested that  $T_4$  deiodination might be a random process (32). Since removal of either

phenolic ring iodine atom from  $T_4$  would result in  $T_3$  formation, provided that the side chain remains unaltered, it is apparent that if  $T_4$  is metabolized by random monodeiodination a maximum of one-half of the  $T_4$  molecules deiodinated will form  $T_3$ . The excellent agreement between the observed rate of  $T_3$  formation and the theoretical maximum for random monodeiodination suggests that random monodeiodination is an obligatory metabolic pathway in  $T_4$  metabolism. This formulation predicts that another iodothyronine, 3,3',5'-triiodothyronine, would also be formed during  $T_4$  metabolism. Since this compound appears to be metabolized at a greater rate than  $T_3$  (33) its detection by current radiochemical techniques would be exceedingly difficult.

The observed conversion ratio also allows estimation of the fraction of the  $T_3$  pool which is derived from  $T_4$  from the following expressions:

$$(\text{turnover } T_3)_{\text{Con}} = \frac{\text{CR}}{100} \times (\text{turnover } T_4),$$

where  $(\text{turnover } T_3)_{\text{Con}}$  represents the  $T_3$  turnover due to conversion.

Since the turnover is equal to the product of the mean plasma concentration,  $[T_3]$ , and the metabolic clearance rate, MCR, it follows that:

$$[T_3]_{\text{Con}} = \frac{\text{CR} \times [T_4] \times \text{MCR}_{T_4}}{100 \times \text{MCR}_{T_3}}.$$

If we now substitute normal values for  $[T_4]$  (80  $\mu\text{g/liter}$ ),  $\text{MCR}_{T_3}$  (23.0 liters/day),  $\text{MCR}_{T_4}$  (1.1 liter/day) and the conversion rate observed in these studies (42.6%) the mean concentration of  $T_3$  in plasma due to conversion = 136 ng/100 ml (range 100–163 ng/100 ml). The close accord of this value with the normal mean  $T_3$  concentration in our laboratory,  $146 \pm 24$  (SD) ng/100 ml (6) suggests that under normal conditions the major portion of  $T_3$  pool is derived from peripheral  $T_4$  metabolism and that the contribution from thyroidal secretion under normal conditions is minor. This is not necessarily the case in iodine deficiency (34) and in pathological conditions such as Graves' disease. These calculations differ from those of Sterling et al. (23) and Pittman et al. (24) who estimated that from 30 to 40% of the  $T_3$  pool is derived from extrathyroidal  $T_4$ . However, our conclusion is supported by recent reports that  $T_3$  constitutes less than 9% of the iodothyronine content of human thyroglobulin (35, 36). Based on this observation and assuming indiscriminate hydrolysis of thyroglobulin, the thyroid secretion in man appears to be primarily  $T_4$ .

As indicated above, the precise concentration of plasma  $T_3$  in euthyroid individuals is still a matter of controversy. If the mean euthyroid plasma  $T_3$  is in the range of 100–110 ng/100 ml as suggested by some

reports (4, 5), the  $T_4$  to  $T_3$  conversion rate in the present studies would be reduced to approximately 30–35%. The lower conversion rate is still of sufficient magnitude to ascribe thyroidal activity in man predominantly to  $T_3$  but is too low to be consistent with the random monodeiodination pathway of  $T_4$  metabolism unless some of the  $T_3$  generated from  $T_4$  is irreversibly metabolized within the cell before entering the plasma. For either conversion rate, however, the  $T_3$  pool would be derived principally from peripheral  $T_4$  and not from thyroidal secretion.

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