3,3'-Diiodothyronine Production, a Major Pathway of Peripheral Iodothyronine Metabolism in Man

LAURENCE A. GAVIN, MARGARET E. HAMMOND, JAMES N. CASTLE, and RALPH R.
CAVALIERI, Medical and Nuclear Medicine Services, Veterans Administration
Hospital and Department of Medicine and Radiology, University of California,
San Francisco, California 94121

A B S T R A C T 3,3'-Diiodothyronine (3,3'-T₂) has been
detected in human serum and in thyroglobulin. How-
ever, no quantitative assessment of its clearance rate
(CR), production rate (PR), or of the importance of ex-
trathyroidal sources of 3,3'-T₂ relative to direct thy-
roidal secretion is yet available. This study examines
these parameters in seven euthyroid subjects, and in
eight athyreotic subjects (H) euematabolic due to thy-
roxine therapy (HT₃) (n = 5) or triiodothyronine re-
placement (HT₃) (n = 3). A highly specific radioim-
unoassay for the measurement of 3,3'-T₂ in whole
serum was developed. Serum 3,3'-T₂ concentrations
were (mean ± SD) 6.0±1.0 ng/100 ml in 13 normal sub-
jects, 9.0±4.6 ng/100 ml in 25 hyperthyroid patients,
and 2.7±1.1 ng/100 ml in 17 hypothyroid patients.
The values in each of the latter two groups were signifi-
cantly different from normal. 3,3'-T₂ was detected regu-
larly in normal concentrations in 11 hypothyroid pa-
tients euematabolic by treatment with synthetic T₄, in
10 euematabolic patients suffering from nonthyroidal
systemic illness, and in 2 subjects with elevated serum
T₄-binding globulin. The 3,3'-T₂-CR was assessed from
data acquired from the ¹³¹I-3,3'-T₂ constant infusion
technique. The 3,3'-T₂-PR was calculated from CR and
and serum concentration of 3,3'-T₂ determined by radio-
immunoassay. In the HT₃ subjects the 3,3'-T₂-CR av-
eraged 840±377 liters/day and 3,3'-T₂ PR 33.9±12.5
µg/day. These results were not significantly different
from those in the control group: 3,3'-T₂-CR 628±218
liters/day and 3,3'-T₂ PR 39.8±19.8 µg/day (all cor-
corrected to 70 kg body wt). In addition to 3,3'-T₂ PR,
T₃ and reverse triiodothyronine (rT₃) were deter-
mined in three of the HT₃ subjects. In each case stud-
ied, the 3,3'-T₂ PR was close to the combined tri-
iothyronine (T₃ + rT₃) PR. The mean molar ratio of
T₂ PR/(T₃ + rT₃) PR was 1.08±0.10. The results ob-
tained in the HT₃ subjects indicate that the production
of 3,3'-T₂ is a major route of T₄ metabolism. The com-
bined studies of 3,3'-T₂, T₃, and rT₃ PR in the HT₃
subjects indicate that both T₃ and rT₃ are major pre-
cursors of 3,3'-T₂. In the HT₃ subjects, the conversion
of T₃ to 3,3'-T₂, determined as the molar ratio of 3,3'-
T₂ PR to T₃ PR, ranged from 0.36 to 0.92, providing
further evidence that T₃ is a precursor of 3,3'-T₂. From
the close agreement between the mean values for 3,3'-
T₂ PR in the euthyroid and HT₃ group it is concluded
that most, if not all of the 3,3'-T₂ produced in normal
humans is derived by extrathyroidal conversion from
T₃ and rT₃.

INTRODUCTION

The presence of 3,3'-diiodothyronine (3,3'-T₂)¹ in
the thyroid gland and the plasma of the rat was de-
monstrated originally by Roche et al. (1). Subsequently,
the same group showed that radioiodine-labeled 3,3',5'-
triiodothyronine (T₃) and 3,3',5'-triiodothyronine (re-
verse T₃[rT₃]) were converted to 3,3'-T₂ in the rat (2).
Flock et al. (3, 4) reported similar findings in the in-
tact dog. Chopra et al. (5) have recently presented evi-
dence in rat liver homogenates that the conversion of
rT₃ and of T₃ to 3,3'-T₂ is enzymatic in nature.

Three groups have described radioimmunoassays
for 3,3'-T₂ in normal human serum, although the re-
ported levels do not agree (6–8). No quantitative esti-
mates have been made, however, of the turnover of
3,3'-T₂ in humans. In an early study, Stanbury and
Morris (9) using ¹³¹I-3,3'-D,L-T₂ administered to nor-

¹Abbreviations used in this paper: ANS, 8-anilino-L-napha-
line sulfonic acid; C, control group; CR, clearance rate; CV,
coefficient of variance; H, hypothroid group; PR, production
rate; 3,3'-T₂, 3,3'-L-diiodothyronine; T₃, triiodothyronine; rT₃,
reverse T₃; T₄, thyroxine; TBG, thyroxine-binding globulin.

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mal subjects in a single bolus injection, found extremely rapid disappearance of tracer from plasma and accumulation of radioiodide in the urine, indicating a high rate of deiodination of this compound.

The present study was initiated to assess the absolute production rate of 3,3'-T₃ in man, using the technique of constant tracer infusion to estimate plasma clearance and a specific radioimmunoassay to measure plasma concentration of this substance. In addition to studies of subjects with intact thyroid glands, measurements of 3,3'-T₃ kinetics were done in athyreotic patients who were maintained euthyroid on L-thyroxine (T₄) to determine the importance of extrathyroidal sources of 3,3'-T₃ relative to direct thyroid secretion. In some of the athyreotic subjects on T₄ therapy, turnover studies of T₂ and rT₃ were performed as well. In three additional cases receiving T₃ as maintenance therapy, T₂ and 3,3'-T₂ kinetics were determined. These studies allowed an assessment of the relative contributions of T₃ and of rT₃ as immediate precursors of 3,3'-T₂ in humans.

METHODS

The subjects participating in this investigation were from the outpatient service of the San Francisco Veterans Administration Hospital. All subjects gave written consent, having been informed of the purpose and nature of the study. Seven male euthyroid control subjects included four normal volunteers and three patients with mild nonthyroidal, nonsystemic illnesses. The eight athyreotic adult males were euthyroid by clinical and laboratory criteria at the time of study. Five subjects were receiving full replacement doses of L-T₄ (Synthroid, Flint Laboratories, Deerfield, Ill.) and three were maintained on L-T₃ (Cytomel, Smith Kline & French Laboratories, Philadelphia, Pa.). None of the patients was taking any drug known to interfere with normal thyroid function or to affect the metabolism of thyroid hormones. The mean T₄ replacement dose in the athyreotics was 145 μg/day.

Radioimmunoassay of 3,3'-T₂. Unlabeled 3,3'-L-T₃ and the various other thyroid hormone analogues and derivatives were obtained through the courtesy of Dr. Eugene Jorgensen, University of California, San Francisco, Calif. The 125I-labeled 1,3,3'-T₂ (labeled presumably in the 3'-position) was prepared from 3,3'-L-T₃ by Abbott Laboratories, North Chicago, Ill. Specific activity ranged from 250 to 350 μCi/μg. At the time of use, each lot of labeled tracer was analyzed for radiochemical purity by Sephadex G-25 column chromatography (by the method described below) and was found to contain <5% of labeled iodide and <1% of labeled rT₃. A conjugate of 3,3'-T₂ and 3,3'-T₃—bovine serum was prepared by the method of Gharib et al. (10). The carbodiimide (1-cyclohexyl-3-carbodiimide metho-p-toluenesulfonate) was obtained from Aldrich Chemical Co., Inc., Milwaukee, Wis., and dimethylformamide from Fisher Scientific Co., Pittsburgh, Pa. Anti-serum was harvested from rabbits immunized with the 3,3'-T₂—bovine serum albumin conjugate emulsified in complete Freund's adjuvant (Miles Laboratories, Inc., Elkhart, Ind.) and pertussis vaccine (El Lilly and Company, Indianapolis, Ind.). The antisera selected for the present study bound 30–35% of tracer amounts of radioactive 3,3'-T₂ in a final dilution of 1:10,000. The binding of ligand to serum proteins was inhibited by 8-anilino-2-naphthalene sulfonic acid (ANS), supplied by Eastman Kodak Co., Rochester, N. Y.

To determine the need for ANS in our assay we compared the percent (% B/T) of ¹²⁵I-3,3'-T₂ bound by a fixed concentration of antisera in barbital buffer (0.07 M barbital, pH 8.6) with that bound in iodothyronine-free serum (11). In the absence of ANS, antibody bound only 8–10% of ¹²⁵I-3,3'-T₂ in serum compared to 50–60% in barbital. From a series of experiments utilizing from 75 to 500 μg ANS per tube, we determined that 450 μg per tube allowed optimum binding (30%) of ¹²⁵I-3,3'-T₂ by our antiserum in iodothyronine-free serum.

Serum 3,3'-T₂ was determined in unextracted serum samples. The various reagents were added in the following order: (a) 200 μl of test sample; (b) 300 μl of 2% normal rabbit serum in barbital buffer (0.07 M barbital, pH 8.6); (c) 100 μl (450 μg) of ANS; (d) 10 μl (5 pg) of ¹²⁵I-labeled 3,3'-T₂; (e) 0.1 μl of 11,250 diluted T₂-binding antisem. The samples were incubated for 24 h at 4°C. A sufficient quantity (50 μl) of previously titered goat anti-rabbit gamma globulin (Antibodies Inc., Davis, Calif.) was then added and the mixture further incubated for 24 h at 4°C. The bound precipitate was separated by centrifugation and aspiration of the supernate. Antibody-bound radioactivity was determined in an automatic well counter. Standards containing stable 3,3'-T₂ (0.5–100 ng/dl) were prepared in iodothyronine-free serum (11) and were processed by the same method. A standard curve was constructed as percent bound ¹²⁵I-3,3'-T₂ vs. concentration of 3,3'-T₂. The 3,3'-T₂ concentration in 0.2 ml of test serum was read from the standard curve and the results were expressed in nanograms of 3,3'-T₂/100 ml. Total serum T₂ and rT₂ were measured by radioimmunoassay as previously described (12). Total serum T₄ and free T₄ index were determined by a competitive binding assay (13).

Preparation of 3,3'-T₂ conjugates. ¹²⁵I-labeled 3,3'-T₂ was injected into the inferior vena cava of an adult male Sprague-Dawley rat. Bile was collected via a biliary cannula for 4 h postinjection. The radioactive components of the bile were separated by thin-layer chromatography of 30 × 20 cm glass-fiber sheets (Gelman Instrument Co., Ann Arbor, Mich.). Chromatograms were developed in butanol-dioxane-2 N ammonia, 4:1:5 (vol/vol/vol), at 25°C for 1½ h, and dried in a stream of nitrogen. The radioactive bands were located by autoradiography of the dried chromatogram and the various components eluted from the chromatogram in methanol-ammonia (99:1). The 3,3'-T₂ glucuronide and sulfo-conjugate zones were identified by enzymatic hydrolysis using glucuronidase (Worthington Biochemical Corp., Freehold, N. J.) and Mylase-P (ICN Pharmaceuticals, Inc., Life Sciences Group, Cleveland, Ohio), respectively (14–16).

Constant-infusion 3,3'-T₂ studies. Subjects were hospitalized on a metabolic ward for the duration of the study. Thyroid radioiodine uptake was minimized in all euthyroid subjects by giving a saturated solution of potassium iodide orally, 10 drops every 8 h before and during the study. ¹²⁵I-3,3'-T₂, diluted in 1% human serum albumin in normal saline to a concentration of 8 μCi/ml, was sterilized by Millipore filtration (Millipore Corp., Bedford, Mass.) before administration to the subjects. A total dose of 200 μCi (25 ml) was administered by intravenous infusion over 8 h at a constant rate (3.1 ml/h) in each subject. The constant infusion was given with a Harvard Infusion Pump (Harvard Apparatus Co., Inc., Millis, Mass.). The accuracy of the rate of infusion was checked on several occasions in control runs by measuring H₂O infusion into a graduated cylinder over 8 h and also during the actual infusions by recording the volume infused per hour from the graduated infusion syringe. We always prepared 30 ml of tracer solution, as extra volume was needed to fill the tubing connecting the pump (syringe) with the patient. Aliquots for standards were
taken from the solution remaining in the tubing at the completion of the infusion. Samples of blood were obtained at time 0 and every 2 h during the infusion.

T₃ and rT₃ kinetics studies. ¹³¹I-T₃ was obtained from Amersham Corp. (Arlington Heights, Ill.) at a specific radioactivity of ≈40 μCi/μg. ¹³¹I-rT₃ was supplied by Abbott Laboratories at a specific radioactivity of 500 μCi/μg. Before administration to human subjects a mixture of ¹³¹I-T₃ and ¹³¹I-rT₃, diluted in 1% human serum albumin-normal saline, was sterilized by Millipore filtration. Tracer doses of ¹³¹I-T₃ (100 μCi) and ¹³¹I-rT₃ (200 μCi), in an accurately measured volume, were injected intravenously. Samples of blood were collected at 15, 30, 60 min and 2, 4, 6, 12 h, and every 12 h thereafter until 72 h after the doses were administered.

In all tracer studies, dose standards were prepared by adding aliquots of tracer doses to undiluted pooled normal serum. These standards were prepared in an identical manner to the plasma samples.

Analysis of sera from kinetics studies. Multiple serum samples, collected during the ¹³¹I-3,3'-T₂ infusion studies, were analyzed by column chromatography employing Sephadex G-25F, by a modification of the method described by Green (17). A column of Sephadex G-25F, bed volume 2.05 x 25 cm, was equilibrated with 0.5 M NaCl-0.054 N NaOH (pH 11.3). Samples and standards (3 ml) were diluted with an equal volume of diluent (1.0 M NaCl in 0.2 M phosphate buffer, pH 6.5) before application to the column. Iodoprotein, iodide, and ¹³¹I-3,3'-T₂ were eluted in succession by 0.005 M NaCl + 0.1 M NaOH, pH 11.3. 20 mM sodium metabisulphite was added to the equilibrating and eluting solution to minimize deiodination. From 140 to 160 fractions (3 ml each) were collected. Each fraction was counted (10 min) in an automatic well-type gamma counter (mean background = 40 cpm). Those fractions included in the ¹³¹I-3,3'-T₂ region (fractions 75-120, approximately) were counted again for longer periods, up to 100 min each, to obtain a relative counting error of 3% or less.

An average of 98.2±4.7% (SD) of the total ¹³¹I in samples and standards applied (40 runs) to the column was recovered in the eluate. Of the total ¹³¹I in the standards prepared from the infusion, 96.6±7.5% was eluted as ¹³¹I-3,3'-T₂. The remainder of the ¹³¹I was in the form of iodide and iodoprotein in approximately equal proportions. The mean ¹³¹I-3,3'-T₂ concentration in two or more samples taken during the steady-state was used in the determination of the clearance rate (CR). ¹³¹I-T₃ and ¹³¹I-rT₃ were separated from other labeled components of serum by means of amion-exchange resin columns (Curtis Nuclear Corp., Los Angeles, Calif.) according to the method described by Nicoloff et al. (18). The experience of our laboratory with this method of analysis for ¹³¹I-T₃ and ¹³¹I-rT₃ has been described (12). All samples and dose standards from a kinetics study were processed at the same time. For each tracer, the results are expressed as the percent of the injected dose per liter of serum.

Calculations. In the constant infusion 3,3'-T₂ study the CR was calculated from the infusion rate divided by the steady-state serum concentration of ¹³¹I-3,3'-T₂ achieved during the infusion (12). The production rate (PR) was calculated from CR multiplied by the serum concentration of stable 3,3'-T₂ (mean of three to five samples taken during infusion). In each single injection (T₃ and rT₃) study, the plasma disappearance curve for each tracer was fitted to a three-exponential function by the method of least squares. From the Y-intercepts and slopes of the exponential components the CR was computed by the method of Tait (19). The production rate was calculated as described for 3,3'-T₂.

Statistical methods. All results have been expressed as mean ± SD. Statistical evaluations were performed by the unpaired Student's t test (20).

RESULTS

Radioimmunoassay for L-3,3'-T₂

Specificity. The reactivities of various compounds relative to 3,3'-T₂ were calculated on the basis of the molar amount that caused 50% inhibition of the binding of ¹²⁵I-3,3'-T₂ to antibody (Table I). The moniodothyronines, 3-T₃ and 3'-T₁, demonstrated the greatest degree of cross-reactivity. T₄ and T₃ demonstrated negligible reactivity, whereas rT₃ showed a cross-reactivity of 0.6%. The calculated total contribution of T₄, T₃, and rT₃ was <5% of the observed 3,3'-T₂ concentration, based on the cross-reactivity values and the known concentrations of these iodothyronines in normal serum.

Analysis of the autoradiographic bands eluted from the thin-layer chromatograph of rat bile demonstrated the presence of two labeled conjugates. The eluted bands containing the conjugates represented 52% of the total biliary counts applied to the chromatograph. Enzymatic hydrolysis of these bands with β-glucuronidase and Mylase-P in separate experiments released ¹²⁵I-3,3'-T₂. Before enzymatic hydrolysis there was no detectable binding by specific 3,3'-T₂-binding antibody of the radioactive conjugates of 3,3'-T₂. In control experiments it was determined that the enzymes did not affect the antibody binding of tracer.

Standard curve. Fig. 1 demonstrates a typical standard curve of 3,3'-T₂ radioimmunoassay. Significant inhibition of the binding of ¹²⁵I-3,3'-T₂ to anti-serum was evident with as little as 20 pg of stable 3,3'-T₂/ml serum (4 pg/tube). The sensitivity of the assay was determined from t-test analyses of data from multiple standard curves performed in triplicate. The data were expressed as percent bound (mean ± SD) and the first point on each standard curve demonstrating a significant difference (P < 0.05) at >2 SD from the

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<th>Table I</th>
<th>Related Cross-Reactivity of Various Iodothyronines with 3,3'-T₂ Antiserum</th>
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<tr>
<td>Compound</td>
<td>Relative cross-reactivity (%)</td>
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<tr>
<td>L-3,3'-T₂</td>
<td>100.0</td>
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<tr>
<td>L-3-T₁</td>
<td>10.3</td>
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<tr>
<td>D,L-3'-T₁</td>
<td>7.3</td>
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<tr>
<td>L-rT₃</td>
<td>0.6</td>
</tr>
<tr>
<td>D,L-3',5'-T₂</td>
<td>0.3</td>
</tr>
<tr>
<td>L-T₃</td>
<td>0.07</td>
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<tr>
<td>Triac*</td>
<td>0.046</td>
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<tr>
<td>L-3,5-T₂</td>
<td>0.014</td>
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<tr>
<td>L-T₄</td>
<td>&lt;0.001</td>
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<tr>
<td>Tetrac*</td>
<td>&lt;0.001</td>
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* Triac, 3,5,3'-L-triiodothyroacetic acid; Tetrac, 3,5,3',5'-L-tetraiodothyroacetic acid.
basal point was taken as the assay sensitivity. Fig. 1 also demonstrates that the binding curve of dilutions (with iodothyronine-free serum) of serum from a hyperthyroid patient was parallel to the standard curve.

Recovery. Treatment of sera with activated charcoal (11) demonstrated that 96–99% of the added 125I-3,3'-T₂ was removed by this procedure. To determine the recovery of 3,3'-T₂ in this assay, various amounts of stable 3,3'-T₂ were added to sera from normal, hypothyroid, hypothyroid subjects, and from euthyroid individuals with idiopathic elevation in T₄-binding globulin (TBG). In 12 separate assays 3,3'-T₂ concentration was determined before and after enrichment of samples with stable 3,3'-T₂. A normal (5.9 ng/100 ml) and a hypothyroid serum (3.0 ng/100 ml) sample were enriched with 3.0 ng/100 ml. The percent recoveries were 112 and 100, respectively. A hyperthyroid (10.0 ng/100 ml) and a high TBG serum (5.0 ng/100 ml) sample were enriched with 9.0 ng/100 ml. The percent recoveries were 107 and 93, respectively. The mean 3,3'-T₂ recovery from the series of experiments was 103%.

Reproducibility. The intra- and interassay reproducibility was examined in samples with variable 3,3'-T₂ concentrations. The intra-assay coefficient of variation (SD/mean × 100) was 6.2%. The mean coefficient of variation of 10 specimens, varying in 3,3'-T₂ concentration from 2.0 to 9.0 ng/100 ml and assayed in duplicate four times in different assays, was 11.6%.

Serum 3,3'-T₂ concentration in health and disease. Table II presents data on serum 3,3'-T₂ concentration in normal subjects and patients with diverse levels of thyroid function as determined by clinical assessment and standard laboratory studies. The mean serum 3,3'-T₂ concentration in 13 healthy euthyroid subjects was

![Figure 1: Standard curve for 3,3'-T₂ radioimmunoassay and the effects of varying dilutions of serum from a hyperthyroid patient on the binding of 125I-3,3'-T₂ by antibody.](image_url)

<table>
<thead>
<tr>
<th>TABLE II</th>
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<tr>
<td><strong>Serum 3,3'-T₂, T₄, T₃, and rT₃ Concentrations in Various Thyroid States</strong></td>
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<tr>
<td><strong>Group</strong></td>
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<tr>
<td>Control subjects (n = 13)</td>
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<td>Hyperthyroidism (n = 25)</td>
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<td>Hypothyroidism (n = 17)</td>
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<tr>
<td>Hypothyroidism treated with T₄ (n = 11)</td>
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<td>Patients with acute nonthyroidal systemic illness (n = 10)</td>
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* Cf. normal P < 0.01.
† Cf. normal P < 0.001.
§ Cf. normal P < 0.05.
6.0±1.0 ng/100 ml with a range from 4.5 to 7.8 ng/100 ml. The mean serum 3,3'-T₂ concentration in 25 untreated hyperthyroid patients was significantly higher than in normal subjects (P < 0.01) but extended over a wide range from 3.0 to 21.0 ng/100 ml. In the 17 untreated hypothyroid patients the mean serum 3,3'-T₂ concentration was significantly lower than in normal subjects (P < 0.001). The mean serum 3,3'-T₂ in 11 hypothyroid patients maintained euthyroid on L-T₄ (100–200 μg/day) was not significantly different from the normal subjects. In 10 patients suffering from acute nonthyroidal systemic illness with decreased total serum T₃ (49±22 ng/100 ml) and elevated rT₃ (43±25 ng/100 ml), the mean 3,3'-T₂ level was within the normal range. These patients had a variety of disorders: three acute hepatitis; three unstable angina; two infectious disorders; one postdiabetic ketoacidosis and one postgastrectomy for peptic ulcer disease. In two subjects with high serum TBG levels the 3,3'-T₂ concentrations were within the normal range. There was no correlation between 3,3'-T₂ concentration and serum T₄, T₃, or rT₃ in any of the groups.

Synthroid and Cytomel tablets ground into powder, dissolved in 0.05 M NaOH, and diluted in iodothyronine-free serum, demonstrated 3,3'-T₂ levels consistent with those anticipated from their respective assay cross-reactivities.

Analyses of stable 3,3'-T₂ concentrations by radioimmunoassay in serum sample collected from controls and athyreotic subjects receiving T₄ (HT₄) or T₃ (HT₃) during the 8-h infusion period of the kinetics study revealed minimal variation in its concentration. All of the samples were stored frozen for an adequate period of time after the tracer infusion period to allow for radioactive decay. The coefficients of variation (CV) of samples collected during the 8-h period for each group studied were: controls (n = 7) 11.9% (mean); HT₄ (n = 5) 18.5%, and HT₃ (n = 3) 15.9%. There was no evidence for a diurnal (24 h) variation in controls (n = 2), HT₄ (n = 2), or HT₃ (n = 2) subjects. Their respective CV on data analyzed from frequent samples collected over 24 h were 14.9, 22.6, and 21.3%.

Fig. 2 demonstrates that it is possible to maintain in an athyreotic subject the serum T₃ concentrations relatively stable over a 24-h period with frequent small doses of L-T₃. This was necessary to obtain steady-state T₃ kinetics studies in these subjects. The CV of T₃ concentration in these samples was 11.9%. The serum 3,3'-T₂ concentration did vary somewhat more but, as reported above, the CV for the two HT₃ cases studied over 24 h was 21.3%, indicating reasonable stability. In the HT₄ subjects (3) the serum T₃ CV was 12.6% over 8 h.

Kinetics studies. The results of Sephadex column chromatographic analyses of one of the serum samples collected during the ¹²³I-3,3'-T₂ infusion in one case are depicted in Fig. 3. Analysis of an aliquot of the infused dose is also shown. In the serum sample and in the infusate, ¹²³I-3,3'-T₂ and iodide were the major labeled constituents. The zone between the ¹²³I⁻ and ¹²³I-3,3'-T₂ peaks contained a number of areas of low radioactivity. These peaks were considered to be metab-

![FIGURE 2](image-url)

**FIGURE 2** Serum concentrations during a 24-h period of T₃ (interrupted line) and 3,3'-T₂ (solid line) in a hypothyroid subject maintained euthyroid on frequent oral doses (10 μg every 3 h) of L-T₃. The arrows (abscissa) indicate the dose times. On the left hand ordinate is the T₃ concentration, and on the right hand ordinate, the 3,3'-T₂ concentration. In each case, the hatched box indicates the mean±2 SD.
olites of $^{125}$I-3,3'-T$_2$. The elution position of some of these metabolites, 3,3'-T$_2$ conjugates and 3'-T$_1$, was determined in separate column analyses. Fig. 3 also demonstrates that the elution profile of stable 3,3'-T$_2$, added to the 6-h sample before analysis on the Sephadex G-25 column, is superimposable on the $^{125}$I-3,3'-T$_2$ peak. This confirms the position of $^{125}$I-3,3'-T$_2$ in the eluted fractions from the column. In each subject studied at least two samples collected during the infusion period were analyzed to determine the $^{125}$I-3,3'-T$_2$ concentration.

The clearance of $^{125}$I-3,3'-T$_2$ was determined by the constant infusion method. In initial studies three separate serum samples collected during the interval from 2 h to the end of the infusion period were analyzed for $^{125}$I-3,3'-T$_2$. Fig. 4 shows that the concentration of $^{125}$I-3,3'-T$_2$ was relatively constant during this interval in all three cases, indicating that a steady state with respect to this tracer was achieved by the 2nd h after infusion. (No priming dose was given.) The CV of the $^{125}$I-3,3'-T$_2$ concentration in these serum samples averaged 8.5%. In the subsequent studies two serum samples collected during each infusion, usually at 4 and at 8 h, were analyzed on the column. The ratio of the $^{125}$I-3,3'-T$_2$ concentration in the final sample to that in the initial sample averaged 0.95±0.08 in the entire group, again confirming that an isotopic steady state had been reached. In every case calculation of the clearance was based on the average of the $^{125}$I-3,3'-T$_2$ concentrations in all serum samples collected beyond the 2-h point of the infusion.

The plasma concentration of 3,3'-T$_2$ and the kinetic parameters obtained in each of the subjects studied are shown in Table III. The mean total serum 3,3'-T$_2$ concentration in the control (C) group ($n=7$) was not significantly different from the T$_4$-treated hypothyroid (H) group ($n=5$). The mean total serum T$_4$ concentrations and free T$_4$ indices (normal 0.5–1.5) were almost identical for the two groups, at 7.7±1.0 μg/100 ml; 0.9±0.2 in C, and 7.9±1.2 μg/100 ml; 0.9±0.3 in H, respectively. The 3,3'-T$_2$ CR averaged 628±218 liters/day in C and 840±377 liters/day in H. 3,3'-T$_2$ PR were not significantly different (39.8±19.8 μg/day in C vs. 33.9±12.5 μg/day in H).

To determine the quantitative significance of 3,3'-T$_2$ as a product of the metabolism of T$_3$ and rT$_3$, the absolute turnover rates of T$_3$, rT$_3$, and 3,3'-T$_2$ were determined in three athyreotic subjects on T$_4$ replacement. Table IV gives the kinetics studies results of each of these iodothyronines in the three subjects. The 3,3'-T$_2$ PR in each case was close to the combined PR of T$_3$ and rT$_3$. The average ratio of 3,3'-T$_2$ PR (nanomoles per day) to the combined triiodothyronine PR (nanomoles per day) was 1.08±0.10.

To estimate the magnitude of conversion of T$_3$ to 3,3'-T$_2$, kinetics studies were conducted on three athyreotic subjects receiving L-T$_3$ replacement at three or four hourly intervals. A single pulse injection of $^{125}$I-T$_3$ and a constant 8-h infusion of $^{125}$I-3,3'-T$_2$ were given. The PR of each was determined as described above. Table V presents the 3,3'-T$_2$ and T$_3$ kinetics results.
### TABLE III

Serum T₄, 3,3′-T₂ Concentrations and Results of Kinetics Studies in Controls and T₄-Treated Hypothyroid Patients

<table>
<thead>
<tr>
<th>Serum T₄</th>
<th>3,3′-T₂</th>
<th>3,3′-T₂</th>
</tr>
</thead>
<tbody>
<tr>
<td>Controls</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Subject</td>
<td>Body wt.</td>
<td>Age</td>
</tr>
<tr>
<td></td>
<td>kg</td>
<td>yr</td>
</tr>
<tr>
<td>1</td>
<td>68</td>
<td>57</td>
</tr>
<tr>
<td>2</td>
<td>85</td>
<td>59</td>
</tr>
<tr>
<td>3</td>
<td>95</td>
<td>53</td>
</tr>
<tr>
<td>4</td>
<td>82</td>
<td>30</td>
</tr>
<tr>
<td>5</td>
<td>77</td>
<td>47</td>
</tr>
<tr>
<td>6</td>
<td>66</td>
<td>31</td>
</tr>
<tr>
<td>7</td>
<td>67</td>
<td>30</td>
</tr>
<tr>
<td>Mean</td>
<td></td>
<td></td>
</tr>
<tr>
<td>±SD</td>
<td>±1.0</td>
<td>±17</td>
</tr>
<tr>
<td>Treated hypo-thyroids</td>
<td>T₄ dose</td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>0.64</td>
<td>±0.13</td>
</tr>
<tr>
<td>±SD</td>
<td>±0.05</td>
<td>±4.3</td>
</tr>
</tbody>
</table>

Concn., serum concentrations.
* Each value is the mean of six or more serum determinations of samples collected.
† Corrected to 70 kg body wt.
‡ Nanomolar ratio of PR.
Significantly different from the control mean: †(P < 0.01), ‡(P < 0.02), **(P < 0.02).

### TABLE IV

Serum T₃, rT₃, 3,3′-T₂ Concentration and Results of Kinetics Studies in T₄-Treated Hypothyroid Patients

<table>
<thead>
<tr>
<th>T₃, rT₃, 3,3′-T₂</th>
<th>T₄</th>
<th>rT₃</th>
<th>3,3′-T₂</th>
<th>(3,3′-T₂, PR)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body wt.</td>
<td>Age</td>
<td>T₄ dose</td>
<td>Conc.</td>
<td>CR₁</td>
</tr>
<tr>
<td>kg</td>
<td>yr</td>
<td>mg/dy</td>
<td>µg/dl</td>
<td>µg/liter</td>
</tr>
<tr>
<td>8</td>
<td>88</td>
<td>62</td>
<td>0.150</td>
<td>0.52</td>
</tr>
<tr>
<td>9</td>
<td>71</td>
<td>64</td>
<td>0.150</td>
<td>0.78</td>
</tr>
<tr>
<td>10</td>
<td>76</td>
<td>79</td>
<td>0.125</td>
<td>0.62</td>
</tr>
<tr>
<td>Mean</td>
<td>0.64</td>
<td>28.9</td>
<td>18.3</td>
<td>0.31</td>
</tr>
<tr>
<td>±SD</td>
<td>±0.13</td>
<td>±6.1</td>
<td>±4.3</td>
<td>±0.05</td>
</tr>
</tbody>
</table>

Concn., serum concentration.
* Each value is the mean of six or more serum determinations of samples collected during the kinetics studies.
† Corrected to 70 kg body wt.
§ Nanomolar ratio of PR.
Significantly different from the control mean: †(P < 0.01), ‡(P < 0.02), **(P < 0.02).
comparison to the control subjects (1.03±0.15 ng/100 ml) (P < 0.001). Although the mean T₃ CR (33.0±4.3 liters/day) was normal, the mean T₃ PR (50.9±13.8 µg/day) was significantly higher (P < 0.005) than the previously reported normal value of 24.2±4.1 µg/day (12). Table V also shows that the PR T₃ in each subject was close to the daily dose of T₃. It thus appears that almost 100% of the oral T₃ was absorbed. This finding agrees with previous studies (21).

The molar ratio of 3,3′-T₂ to T₃ PR varied among the three subjects from 0.36 to 0.92, indicating that 3,3′-T₂ is an important product of T₃ deiodination.

**DISCUSSION**

The present study confirms the presence of 3,3′-T₂ in normal human serum. The mean normal serum 3,3′-T₂ concentration obtained in our study (6.0±1.0 ng/100 ml) is intermediate between the value of 7.6±2.4 ng/100 ml reported by Wu et al. (6), who employed ethanolic extraction of serum and the recently reported value, 4.3±2.0 ng/100 ml, from Burger and Sakoloff (8), who analyzed unextracted serum. The presence of 3,3′-T₂ in normal whole serum at a slightly higher concentration (17.0±1.0 ng/100 ml) measured by radioimmunoassay has also been reported by Burman et al. (7).

In hyperthyroidism, serum 3,3′-T₂ levels are increased, with the T₃ PR elevated on the average. There was, however, a wide range of values from moderate elevation to markedly high serum 3,3′-T₂ levels. The absence of a positive correlation between the serum total T₄ and the 3,3′-T₂ levels has been noted previously (22). It may be due to an increased 3,3′-T₂ CR, as preliminary studies in our laboratory demonstrate increased 3,3′-T₂ CR in hyperthyroidism (23). The normal serum 3,3′-T₂ levels in patients with elevated TBG concentration is consistent with the reported low binding affinity of 3,3′-T₂ for TBG, compared with T₄ (24), and normal levels in pregnancy (6).

The finding of normal serum 3,3′-T₂ levels in athyreotic subjects maintained eumetabolic on T₄ replacement confirms a previous report (22) and suggests that the major source of circulating 3,3′-T₂ is extrathyroidal production.

The original analysis of rat thyroglobulin by Roche et al. (1) suggested that 3,3′-T₂ was a major component of the iodothyronines present. This finding in the rat was not confirmed by the recent studies of Taurog et al. (25), who demonstrated that 3,3′-T₂ comprised <2% of the total ¹³¹I in the thyroids of severely iodine-deficient rats and even less in the iodine-replete animal. In a recent report Burman et al. (7) found a 3,3′-T₂ concentration of 0.4±0.03 µg/ml in normal thyroid tissue and a lack of a serum 3,3′-T₂ response secondary to thyrotropin-releasing hormone stimulation, findings indicative of a minimal rate of thyroidal secretation. That the thyroid gland is not a major source of circulating 3,3′-T₂ is confirmed by the similar 3,3′-T₂ PR in the euthyroid control group and in the athyreotic subjects in whom the only source of 3,3′-T₂ was exogenous T₄.

There are no other data available at present to compare with the PR of 3,3′-T₂ obtained in this study.

The rate of disappearance from the blood of tracer 3,3′-T₂ (as the racemic mixture) in man has been examined in a previous study and found to be extremely rapid (9). The achievement of a constant plasma level of ¹³¹I-3,3′-T₂ during the first few hours of a constant infusion is consistent with this finding. However, quantitative estimates of 3,3′-T₂ CR have not been reported previously.

The metabolism of 3,3′-T₂ would be expected to yield monoiodothyronines, thyronine itself, deaminated derivatives, and conjugated forms. The glucuronic acid conjugate of 3,3′-T₂ has been identified in the bile of the rat after the injection of labeled 3,3′-T₂ (26) and the sulfate conjugate of 3,3′-T₂ in the bile and urine of the dog after the administration of labeled 3,3′-T₂ (16). Stanbury and Morris (9) demonstrated

**Table V**

*3,3′-T₂ Concentration and Results of Kinetic Studies in T₃-Treated Hypothyroid Subjects*

<table>
<thead>
<tr>
<th>Treated hypothyroid</th>
<th>Body wt.</th>
<th>Age</th>
<th>T₃ dose</th>
<th>T₃</th>
<th>3,3′-T₂</th>
<th>(T₃ PR)/</th>
<th>3,3′-T₂ PR/</th>
</tr>
</thead>
<tbody>
<tr>
<td>kg yr</td>
<td>µg/liter</td>
<td>liter/day</td>
<td>µg/day</td>
<td>µg/liter</td>
<td>liter/day</td>
<td>µg/day</td>
<td>(T₃ PR)</td>
</tr>
<tr>
<td>9 69 64</td>
<td>1.24</td>
<td>33.4</td>
<td>41.4</td>
<td>20</td>
<td>599</td>
<td>12.0</td>
<td>1.03</td>
</tr>
<tr>
<td>11 71 60</td>
<td>1.57</td>
<td>28.6</td>
<td>44.8</td>
<td>41</td>
<td>816</td>
<td>33.5</td>
<td>0.75</td>
</tr>
<tr>
<td>13 85 65</td>
<td>1.79</td>
<td>37.1</td>
<td>66.4</td>
<td>41</td>
<td>544</td>
<td>22.3</td>
<td>1.01</td>
</tr>
</tbody>
</table>

Conc., serum concentration.
* Each value is the mean of six or more serum determinations on samples collected during the kinetics studies.
† Corrected to 70 kg body wt.
‡ Uncorrected for body wt.
§ Nanomolar ratio of PR.

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the presence of two unidentified compounds in some serum samples chromatographed after the administration of $^{131}$I-3,3′-T$_2$. The Sephadex column employed in the present study separates 3,3′-T$_2$ conjugates and 3′-T$_1$ from 3,3′-T$_2$. Even if the 3,3′-T$_2$-containing fractions from our columns did include labeled metabolites, however, the calculated CR would have underestimated the true CR in our subjects.

The magnitude of 3,3′-T$_2$ PR suggests that it is a major metabolite of the deiodination pathways. Tracer studies in both the rat (2, 27, 28) and the dog (4, 16) demonstrated that both T$_3$ and rT$_3$ are precursors of 3,3′-T$_2$.

Recent in vitro studies by Chopra et al. (5) in rat liver homogenates have demonstrated that both T$_3$ and rT$_3$ are converted to 3,3′-T$_2$. The finding of 5′-deiodination (phenolic ring) of rT$_3$ to 3,3′-T$_2$ by liver homogenates confirms the previous reports by Flock and Owen (29) who employed perfused rat liver and a recent study of the metabolism of thyroid hormones by cultured monkey hepatocarcinoma cells (30).

There has been no information, however, on the quantitative significance of 3,3′-T$_2$ as a metabolite of T$_3$ or rT$_3$ until the present study in humans. The magnitude of 3,3′-T$_2$ PR indicates that it is a major metabolite of both triiodothyronines. In all cases studied, the 3,3′-T$_2$ PR was close to the combined (T$_3$ + rT$_3$) PR, accounting for 90–110% of total turnover. This finding is supported by the demonstration that the conversion of T$_3$ to 3,3′-T$_2$ (in T$_3$-replaced subjects) was a major pathway of T$_3$ metabolism.

The finding that both T$_3$ and rT$_3$ are major precursors of 3,3′-T$_2$ and that the magnitude of their contribution may be variable could explain the observation of a normal serum 3,3′-T$_2$ concentration in acute nonthyroidal systemic illness with low serum T$_3$ but high serum rT$_3$.

Previous studies in normal humans have demonstrated that 5′-deiodination of T$_4$ to rT$_3$ is at least as active as 5′-deiodination to T$_3$, the total activity of both pathways accounting for most of the T$_4$ metabolized (12, 31). The present finding that 3,3′-T$_2$ is the principal product of the metabolism of both T$_3$ and T$_3$ indicates the importance of 3,3′-T$_2$ as an intermediate in T$_4$ metabolism. Furthermore, the finding implies that the other diiodothyronines, 3,5-T$_2$ and 3′,5′-T$_2$, are of only minor quantitative significance.

Early studies by Roche et al. (1) demonstrated that 3,3′-T$_2$ was calorigenically active but this has not been confirmed by subsequent in vivo studies (25, 32–34). Recent studies have shown the ability of 3,3′-T$_2$ to enhance the uptake of radioecilune by thymocytes in culture, but the effect was seen only at high concentrations and so its biological significance is not clear (35). Even if it is not biologically active, 3,3′-T$_2$ must be regarded as a major intermediate in the deiodination of iodothyronines. A recent report by Chopra (36) shows that 3,3′-T$_2$ is a weak inhibitor of the conversion of T$_3$ to T$_2$ in rat liver homogenates. This raises the possibility that 3,3′-T$_2$ may regulate the monodeiodination of T$_4$. Studies are presently in progress in our laboratory to elucidate further the significance of this intermediate in the peripheral metabolism of iodothyronines.

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REFERENCES

conversion of thyroxine to 3,3',5'-triiodothyronine (reverse-T3) and to 3,5,3'-triiodothyronine (T3) in humans.


