Conversion of Thyroxine (T4) to Triiodothyronine (T3) in Athyreotic Human Subjects

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ABSTRACT Studies of the possibility that thyroxine (T4) is converted to 3,5,3′-triiodo-L-thyronine (T3) in the extrathyroidal tissues in man have been conducted in 13 patients, all but two of whom were athyreotic or hypothyroid, and all of whom were receiving at least physiological replacement doses of synthetic sodium-L-thyroxine.

T3 was found in the sera of all patients, in concentrations ranging between 243 and 680 ng/100 ml (normal range 170–270 ng/100 ml). These concentrations were far in excess of those which would have been expected on the basis of the T3 contamination of the administered T4, as measured by the same technique employed in the analysis of serum. When oral medication was enriched with ^{125}I-labeled T4 for 8 or more days, labeled T3 and tetraiodothyroacetic acid (Tetrac or TA_4) were found in the serum to the extent of approximately 2–5% of total radioactivity, as assessed by unidimensional paper chromatography. The same results were obtained with a specially purified lot of radioactive T4 containing less than 0.1% T3 as a contaminant. The identities of the ^{125}I-labeled T3 and TA_4 were verified by two-dimensional chromatography as well as by specific patterns of binding in serum. The labeled T3 isolated was bound by albumin and by T4-binding globulin (TBG), but not by T4-binding prealbumin (TBPA); in contrast the labeled TA_4 was bound by albumin and TBPA, but not by TBG.

To exclude the possibility that the conversion of T4 to T3 was a peculiarity of the oral route of administration, the sera of two additional patients were obtained 48 hr after 7-day courses of daily intravenous injections of a mixture of stable and ^{125}I-labeled T4. Both stable and labeled T3 were likewise found in these sera.

In contrast to earlier experiments in humans in which ^{131}I-labeled T3 was not definitively demonstrated in serum after a single intravenous injection of ^{131}I-labeled T4, the present findings are taken to provide conclusive evidence of the extrathyroidal conversion of T4 to T3 in man. These results raise once again the question of the extent to which the metabolic effect of T4 is mediated through the peripheral generation of T3.

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INTRODUCTION

Since 1952, when 3,5,3′-triiodo-L-thyronine (T3) was found in human plasma by Gross and Pitt-Rivers (1) the thyroid gland, T3 having been found in the thyroid metic potency of thyroxine (T4), a number of questions concerning these hormones have been under consideration. Among these are: (*a*) what are the relative rates of secretion of T4 and T3 by the thyroid gland, (*b*) what are their relative contributions to the over-all metabolic state of the patient, (*c*) to what extent is T4 converted to T3 in the peripheral tissues, and (*d*) to what extent does the metabolic activity of T4 depend upon its conversion to the more highly potent T3.

There is no doubt whatsoever that at least a portion of the T3 in the blood results from direct secretion by the thyroid gland, T3 having been found in the thyroid venous effluent, and in concentrations substantially higher than in the concurrently sampled arterial blood (4, 5). What has remained uncertain, however, is what fraction, if any, of the T3 in the blood arises from the

peripheral monodeiodination of T4. Evidence for the operation of this pathway in animal tissues has been obtained in some experiments conducted in vivo or in vitro, but not in the majority. The situation in humans has been even more clouded. Previous studies have consisted mainly of efforts to identify ^{131}I-labeled T3 after a single injection of ^{131}I-labeled T4 (6) and have led to equivocal results, the most recent study having provided negligible evidence of such conversion (7).

The development of a sensitive and reproducible method for measuring stable T3 in serum prompted the reinvestigation of this problem which is the subject of this report. Stable T3 was sought in the serum of hypothyroid or athyreotic patients being maintained with synthetic sodium-L-thyroxine (Synthroid).[1] In addition, ^{125}I-labeled T3 was sought in their serum after repeated daily administration of ^{125}I-labeled T4. Evidence of the extrathyroidal conversion of T4 to T3 was obtained by both methods.

METHODS

Clinical material

Studies were performed in 13 patients receiving L-T4 therapy for long periods. In all but two, severe hypothyroidism or athyreosis had either been demonstrated by clinical findings and laboratory tests or can be presumed to have been present because total surgical thyroidectomy for carcinoma had been performed. Pertinent clinical and laboratory data of 11 patients are recorded in Table I. The sera from the last five patients were kindly provided by Dr. Carl Feind and all were considered as proven to be athyreotic, except for F.W. who may well have been hypothyroid, although this was not documented.

Two additional patients were subsequently studied while receiving intravenous L-T4 therapy. G.T., a 50 yr old woman with nonfunctioning thyroid adenoma, had a serum PBI of 6.8 μg/100 ml before institution of suppression therapy. On long-term oral L-T4 therapy, 300 μg daily, the 24 hr thyroidal uptake of ^{131}I was 2%, indicating almost complete suppression of thyroid function. J.M., a 25 yr old woman, had a total thyroidectomy and radical neck dissection for thyroid carcinoma, after which she was maintained on 300 μg of L-T4 by mouth per day.

Laboratory techniques

Analyses for the concentration of stable T3 in serum were carried out by the method of Sterling, Bellabarba, Newman, and Brenner (8). Briefly, this procedure consists of (*a*) removal of the thyroid hormones from serum with cation exchange resin columns; (*b*) separation of T3 from T4 by descending paper chromatography with a hexane–tertiary amyl alcohol–ammonia solvent system, and (*c*) measurement of the eluted T3 by the binding displacement technique. This procedure was employed with appropriate adaptations for varying experiments described below.

Analyses for stable T4 concentration in human sera were carried out by the Boston Medical Laboratory, Waltham, Mass., which employs a modification of the method of Murphy and Pattee (9, 10) that achieves a recovery of T4 from serum of approximately 95%.

The labeled T4 and T3 (^{131}I and ^{125}I) were provided by Abbott Laboratories in shipments to which 0.2% cysteine had been added to minimize self-irradiation and deiodination.[2]

Radioactive scanning of paper chromatographic strips was carried out with a modified Nuclear-Chicago Actigraph III, model 1004. Actual quantitation of radioactivity was carried out by cutting the paper strips into 0.5 cm segments which were counted in a Packard Auto-Gamma well-type scintillation spectrometer. The histograms so obtained revealed peaks that were correlated with the scans, but yielded clearer definition of the radioactive compounds and better quantitative data because of the higher counting rates provided by well counting.

By the process employed in its manufacture, the T4-^{125}I is labeled only in its outer (phenolic) ring. Furthermore, in view of the degree of radioiodination achieved by the exchange reaction, only one of the two iodine atoms in the outer ring was labeled (11). Hence, monodeiodination of T4 would yield only one molecule of T3 containing radioiodine for each two molecules formed. Hence, to make data comparable to those for stable T3, measured percentages of T3-^{125}I hereafter described, as well as T3-^{125}I : T4-^{125}I ratios, have been "corrected" by multiplying values for T3-^{125}I by two.

Methodological control experiments

Experiment 1: T3 contamination in administered stable T4. Extensive quantitative analyses were carried out on all lots of stable and radioactive T4 administered, since it was obviously essential to assess T3 contamination in the administered T4. The approach to this problem was a modification of the analytic procedure previously developed (8), including the improved paper chromatographic separation of T3 and T4.

For analyses of a given lot of stable T4, it was found convenient to prepare gravimetrically an alkaline 2 mg/ml stock solution, making appropriate correction for water of crystallization of the sodium salt. This solution was then enriched with a minute tracer of high specific activity (over 50 mCi/mg) T3-^{131}I solution, so that an insignificant amount of T3 (less than 2 μg) was added to the T4 solution. After aliquots had been taken for radioactive assay, the material was subjected to descending chromatography in duplicate and the paper strips were scanned with the modified Actigraph III. The T3 areas were cut out and eluted in methanol-ammonia, dried with nitrogen, and subjected to a second descending paper chromatographic separation. The T3 areas of the second run were eluted, and various aliquots were dried down, assayed for T3-^{131}I recovery, and analyzed by competitive binding displacement. The amounts of stable T3 measured could then be related to the original solution of T4 by using the T3-^{131}I recovery figures.

In practice, the approximate amount of T3 was measured in a preliminary fashion. This was followed by quadruplicate determinations on at least 2 separate days with the usual T3 standards in the nanogram range. According to the manufacturer,[3] Flint's oral Synthroid used in the present

[1] Hereafter referred to as L-T4.

[2] During the course of the work, Abbott Laboratories has adopted the routine addition of cysteine to all lots of labeled T4 and T3 for general use.

[3] Baxter Laboratories, Inc., Morton Grove, Ill. Information supplied by Dr. Leonard G. Ginger.

TABLE I
Clinical Data and Results

Patient, age and sex	Diagnosis	Daily T4 maintenance dose	Basal serum PBI before replacement therapy	Thyroidal uptake of ^{131}I	Simultaneous study values		T4:T3 ratio
					T4	T3	
		μg	μg/100 ml	%	μg/100 ml	ng/100 ml	
H. D., 45, F	Myxedema after ^{131}I therapy for Graves' disease	300	1.6	1	13.5	421 (427*)	32.1:1
L. K., 42, M	Hypothyroidism and mongolism	50	1.5	7	7.0	331 (242*)	21.1:1
R. M., 20, F	Total thyroidectomy for thyroid carcinoma	300	—	—	14.5	655 (639*)	22.1:1
L. O., 22, F	Total thyroidectomy for thyroid carcinoma	300	—	—	15.0	273 (268*)	54.9:1
A. R., 47, F	Myxedema after ^{131}I therapy for Graves' disease	400	1.4	—	18.0	473 (601*)	38.1:1
V. R., 48, F	Myxedema after ^{131}I therapy for Graves' disease	300	1.0	9	15.5	269 (698*)	57.6:1
J. J., 48, F	Total thyroidectomy for thyroid carcinoma	600	—	<1.0‡	14.0	392	35.7:1
E. C., 28, F	Total thyroidectomy for thyroid carcinoma	600	1.0	0.6‡	15.5	392	39.5:1
H. R., 37, F	Total thyroidectomy for thyroid carcinoma	600	1.0	1.3‡	19.0	561	33.8:1
G. W., 43, M	Total thyroidectomy for thyroid carcinoma followed by 100 mCi ^{131}I	600	1.1§	0.5‡	14.0	680	20.6:1
F. W., 50, F	Subtotal thyroidectomy for nontoxic nodular goiter with occult carcinoma	450	—	—	11.5	521	22.1:1
	Mean				14.3	452	34.3:1
	Standard deviation				3.1	104	12.9:1

* These values indicate T3 concentrations by gas-liquid chromatography kindly performed by Dr. Charles S. Hollander.
‡ The thyroidal uptakes were determined 48 hr after administration of 1 mCi of ^{131}I and before institution of T4 replacement therapy. The low values obtained indicated the success in ablation of the thyroid gland. In contrast, the other three uptake values were at 24 hr, and were carried out during T4 replacement therapy.
§ Simultaneous T4 iodine by column was 0.4 μg/100 ml (Bio-Science Laboratories).

studies was all derived from the parent Baxter lot No. FE-8B. The analyses of this material showed it to contain 1.00 ±0.13% of stable T3 (mean ±SD). In contrast, the injectable Synthroid, derived from Baxter's lot No. FE-6, was found to contain 0.22% ±0.018% of stable T3 contaminant.

Experiment 2: Influence of tetraiodothyroacetic acid (Tetrac or TA_4) upon the displacement assay for T3. Because of the position of Tetrac in the zone between T4 and T3 on descending paper chromatography, it was important to evaluate the possible influence of this compound on the binding displacement analysis for T3. Studies of this question revealed Tetrac to have no detectable effect, even at concentrations equivalent to a serum concentration of 6 μg/100 ml.

Experiment 3: Conversion of T4 to T3 in analytic systems. Purified T4 preparations, both radioactive and stable, were found to give rise to no detectable T3 during the course of descending chromatography, as evaluated by radioactive scanning or by displacement assay. In other experiments, stable T4 was added to serum which was then subjected to the complete analytic procedure. When sera were enriched with concentrations of T4 as much as sixtyfold greater than their concentration of T3, no significant elevation of the T3 concentrations in these sera was detected.

RESULTS

Experiment 1: Measurements of stable T3 in sera. Stable T3 (T3-^{127}I) was detected in the sera of all sub-

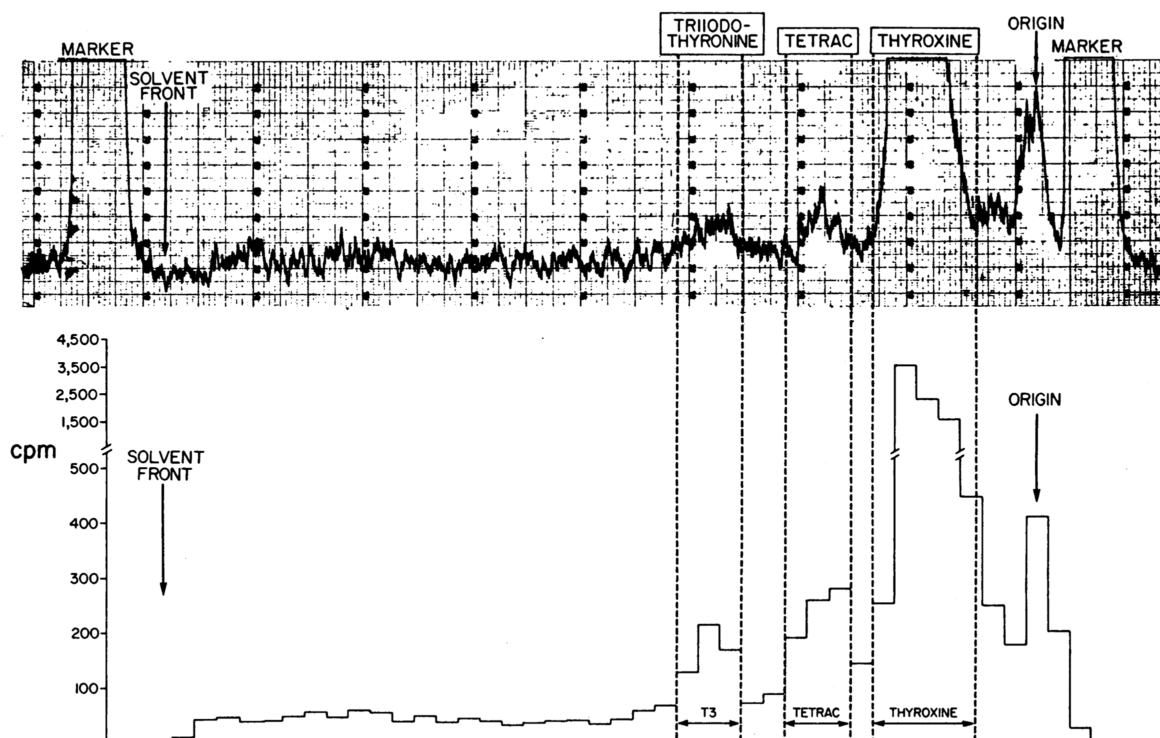

FIGURE 1 Radioactive scan and histogram of paper chromatogram of serum of H. D. after receiving oral stable and radioactive (^{125}I-labeled) T4. In the upper portion, in addition to the large truncated T4 peak and the origin peak, small peaks in the T3 and Tetrac zones are recognizable. Scanning carried out at 7.5 cm/hr with a 6 mm slit width.

In the histogram in the lower portion, the break in the ordinate scale permitted plotting of the large T4 peak. The counts were made on 0.5 cm segments of the paper strip.

jects receiving oral L-T4 (Table I). Serum T4 concentrations varied between 7 and 19 μg/100 ml, and averaged 14.3 μg/100 ml. Serum T3 concentrations varied between 269 and 680 ng/100 ml and averaged 452 ng/100 ml. The T3 values, therefore, were mainly above the normal range (170–270 ng/100 ml) and normal mean ±SD (220 ±27 ng/100 ml) (8). The mean value of the T4: T3 ratio in the sera of patients receiving oral L-T4 was 34:1 (Table I).

Sera of five athyreotic patients on T4 maintenance, kindly supplied by Dr. Samuel P. Asper, Jr., were also studied, and these too revealed T3 in all instances. The values ranged between 396 and 735 ng/100 ml, the latter in a patient maintained on 450 μg of L-T4 daily. The figures from Dr. Asper's sera have not been tabulated because of lack of simultaneous serum T4 measurements.

Confirmation of the presence of stable T3 in six of the initial eleven sera was kindly provided by Dr. Charles S. Hollander, who has employed gas-liquid chromatography (GLC) for the analysis of T3 in serum (12, 13). The values obtained by GLC are indicated in Table I and agree remarkably well with those obtained by displacement analysis in five of six instances.

Experiment 2: Measurement of stable and radioactive T3 in serum after the addition of T4-^{125}I to the daily oral L-T4 regimen. To obtain further verification of the apparent conversion of T4 to T3, additional studies were carried out in three of the foregoing patients in whom T4-^{125}I was added to the daily oral dose of stable T4. The total daily dose of L-T4 was placed within a gelatin capsule which had been partially filled with lactose, and ^{125}I-labeled T4 (15–20 μCi) was pipetted into the mixture. The capsules were sealed and refrigerated until taken orally. Samples of serum were obtained from patients V. R., H. D., and L. O. 2 days after a 20 day course of therapy had been temporarily discontinued.

A ^{125}I-labeled compound having the chromatographic mobility of T3 was found in all the sera tested.

In addition to a moderate peak at the origin and a very large T4 peak, scans of unidimensional chromatograms of serum extracts revealed small but distinct peaks of radioactivity in the areas occupied by tetraiodothyroacetic acid (TA_4, Tetrac) and by T3. The mo-

bility of the T3 peak was verified both by separate chromatograms in which T3-^{131}I was added at the origin to the hormones extracted from serum and by parallel standard strips containing tracer T3 and T4. A typical scan of the unidimensional chromatogram of radioactive compounds extracted from the serum of a patient (H. D.) is shown in Fig. 1.

On the basis of the histogram depicting the ^{125}I content of successive segments of chromatographic strips (Fig. 1), from 1.5 to 5.4% of the total serum radioactivity was present as Tetrac, while from 2.0 to 4.6% (corrected) of the total radioactivity was comprised of T3 (Table II).

Experiment 3: Measurement of stable and radioactive T3 in serum after addition of purified T4-^{125}I to daily oral L-*T4 maintenance.* T4-^{125}I was provided as a special lot which had been dried down from a methanol-ammonia solution before shipment from Abbott Laboratories. This facilitated further chromatographic purification. Initial chromatographic analysis revealed 4.6% T3 contamination. After repurification by descending paper chromatography, T3 contamination was decreased to less than 0.1%. As before, the tracer T4-^{125}I was added to the daily dose of L-T4 placed within gelatin capsules.

Subjects R. M. and A. R. received L-T4 containing T4-^{125}I daily for 8 days and serum samples were obtained 48 hr after the last dose. As in experiment 2, ^{125}I-labeled compounds with the chromatographic mobility of T3 and Tetrac were observed, and in proportions at least as large as those found with the less highly purified T4-^{125}I used in experiment 2 (Table II).

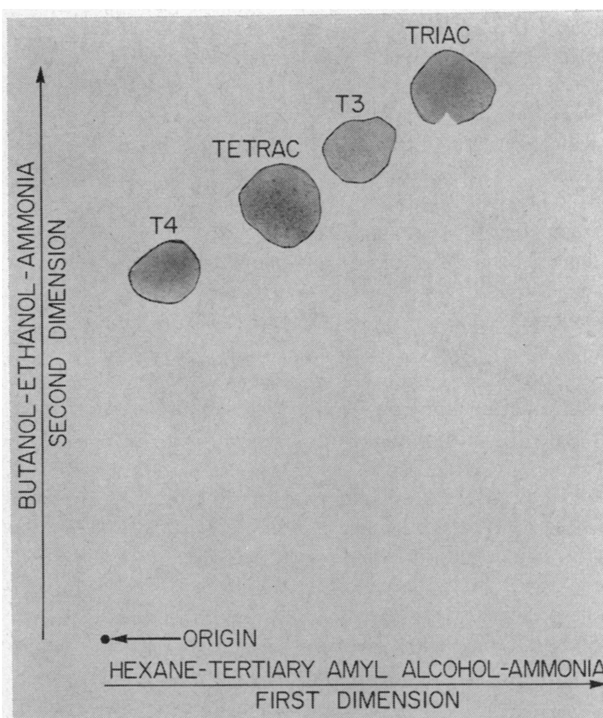

FIGURE 2 Migration of carrier compounds during two-dimensional chromatography. The photograph illustrates the separation between the nonradioactive carriers, which were stained by spraying lightly with 4-aminoantipyrene and potassium ferricyanide. In this chromatogram, the material applied at the origin was eluate containing ^{125}I-labeled Tetrac recovered from the second of two unidimensional chromatographic separations of this zone from an extract of the serum of patient A. R.

TABLE II
Metabolic Products of Labeled Thyroxine

	% Total ^{125}I	
Patient	Tetrac	T3 (corrected)*
V. R.	2.6	3.2
H. D.	3.1	2.7
L. O.	1.5	2.0
R. M.‡	4.1	4.5
A. R.‡	5.4	4.6
G. T.§	2.0	4.1
J. M.§	2.0	3.7
Mean	2.9	3.5

* Values for T3-^{125}I corrected by multiplying measured values by two, since monodeiodination of T4-^{125}I would yield only one molecule of T3-^{125}I for each two molecules of T3 formed.
‡ Subjects received the purified T4-^{125}I which contained less than 0.1% T3 (experiment 3).
§ Received the stable and radioactive T4 by the intravenous route (experiment 4).

The identification of the T3 and Tetrac present in the serum of these patients was verified by two-dimensional chromatography. After a unidimensional run, the appropriate zones of the papers were excised, eluted, and rechromatographed. The major peaks were then subjected to two-dimensional chromatography together with four carrier compounds: T4, T3, Tetrac, and Triac (triiodothyroacetic acid). The first dimension was hexane–tertiary amyl alcohol–ammonia and the second dimension was butanol-ethanol-ammonia. The position of the carriers was localized by lightly spraying with 2% 4-aminoantipyrine solution in 2% sodium carbonate followed by light spraying with 2% potassium ferricyanide (14, 15). Adequate separation of carrier components was obtained (Fig. 2). The carrier zones were cut out and counted; the results obtained in serum from R. M., one of the two samples studied, afforded strong corroboration. The result of the two-dimensional analysis of ^{125}I in the unidimensional T3 zone revealed that 76% of the counts were localized in the two-dimensionally separated T3 spot, rather small proportions

(6.4, 10.8, and 6.8%), begin found in the Triac, Tetrac, and T4 spots, respectively. In the comparable analysis of the Tetrac spot, 91% of ^{125}I was found in the two-dimensionally separated Tetrac spot, with quite small proportions (0.0, 2.7, and 6.6%) in the other areas (Triac, T3, and T4, respectively). The findings in A. R. were similar: 74% of the radioactivity in the two-dimensionally separated T3 spot, and 85% in the Tetrac spot. The small or almost undetectable amounts of radioactivity in the other areas were ascribed to "trailing," which is apparently unavoidable in two-dimensional chromatography.[4]

Finally, the Tetrac obtained from the serum of A. R. by chromatography was subjected to an additional single-dimension chromatographic separation. The concentrated, purified Tetrac from two such procedures was pooled to afford a sufficient counting rate. This material was spotted at the origin of a dry paper strip. After the strip had been saturated with the glycine acetate system used for paper electrophoresis (16, 17), normal human serum (8 µl) was also added at the origin. Aliquots of the same serum were enriched with tracer T3-^{131}I or T4-^{131}I and all strips were subjected to electrophoresis simultaneously. Radioactive scans revealed the labeled material from the myxedematous subject's serum to be bound to albumin and T4-binding prealbumin (TBPA), but not to the T4-binding globulin (TBG). This pattern of binding is that which is characteristic of the deaminated derivatives of T4 and T3 (18, 19). Similar studies were carried out with pooled, twice chromatographed T3 from sera of G. T. and J. M. In this instance, the radioactive material from the subjects' sera was bound by TBG and albumin, but not by TBPA, as is compatible with the behavior of T3 (18, 19).

Experiment 4: Measurement of stable and radioactive T3 in serum in patients receiving L-*T4 and T4-^{125}I intravenously.* The foregoing data, which strongly indicate that T4 undergoes peripheral conversion to T3 and Tetrac, were all derived from patients who were receiving chronic T4 therapy by the oral route. It seemed possible that conversion of T4 to T3 might take place within the lumen of the gut, a process that could have little relevance to the fate of endogenously secreted hormone. Furthermore, after oral administration, hepatic metabolism of administered T4 could conceivably be accentuated, with the result that more T3 might be found than would be the case with T4 of endogenous origin.

Consequently, studies were performed in two patients (G. T. and J. M.) in whom oral L-T4 therapy was replaced by a 7 day course of daily intravenous injections of 200 µg of stable T4 and 30 µCi of T4-^{125}I. After a 48 hr period in which neither oral nor intravenous therapy was given, sera were obtained for analysis. In both patients, stable T3 was found in the serum, 541 ng/100 ml in G. T. and 416 ng/100 ml in J. M. Moreover, radioactive scans of chromatograms of serum extracts revealed labeled T3 and Tetrac even more clearly than had been the case in studies of patients receiving oral therapy (Fig. 3). In the two patients, labeled Tetrac was present to the extent of 2.0% of total radioactivity in both, and T3-^{125}I to corrected percentages of 4.1 and 3.7.

[4] Sterling, K., M. A. Brenner, and E. S. Newman. 1968–69. Unpublished observations.

DISCUSSION

The present studies provide strong evidence that significant quantities of T4 are converted to T3 in the peripheral tissues of man. This conclusion is based on the finding of both stable and radioiodine-labeled T3 in the serum of patients lacking thyroid function who were given mixtures of stable and ^{125}I-labeled T4. Other than peripheral conversion of T4 to T3, two major possible explanations for this finding warranted consideration. First, T3 may have been present in the serum as a result of contamination of the administered T4 by T3. Several lines of evidence seem adequate to exclude this possibility. As judged from findings in patients with normal thyroid function, the rate of clearance of T3 from the plasma is about 20 times that of T4 (20–25). Hence, even if the stable T4 administered were contaminated with 1% of T3, as was the case with the oral preparation, a T4:T3 ratio of approximately 2000:1 would have been anticipated at equilibrium. In the case of the intravenous preparation of L-T4, a lesser degree of contamination was found, and an even higher T4:T3 ratio in the blood would have been expected (approximately 10,000:1). Moreover, analyses were performed on sera obtained 48 hr after the last dose of T4; hence, the far more rapid rate of clearance of T3 than of T4 should have raised the T4:T3 ratio still further. In fact, for all of the subjects studied, the observed T4:T3 ratio averaged only 34:1. Similar considerations apply to the data with respect to the ^{125}I-labeled T4:T3 ratios, which were also far less than would have been expected from the measured contamination, particularly since one batch of T4-^{125}I employed was almost totally devoid of contaminating T3 (experiment 3).

A second possibility is that the T3 found in the serum arose as the result of artefactual deiodination of T4, either during storage of sera or during the separative or analytic procedures. This possibility also seems remote. Purified T4-^{125}I containing less than 0.1% T3-^{125}I remained free of T3-^{125}I in repeated chromatographic studies carried out during a 10 day period of storage. This was also true of the ^{125}I-labeled materials

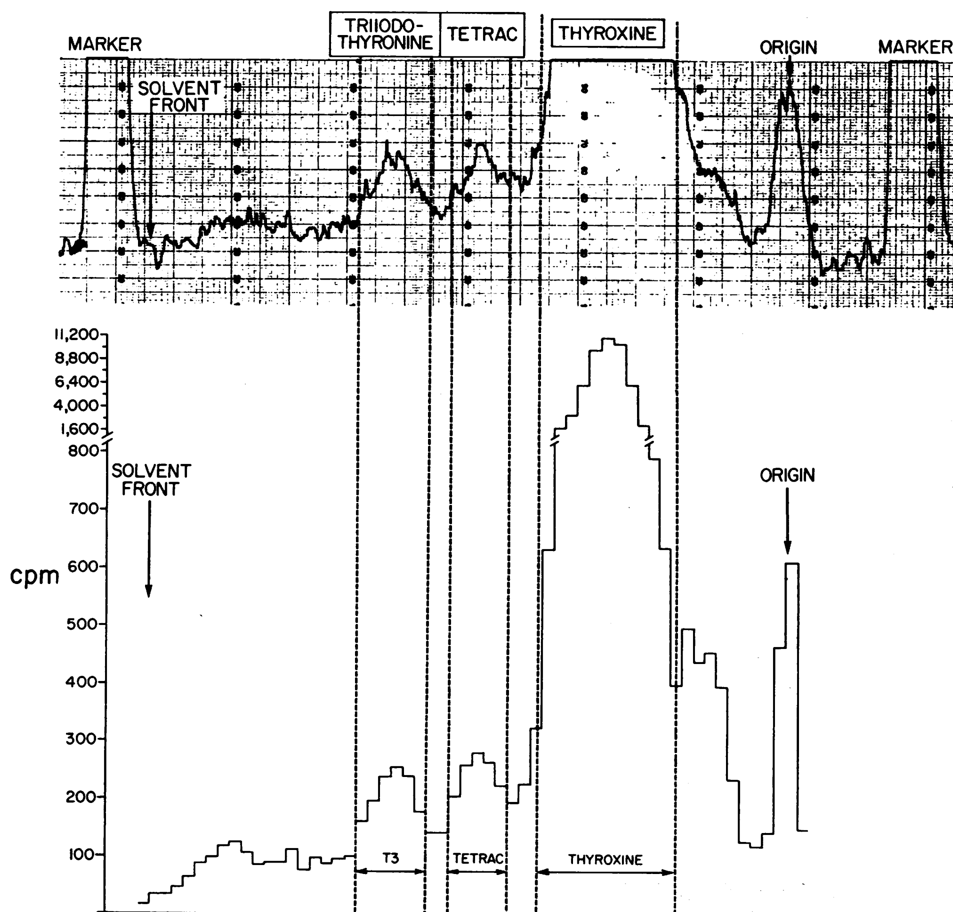

FIGURE 3 Radioactive scan and histogram of paper chromatogram of extract of serum of Patient G. T. The patient received a mixture of stable and ^{125}I-labeled T4 intravenously for 7 days and serum was obtained 2 days later. In the upper portion, in addition to the large truncated T4 peak and the origin peak, small peaks of T3 and Tetrac are clearly evident. Scanning carried out at 7.5 cm/hr with a 12 mm slit width.

In the histogram in the lower portion, the break in the ordinate scale permitted plotting of the large T4 peak. The counts were made on 0.5 cm segments of the paper strip.

contained in the capsules prepared for oral administration and studied again after periods of refrigeration longer than 20 days. Furthermore, when subjected to the same chromatographic procedure, the original lots of stable L-T4 contained smaller proportions of T3 than did the sera of patients. Moreover, the addition of high concentrations of stable T4 to serum which was then carried through the entire analytic procedure resulted in no detectable increase from the previously measured T3 concentration.

Further evidence has been provided by a current investigation by Sterling, Brenner, Newman, Read, and Pittman (26) in which purified ^{3}H-labeled T4 was added to serum and run through the analytic procedure. The absence of significant ^{3}H in the T3 zone of chromatographic strips confirms the lack of artefactual T3 formation. Finally, in five of six instances, values for the concentration of stable T3 in the serum measured by gas chromatographic analysis agreed closely with those obtained by the binding displacement technique routinely used in the present studies. Hence, it would be difficult indeed to ascribe the T3 found in the serum of athyreotic patients receiving T4 to artefactual deiodination of the latter hormone.

Thus, it seems clear that the T3 found in the serum was formed in vivo. It also seems the case that it was not the result of a peculiarity in the metabolism of orally administered T4, since both stable and radioactive T3 were found in the sera of patients given stable and ^{125}I-labeled T4 intravenously, and in amounts similar to those present after oral administration.

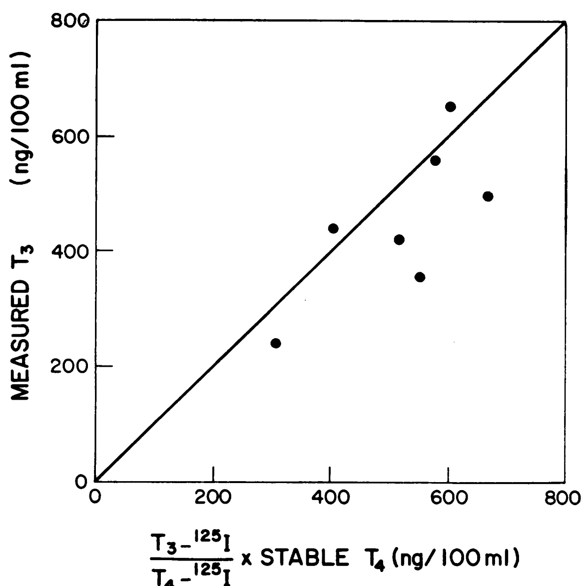

FIGURE 4 Concordance between measured and calculated T3 concentrations. The points plotted represent values obtained simultaneously by stable and radioactive T3 assays. The T3-^{125}I : T4-^{125}I ratio shown is a corrected ratio derived by multiplying values for T3-^{125}I by 2, since monodeiodination of T4-^{125}I would yield only one labeled T3 molecule for each two molecules of T3 formed. The diagonal line is the line of equivalence.

A variety of in vitro studies have suggested that T4 may be converted to T3 by peripheral tissues (14, 27–29). Previous studies of this question in man have been few in number and contradictory in results (6, 7). These studies differed in approach from those presently described in that labeled T3 was sought in serum after a single intravenous injection of labeled T4. This experimental design would tend to minimize the likelihood of detecting any labeled T3 formed.

It would appear, in contrast, that the present experimental approach, in which both stable and labeled T4 were administered chronically, not only allowed demonstration of T3, but permitted the attainment of isotopic equilibrium.

The relatively close agreement between stable and radioactive T4 : T3 ratios is depicted in Fig. 4, wherein the measured stable T3 concentration is plotted against a value for stable T3 calculated as the product of the measured serum T4 concentration and the corrected T3-^{125}I : T4-^{125}I ratio. Considering the potential errors in all the measurements involved, the satisfactory agreement was considered to afford convincing corroboration that an isotopic equilibrium state had been achieved.

Although greater interest may attach to the generation of T3, owing to its greater metabolic potency, the present studies seem clearly to demonstrate that Tetrac arises from T4 in vivo. This pathway of T4 metabolism has been shown to be operative in animals (30–33), but, to our knowledge, has not hitherto been shown to function in man. The proportions of labeled Tetrac and T3 found in the serum after administration of labeled T4 were quite similar. Hence, it would appear that relatively more T3 than Tetrac is generated from T4, since the rate of clearance of Tetrac from plasma is normally much less than that of T3 (34). However, it is unknown to what extent each compound may be either deiodinated, conjugated, or otherwise degraded at its site of formation, preventing entry into the plasma compartment. As a result, reliable estimates of the relative rates of generation of Tetrac and T3 cannot be made.

One disturbing feature of the present observations is the question of why patients in the present study were not frankly thyrotoxic. Some of the patients were receiving 600-μg daily doses of T4 for maintenance above the normal level in the effort to minimize the risk of recurrence of thyroid malignancy. Despite a few borderline toxic manifestations, the general clinical appearance indicated definitely euthyroid status in the majority of cases. Despite the high concentrations of both hormones, one cannot assume markedly elevated removal rates in these patients in the absence of kinetic data. Even after many months of replacement therapy, persistently prolonged biologic half-time of T4 has been observed in myxedema (35). It has generally been assumed that maintenance of a euthyroid state with L-T4 requires doses sufficient to raise the serum T4 concentration well above the normal range, since the metabolic contribution normally afforded by T3 is lacking. On superficial examination, the findings and conclusions of the present study would seem in conflict with this view, since both T4 and T3 concentrations in serum were often within the thyrotoxin range. What is most relevant to the metabolic state of the patient, however, is probably not the serum concentrations of T4 and T3, but their over-all rates of production and disposal. In frankly thyrotoxic patients, fractional rates of turnover of both hormones are increased (20, 36, 37); as a result, relative to the normal, disposal rates are underestimated from an examination of serum concentrations alone. No data are available, however, concerning the rates of clearance of T4 and T3 from the blood in patients receiving maintenance therapy with L-T4. In euthyroid subjects, elevation of the serum T4 concentration by an acute intravenous load of T4 results in slowing of the fractional rate of T3 turnover (38), and it is possible that a similar mechanism is operative in the present patients. If so, then the over-all rate of T3 production and disposal in these patients would be far less than that in thyrotoxic patients at the same

concentration of T3 in the serum. Clearly, resolution of this question requires studies of the rates of peripheral turnover of T4 and T3 in patients receiving L-T4 therapy, and such are currently in progress.

If one assumes that in patients receiving L-T4, as in normals, T3 is cleared from the plasma about 20 times faster than T4, then the present data would suggest that a substantial fraction, perhaps as much as half, of T4 is converted to T3. However, since the validity of this assumption is uncertain and since the compartmental metabolism of T3 generated from T4 is unknown, estimates of the proportion of T4 which gives rise to T3 must be quite speculative. Despite this, the presently demonstrated peripheral conversion of T4 to T3 raises once again questions as to the ultimate relative contributions of T4 and T3 to the metabolic state of the patient and as to whether T4 itself has a primary action or exerts its effects only after transformation to T3.

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