JCI The Journal of Clinical Investigation

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

Martin I. Surks, … , Jerrold M. Stock, Jack H. Oppenheimer

J Clin Invest. 1973;52(4):805-811. https://doi.org/10.1172/JCI107244.

Research Article

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±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 […]

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±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.

Received for publication 7 August 1972 and in revised form 31 October 1972.

INTRODUCTION

The recent development of accurate methods for the determination of plasma L-triiodothyronine (T_3)[1] 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 noncompartmental 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

[1] *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 μU/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 $-20°$ until the assays were performed.

L-thyroxine labeled with ^{125}I (Tetramet-^{125}I), specific activity = 40–60 μCi/μg, and L-triiodothyronine, labeled with either ^{125}I (Triomet-^{125}I), specific activity = 70–90 μCi/μg, or ^{131}I (Triomet-^{131}I), specific activity = 30–50 μCi/μg, were obtained from Abbott Laboratories, North Chicago, Ill. For the determination of metabolic clearance rates 20 μCi of either [^{125}I]T_4 or [^{125}I]T_3, or a combined dose of 20 μCi [^{125}I]T_4 and 40 μCi [^{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 nonextractable 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 statistical precision of ±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 μg T_4/day (Table I). The range of values for individual samples collected during a 12 day period was ±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 ±15% of the mean T_3 concentration for each patient (Table I). The mean plasma T_3 concentration for all of the subjects, 172±9.3 (SE) ng/100 ml, was somewhat higher than the mean of untreated euthyroid individuals in our laboratory, 146±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:

$$\text{iodothyronine turnover, } \mu\text{g/day} = \text{iodothyronine absorbed, } \mu\text{g/day,}$$

and

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

During treatment with T_4 from 39.8 to 54.6% of the ingested T_4 was absorbed in these patients (mean 48.0± 2.6%) (Table I). Similar calculations of T_3 absorption were made in four patients during a separate treatment period with 50–75 μg 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.[2] The

[2] 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**

Patient	Body weight	T_4 Plasma‡ T_4	T_4 Metabolic§ clearance rate	T_4 Turnover		T_4 Absorption‖	T_3 Plasma‡ T_3	T_3 Metabolic§ clearance rate	T_3 Turnover¶		Conversion** rate
	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 × T_4 turnover, μg day^{-1}/T_4 ingested, μg day^{-1}.

¶ 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^{-1}/T_4 turnover, μmol day^{-1}.

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[3] 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± 3.1% (range 30.7–50.8%) (Table I).

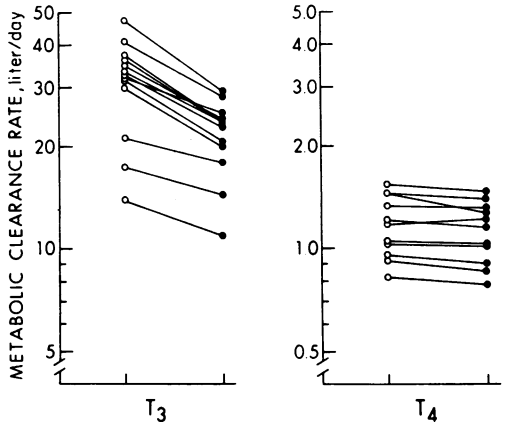

FIGURE 1 Comparison of T_3 and T_4 metabolic clearance rate calculations by single compartment kinetics (open circles) and by an integral (noncompartmental) approach (filled circles). In addition to the seven subjects described in this report, data from eight euthyroid subjects who were either normal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

[3] 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‡
	μg/day	*ng/100 ml*	*liter/day*	*μg/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±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 magnitude (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±9.3 ng/100 ml, is higher than that of euthyroid subjects in our laboratory (6). Similar data have been reported by Lieblich 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 difficulties 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:

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

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

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

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

If we now substitute normal values for $[T_4]$ (80 μg/liter), MCR_{T_3} (23.0 liters/day), 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±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.

ACKNOWLEDGMENTS

The authors are indebted to Mr. Kenneth Gans for expert technical assistance. We also appreciate the secretarial help of Ms. Maria Morel and Mary Ann Mullen. The figure was drawn by Mr. Barry Shapiro.

This work was supported by U. S. Public Health Service Grant 9 RO1 AM 15421-12 and U. S. Army Contract DA-49-193-MD-2967.

REFERENCES

1. Gharib, H., W. E. Mayberry, and R. J. Ryan. 1970. Radioimmunoassay for triiodothyronine: a preliminary report. *J. Clin. Endocrinol. Metab.* **31**: 709.
2. Mitsuma, T., N. Nihei, M. C. Gershengorn, and C. S. Hollander. 1971. Serum triiodothyronine: measurements in human serum by radioimmunoassay with corroboration by gas-liquid chromatography. *J. Clin. Invest.* **50**: 2679.
3. Leiblich, J., and R. D. Utiger. 1972. Triiodothyronine radioimmunoassay. *J. Clin. Invest.* **51**: 157.
4. Chopra, I. J., and R. W. Lam. 1972. Use of 8-anilino-*l*-naphthalene-sulfonic acid (ANS) in radioimmunoassay (RIA) of triiodothyronine in unextracted serum. *Clin. Res.* **20**: 216. (Abstr.)
5. Larsen, P. R. 1972. Direct immunoassay of triiodothyronine in human serum. *J. Clin. Invest.* **51**: 1939.
6. Surks, M. I., A. R. Schadlow, and J. H. Oppenheimer. 1972. A new radioimmunoassay for plasma L-triiodothyronine: measurements in thyroid disease and in patients maintained on hormonal replacement. *J. Clin. Invest.* **51**: 3104.
7. Oppenheimer, J. H., H. L. Schwartz, H. C. Shapiro, G. Bernstein, and M. I. Surks. 1970. Differences in primary cellular factors influencing the metabolism and distribution of 3,5,3'-L-triiodothyronine and L-thyroxine. *J. Clin. Invest.* **49**: 1016.
8. Oppenheimer, J. H., H. L. Schwartz, and M. I. Surks. 1972. Propylthiouracil inhibits the conversion of L-thyroxine to L-triiodothyronine. An explanation of the antithyroxine effect of propythiouracil and evidence supporting the concept that triiodothyronine is the primary thyroid hormone. *J. Clin. Invest.* **51**: 2493.
9. Odell, W. D., J. F. Wilber, and R. D. Utiger. 1967. Studies of thyrotropin physiology by means of radioimmunoassay. *Recent Progr. Horm. Res.* **23**: 47.
10. Murphy, B. E. P., and C. J. Pattee. 1964. Determination of thyroxine utilizing the property of protein-binding. *J. Clin. Endocrinol. Metab.* **24**: 187.
11. Pileggi, V. J., N. D. Lee, O. J. Golub, and R. J. Henry. 1961. Determination of iodine compounds in serum. I.

Serum thyroxine in the presence of some iodine contaminants. *J. Clin. Endocrinol. Metab.* **21**: 1272.
12. Tait, J. F. 1963. The use of isotopic steroids for the measurement of production rates in vivo. *J. Clin. Endocrinol. Metab.* **23**: 1285.
13. Blackburn, C. M., W. M. McConahey, F. R. Keating, Jr., and A. Albert. 1954. Calorigenic effects of single intravenous doses of L-triiodothyronine and L-thyroxine in myxedematous persons. *J. Clin. Invest.* **33**: 819.
14. Gharib, H., R. J. Ryan, W. E. Mayberry, and T. Hockert. 1971. Radioimmunoassay for triiodothyronine (T_3). I. Affinity and specificity of the antibody for T_3. *J. Clin. Endocrinol. Metab.* **33**: 509.
15. Sterling, K., and R. B. Chodos. 1956. Radiothyroxine turnover studies in myxedema, thyrotoxicosis, and hypermetabolism without endocrine disease. *J. Clin. Invest.* **35**: 806.
16. Nicoloff, J. T., J. C. Low, J. H. Dussault, and D. A. Fisher. 1972. Simultaneous measurement of thyroxine and triiodothyronine peripheral turnover kinetics in man. *J. Clin. Invest.* **51**: 473.
17. Cavalieri, R. R., M. Steinberg, and G. L. Searle. 1971. Metabolic clearance rate of L-triiodothyronine in man: a comparison of results by single-injection and constant infusion methods. *J. Clin. Endocrinol. Metab.* **33**: 624.
18. Oddie, T. H., D. A. Fisher, and D. Epperson. 1965. Effect of exogenous thyroxine on thyroid accumulation and secretion in euthyroid subjects. *J. Clin. Endocrinol. Metab.* **25**: 1196.
19. Hays, M. T. 1968. Absorption of oral thyroxine in man. *J. Clin. Endocrinol. Metab.* **28**: 749.
20. Hays, M. T. 1970. Absorption of triiodothyronine in man. *J. Clin. Endocrinol. Metab.* **30**: 675.
21. Gross, J., and R. Pitt-Rivers. 1953. 3:5:3′-triiodothyronine. 2. Physiological activity. *Biochem. J.* **53**: 652.
22. Braverman, L. E., S. H. Ingbar, and K. Sterling. 1970. Conversion of thyroxine to triiodothyronine in athyreotic human subjects. *J. Clin. Invest.* **49**: 855.
23. Sterling, K. S., M. A. Brenner, and E. S. Newman. 1970. Conversion of thyroxine to triiodothyronine in normal human subjects. *Science (Wash. D. C.).* **169**: 1099.
24. Pittman, C. S., J. B. Chambers, Jr., and V. H. Read. 1971. The extrathyroidal conversion rate of thyroxine to triiodothyronine in normal man. *J. Clin. Invest.* **50**: 1187.
25. Schwartz, H. L., M. I. Surks, and J. H. Oppenheimer. 1971. Quantitation of extrathyroidal conversion of L-thyroxine to 3,5,3′-triiodo-L-thyronine in the rat. *J. Clin. Invest.* **50**: 1124.
26. Schadlow, A. R., M. I. Surks. H. L. Schwartz, and J. H. Oppenheimer. 1972. Specific triiodothyronine binding sites in rat anterior pituitary. *Science (Wash. D. C.).* **176**: 1252.
27. Oppenheimer, J. H., D. Koerner, H. L. Schwartz, and M. I. Surks. 1972. Specific nuclear triiodothyronine binding sites in rat liver and kidney. *J. Clin. Endocrinol Metab.* **35**: 330.
28. Berson, S. A., and R. S. Yalow. 1954. Quantitative aspects of iodine metabolism. The exchangeable organic iodine pool, and the rates of thyroidal secretion, peripheral degradation, and fecal excretion of endogenously synthesized organically bound iodine. *J. Clin. Invest.* **33**: 1533.
29. Ingbar, S. H., and N. Freinkel. 1955. Simultaneous estimation of rates of thyroxine degradation and thyroid hormone synthesis. *J. Clin. Invest.* **34**: 808.
30. Oddie, T. H., D. A. Fisher, and C. Rogers. 1964. Whole-body counting of ^{131}I-labeled thyroxine. *J. Clin. Endocrinol.* **24**: 628.
31. Blomstedt, B., and L. O. Plantin. 1965. The extrathyroidal distribution of ^{131}I-thyroxine. *Acta Endocrinol.* **48**: 536.
32. Surks, M. I., and J. H. Oppenheimer. 1971. Metabolism of phenolic- and tyrosyl-ring labeled L-thyroxine in human beings and rats. *J. Clin. Endocrinol. Metab.* **33**: 612.
33. Dunn, J. T., and J. B. Stanbury. 1958. The metabolism of 3:3′:5′-triiodothyronine in man. *J. Clin. Endocrinol. Metab.* **18**: 713.
34. Greer, M. A., Y. Grimm, and H. Studer. 1968. Qualitative changes in the secretion of thyroid hormones induced by iodine deficiency. *Endocrinology.* **83**: 1193.
35. Chopra, I. J., and G. N. Beall. 1971. Triiodothyronine (T_3) and thyroxine (T_4) ratios (T_3/T_4) in thyroid glands in health and disease. *Clin. Res.* **19**: 181. (Abstr.)
36. Nagataki, S., H. Uchimura, Y. Masuyama, K. Nakao, and K. Ito. 1972. Triiodothyronine and thyroxine in thyroid glands of euthyroid Japanese subjects. *J. Clin. Endocrinol. Metab.* **35**: 18.