Iodothyronine Metabolism in Rat Liver Homogenates

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ABSTRACT To investigate mechanisms of extrathyroidal thyroid hormone metabolism, conversion of thyroxine (T4) to 3,5,3'-triiodothyronine (T3) and degradation of 3,3',5'-triiodothyronine (rT3) were studied in rat liver homogenates. Both reactions were enzymatic. For conversion of T4 to T3, the K_m of T4 was 7.7 μM, and the V_max was 0.13 pmol T3/min per mg protein. For rT3 degradation, the K_m of rT3 was 7.5 nM, and the V_max was 0.36 pmol rT3/min per mg protein. Production of rT3 or degradation of T3 or T4 was not detected under the conditions employed. rT3 was a potent competitive inhibitor of T4 to T3 conversion with a K_i of 4.5 nM; 3,3'-diiodothyronine was a less potent inhibitor of this reaction. T4 was a competitive inhibitor of rT3 degradation with a K_i of 10.2 μM. Agents which inhibited both reactions included propylthiouracil, which appeared to be an allosteric inhibitor, 2,4-dinitrophenol, and iopanoic acid. Sodium diatrizoate had a weak inhibitory effect. No inhibition was found with α-methylparathyroisine, Fe^{3+}, Fe^{4+}, reduced glutathione, β-hydroxybutyrate, or oleic acid.

Fasting resulted in inhibition of T4 to T3 conversion and of rT3 degradation by rat liver homogenates which was reversible after refeeding. Serum T4, T3, and thyrotropin concentrations fell during fasting, with no decrease in serum protein binding as assessed by a T3-charcoal uptake. There was no consistent change in serum rT3 concentrations. Dexamethasone had no effect in vitro. In vivo dexamethasone administration resulted in elevated serum rT3 concentrations after 1 day, and after 5 days, in inhibition of T4 to T3 conversion and rT3 degradation without altering serum T4, T3, or thyrotropin concentrations. Endotoxin treatment had no effect of iodothyronine metabolism in liver homogenates. In kidney homogenates the reaction rates and response to propylthiouracil in vitro were similar to those in liver. No significant T4 to T3 conversion or rT3 production or degradation could be detected in other tissues.

These data suggest that one iodothyronine 5'-deiodinase is responsible for both T4 to T3 conversion and rT3 degradation in liver and, perhaps, in kidney. Alterations in serum T3 and rT3 concentrations induced by drugs and disease states may result from decreases in both T3 production and rT3 degradation consequent to inhibition of a single reaction in the pathways of iodothyronine metabolism.

INTRODUCTION

Deiodination is a major mechanism of thyroxine (T4) disposal in the human and the rat (1, 2). The active thyroid hormone, 3,5,3'-triiodothyronine (T3) is produced by 5'-deiodination of T4, whereas a calorigenically inactive compound, 3,3',5'-triiodothyronine (reverse-T3, rT3), is produced by 5-deiodination. Rates of removal of the 5'- and 5-iodine of T4 are approximately equal in humans and rats (3, 4), but these processes do not occur randomly. Decreased serum T3 concentrations, resulting from decreased peripheral 5'-deiodination of T4, are found in patients with a variety of illnesses, during fasting and in fetal life (4–21). In these situations, serum rT3 concentrations are usually elevated, suggesting diversion of T4 to the inactivating 5-deiodinating pathway (4, 7, 8, 10, 12, 15, 16, 18, 20, 21). However, elevated serum rT3 concentrations in two such situations, hepatic cirrhosis and fasting, were recently shown (4, 22) to result from decreased rT3 degradation rather than increased rT3 production. In fetal sheep, the metabolic clearance rate of rT3 is low, relative to adult sheep (23), and, although the production rate of rT3 is elevated, it is not markedly different from the adult rate if expressed as a fraction of the daily T4 production (0.32 for the fetal sheep vs. 0.25 for the adults).

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Many tissues, including liver, kidney, heart, skeletal muscle, leukocytes, and fibroblasts, are capable of deiodinating T₄ in vitro (24–39). The techniques used to demonstrate T₄ deiodination in these studies included incubation of tissue homogenates, tissue slices, dispersed cells, and whole organ perfusion. T₃ has been identified as a deiodination product of T₄ (24, 26, 27, 29, 31, 35, 36). At the subcellular level, both T₄ deiodination and T₃ to T₂ conversion were localized to the microsomal fraction of liver homogenates (37–39), whereas production of rT₃ from T₄ has been found in the mitochondrial and soluble fractions of a liver homogenate preparation (36). Most of these reports contain relatively little data concerning the characteristics and kinetics of the reactions studied or information about T₄ metabolism in vitro in situations such as starvation or treatment with drugs which alter extrathyroidal T₄ or rT₃ metabolism in man.

The original aim of this study of T₄ metabolism in rat liver homogenates was to obtain information about T₃ and rT₃ production, inasmuch as the nature of these processes and their regulation are undefined. It soon became clear that, if rT₃ was being produced, it was also being destroyed too rapidly for its production to be observed. Further experiments showed that factors which inhibited T₄ to T₃ conversion also inhibited rT₃ degradation. In light of these observations and those of Cavalieri et al. (36) and Chopra et al. (40), who have reported that 5′-deiodination is a major pathway of rT₃ degradation, the experiments were extended to test the hypothesis that T₄ to T₃ conversion and rT₃ degradation reflect activities of the same enzyme. The results support this hypothesis. This report, therefore, describes biochemical characterization of T₄ to T₃ conversion and rT₃ degradation reactions in homogenates of rat liver and other tissues and studies of these reactions in rats in situations of altered extrathyroidal iodothyronine metabolism in vivo.

METHODS

Materials. rT₃ was a gift of Dr. R. Meltzer, Warner-Lambert Research Institute, Morris Plains, N. J.; 3,3′-diiodothyronine (3,3′-T₂) was a gift of Dr. H. Cahman, National Institutes of Health, Bethesda, Md.; 3,5-diiodothyronine (3,5′-T₂) was obtained from Travenol Laboratories, Inc., Morton Grove, Ill.; iopanoic acid was a gift of Dr. W. Blakemore, Sterling-Winthrop Research Institute, Rensselaer, N. Y.; and 131I-rT₃ was purchased from Abbott Laboratories, Chemical Div., North Chicago, Ill.

Preparation of homogenates. Liver homogenates were prepared by a modification of the method of Visser et al. (26). Rats were sacrificed by decapitation. Serum was separated from the trunk blood and stored at −4°C. The livers were removed, minced in cold 0.05 M Tris, pH 7.6, washed twice and homogenized in 3 vol of the same buffer (w/vol) with three to four strokes in a glass homogenizer with a motor-driven Teflon pestle. In one group of experiments, a series of different buffers were used (see below). The crude homogenate was centrifuged at 2000 g, the sediment was discarded, and the supernate, referred to hereafter as the liver homogenate, was used for subsequent incubations. It was assumed to contain cytosol, micromesosomes, mitochondria, and membrane fragments, but not whole cells or nuclei. Homogenates of other tissues were prepared by the same procedure. The liver homogenates had a protein content of 28±1 mg/ml, SE, and that of the kidney homogenates was 18±1 mg/ml. All incubations were performed immediately after preparation of the homogenates.

Incubation procedure. Incubations were carried out with 2 ml homogenate per incubation tube in a 37°C water bath (except for the temperature dependence studies) after preincubation for 5 min at 37°C before addition of any reagents. and those at later times, specified below, were used. Addition of reagents, mixing, and sampling took ~7 s. This was taken as zero in the T₄ to T₃ conversion experiments, but was taken as 7 s in the rT₃ degradation experiments, with initial concentrations calculated by extrapolation. Initial extracts taken after addition of T₄ had immunoreactivity for T₄ and rT₃ which increased in proportion to the added T₄. It cannot be determined how much of this activity was due to contamination of the T₄ preparation and how much was intrinsic T₃ cross-reactivity. In that the T₃ immunoreactivity did not change over 120 min when T₄ was incubated with buffer or heat inactivated
homogenate, it was felt that subtracting the initial concentration from subsequent ones gave a valid measurement of T3 production. The initial T3 immunoreactivity limited the range of added T4 concentrations which could be used, because, at high initial T4 concentrations, the change in T3 concentrations became a progressively smaller fraction of the initial T3 immunoreactivity. For this reason, the concentration of T4 used in the inhibition and in vivo studies had to be less than the $K_0$ of T4.

The rT3 immunoreactivity after addition of T4 to homogenates did not change over 120 min. In that rT3 was degraded so rapidly (see below), this was considered to represent T4 cross-reactivity in the rT3 assay, and thus did not invalidate measurements of changes in rT3 concentrations in the presence of T4. A similar interaction of 3,3',T2 was noted in the rT3 assay.

Endogenous T3 concentrations in rat liver have been estimated to be 11.09±1.63 ng/g whole liver (41), which would be <5 mmol/liter of homogenate, <1% of the T4 added in the present experiments. No information is available about endogenous hepatic rT3 concentrations. They are probably very low, even with the unlikely assumption that the entire difference between blank liver extract and buffer in the rT3 assay was due to endogenous rT3, the endogenous rT3 concentration would contribute <0.8 mmol/liter homogenate. Once again, this concentration is small compared to those used in these experiments. For the above reasons, endogenous T4 and rT3 were felt to be negligible in the kinetic calculations and were ignored.

The determination of $K_0$ and $K_1$ values for rT3 was complicated by the substantial decrease in rT3 concentration during the reaction. This problem was managed by keeping the incubation periods as short as possible in the experiments which measured these constants, and by using the mean of the initial and final rT3 concentrations in the kinetic plots as suggested by Segel (42).

$T_3$, $T_3$, and rT3 determinations in serum. Double antibody assays were performed as previously described (43-45). The anti-T3 antibody was purchased from Endocrine Sciences, Tarzana, Calif. The rT3 assay was modified from the assay for rT3 in human serum described elsewhere (45), using 200 μl serum, 75 μl anti-rT3 antiserum diluted 1:5000, 141I-T3 of high specific activity (~800 μCi/μg), and 200 μg 8-anilino-1-naphthalenesulfonic acid in each assay tube. Sensitivity was 2-4 pg/tube or 1-2 ng/dl of serum. Cross-reactivity with T4 was 0.04%, and cross-reactivity with T3 was <0.01%. There was rT3 immunoreactivity in all but one of 138 rat serum samples tested. In these 137, the measured rT3 concentrations ranged from 1.6 to 7.2 ng/dl, with a mean of 4.0 ng/dl. Because 0.04% T3 cross-reactivity combined with a serum T3 concentration of 5 μg/dl accounts for 2 ng rT3/dl, a substantial fraction of the total, the measured rT3 concentrations were corrected by subtracting 0.04% of the T3 concentration in each serum. The corrected mean normal rat serum rT3 concentration was 1.8±0.9 ng/dl SD. Serum thyrotropin (TSH) was also measured by radioimmunoassay, using reagents supplied by the National Pituitary Agency (NPA). Results are expressed in nanograms of the NPA RP-1 rat pituitary TSH standard/milliliter serum. Protein was measured by the method of Lowry et al. (46), using BSA as a standard.

Serum T3 concentrations in the normal rats were higher than reported by others (47). The assay method used in these studies often yielded lower results in other normal rats, but the values reported here were consistently obtained when the sera were measured in two to three different assay runs. Serum rT3 concentrations in the range of 2 ng/dl are consistent with a recent report (48) of immeasurable rat serum rT3 using an assay with a sensitivity of 6 ng/dl. The low values and the necessity of using a substantial correction for T4 cross-reactivity require that the rT3 concentrations be interpreted very cautiously.

To assess serum protein binding of T3, a T3-charcoal uptake was performed. The method was adapted for rat serum from that of Bermudez et al. (6). 100 μl serum was incubated with 7-8,000 cpm 125I-T3 in 50 μl 0.1% BSA, 0.075 M Na PO4, pH 7.4, for 30 min at 37°C. 700 μl of 0.025% dextran T-70 (Pharmacia Fine Chemicals, Div. of Pharmacia, Inc., Piscataway, N.J.) and 0.05% charcoal in the same buffer was added, and the mixture was incubated at room temperature for 20 min and centrifuged at 2,000 rpm for 15 min. The supernate was then aspirated, and the charcoal was counted. This method was validated by comparing normal rat serum (total serum T3 5.6±1.6 μg/dl SD) with serum from rats made hyperthyroid by injection of T4, 10 μg/100 g/day s.c. for 12 days (total serum T3 8.9±2.1 μg/dl) and with serum from thyroidectomized rats (total serum T3 1.7±0.3 μg/dl). The mean T3-charcoal uptakes were: normal, 39.1±3.5% SD; T3-treated, 93±2.2%; and thyroidectomy 26.5±3.8%.

In vivo studies

Fasting. Groups of five 200-250-g male Sprague-Dawley rats were fasted for 1-4 days with free access to tap water. Other groups were fasted for 3 days, then fed with Wayne Lab-Blox (Allied Mills, Inc., Chicago, Ill.) for 1-6 days before sacrifice. A control group of five fed rats from the same animal shipment as each experimental group was sacrificed simultaneously. Fasting was begun at 9 A.M., and the animals were sacrificed at the same time. Liver homogenates from each rat were incubated with 1.3 μM T4, and aliquots were removed and extracted with ethanol at 0, 15, and 30 min. In this period the reaction rate was reasonably constant (see Results). Liver homogenates from each rat were also incubated with 1.54 nM T3, aliquots being taken at 0 and 3 min. With these incubation conditions rT3 degradation was reliably measurable. The concentration of rT3 was chosen to avoid extremes of the assay curve in a 3-min incubation, and the time was chosen as a compromise because small concentration changes, difficult to measure reliably, resulted from shorter incubations, and gross nonlinearity in the reaction rate was found after longer incubations (see below). The same conditions were used in the following studies.

Dexamethasone treatment. 300-g male rats were treated with 1.5 mg/kg dexamethasone phosphate in 0.5 ml 0.15 M NaCl i.p. Five rats were given one injection and sacrificed 24 h later. Five rats were given five injections at 24-h intervals and sacrificed 24 h after the last injection. Control rats were given one or five injections of 0.5 ml 0.15 M NaCl i.p.

Endotoxin treatment. Seven male rats were injected i.p. with 5 mg/kg endotoxin (lipopolysaccharide B, Escherichia coli 055:B5, Difco Laboratories, Detroit, Mich.) in 0.5 ml 0.15 M NaCl. Seven control rats were injected with 0.5 ml 0.15 M NaCl i.p. The rats were sacrificed 15 h after injection in one experiment and 24 h after injection in another and had access to food and water during that interval. 3 out of 14 rats injected with endotoxin died.

Statistical methods. Mean values from experimental groups were compared to controls using Student's t test for unpaired data. In the in vivo experiments the results of the liver homogenate incubations are given as percent of the mean value from control animals to allow results from different groups of rats and data from different assays to be compared.

RESULTS

Time-course of the reactions. Fig. 1 shows the production of T3 as a function of time when varying...
The rate of degradation of rT₃ (Fig. 2A) diminished after 2–3 min. At low initial concentrations (15.4 and 38.5 nM), most of the rT₃ disappeared in 10 min. At higher rT₃ concentrations (77 and 154 nM), degradation could be measured for up to 60 min (data not shown). When T₄ and T₃ were incubated at similar low initial concentrations, there was no detectable degradation of either in 120 min as shown in Figs. 2B and 2C.

**Reaction conditions.** No significant differences were found in the rate of T₃ production from T₄ or rT₃ degradation when portions of the same livers were homogenized in 0.05 M Tris, 0.05 M Tris + 0.25 M sucrose, 0.05 M Tris + 0.25 M glucose, 0.05 M Na₂HPO₄ – 0.15 M NaCl, or 0.05 M Na₂HPO₄ – 0.15 NaCl + 0.25 M glucose, all at a buffer pH of 7.4.

The rate of T₄ to T₃ conversion at 22°C was 32% of the rate at 37°C; at 4°C it was 10% of the 37°C rate. The rate of rT₃ degradation at 22°C was 28%, and the rate at 4°C was 12% of the rate of degradation at 37°C. Heating the homogenate at 56°C for 30 min before incubation at 37°C completely abolished both activities.

The pH of the homogenates was initially 0.2 pH units less than that of the buffer, and it decreased by 0.1–0.2 pH units during the course of a 2-h incubation. There was no variation in reaction rate for T₄ to T₃ conversion or rT₃ degradation using 0.05 M Tris between pH 6.8 and 7.6 (measured at zero time directly in the homogenate). At pH 8.1 and pH 6.6, the degradation of rT₃ was 50% of the rate at pH 7.2–7.6. A homogenate pH of 7.4 (pH of the Tris buffer being 7.6) was used in all other experiments.

**FIGURE 1** T₃ concentrations±SE in liver homogenate during 240-min incubations with initial T₄ concentrations of 0.6, 1.3, and 2.6 μM. No T₃ was added.

amounts of T₄ (0.6–2.6 μM) were incubated with liver homogenate. With each dose of T₄ the rate of T₃ production declined after 30–60 min. With initial T₄ concentrations of 0.65, 1.3, and 2.6 μmol/liter, the net production of T₃ in 240 min was 22.1, 27.7, and 23.2 nmol/liter, representing conversion of 3.4, 2.1, and 0.9%, respectively, of the initial T₄ added. No decline in directly measured T₄ concentrations was detectable in this period. With no added T₄, there was no increase in homogenate T₃ concentration over 120 min. No measurable production of rT₃ from T₄ occurred.

**FIGURE 2** (A) rT₃ concentrations±SE in liver homogenate during 15-min incubations with initial rT₃ concentrations of 15.4 (○) and 30.8 (■) nM. (B) T₃ concentrations±SE in liver homogenate during 60- and 120-min incubations with initial T₃ concentrations of 15.4 (○) and 45 (●) nM. (C) T₄ concentrations±SE in liver homogenate during 120-min incubations with an initial T₄ concentration of 12.9 nM (x).
Reaction kinetics. Fig. 3 shows a Lineweaver-Burk plot for the conversion of T₄ to T₃. Values for V were calculated in picomoles T₃/minute per milligram protein using the 0- and 15-min time points, i.e., during the period when the reaction rate was constant. The line of best fit using the least squares method yielded a Kₘ for T₄ of 7.7 μM and a Vₘₐₓ of 0.13 pmol T₃/minute per mg protein. With an initial T₄ concentration of 1.3 μM, the concentration used in most of the subsequent studies, the mean reaction rate was 0.025±0.003 pmol SE T₃/minute per mg protein. Fig. 4 shows a Lineweaver-Burk plot for rT₃ degradation. V was calculated in picomoles rT₃/minute per milligram protein from the 7-s, 0.5- and 1-min time points. The least squares line of best fit yielded a Kₘ for rT₃ of 7.5 nM and a Vₘₐₓ of 0.36 pmol rT₃/minute per mg protein. The difference in the Kₘ values for T₄ and rT₃ implies that, if there is a single 5'-deiodinase, its affinity for rT₃ is about 1,000-fold greater than that for T₄.

Interactions of iodothyronines. Table I shows that conversion of T₄ to T₃ was not inhibited by addition of T₃ or of 3,5-T₂, but was inhibited in a dose-dependent manner by the addition of rT₃ and 3,3'-T₂. rT₃ was ≈ four times as potent an inhibitor as 3,3'-T₂ on a molar basis. A Dixon plot of rT₃ inhibition of T₄ to T₃ conversion is shown in Fig. 5. As mentioned above, the mean of the initial and final rT₃ concentrations was used in the calculations. Straight lines were fitted to the points shown by the least squares method. The intersection of the lines above the x axis, and the reasonable fit of the points to straight lines, suggest that rT₃ is a competitive inhibitor of T₄ to T₃ conversion, with a Kᵢ of 4.5 nM, close to the Kₘ of rT₃ (7.5 nM) in the rT₃ degradation reaction.

Table II shows that T₄ inhibited rT₃ degradation in a dose-dependent fashion, but that the other iodothyronines did not. In particular, 3,3'-T₂ at a 0.15 μM concentration had no effect in inhibiting rT₃ degradation, whereas the same 3,3'-T₂ concentration substantially inhibited T₄ to T₃ conversion (cf. Table I). Fig. 6 is a

**Figure 3** Lineweaver-Burk plot of T₄ to T₃ conversion. Values shown are mean±SE results from four incubation mixtures.

**Figure 4** Lineweaver-Burk plot of rT₃ degradation. Each point represents the mean of five incubations of 1 min duration. The y axis shows 1/V±SE. The x axis shows the means of the reciprocals of the average [rT₃] (1/8 [added rT₃ + measured rT₃ after 1 min]) during the incubation. The standard errors of 1/[rT₃] (not shown) ranged from 0.001 to 0.005 nM⁻¹.
TABLE I
Inhibition of T₄ to T₃ Conversion by Iodothyronines

<table>
<thead>
<tr>
<th>Iodothyronine</th>
<th>Concentration (μM)</th>
<th>(Inhibitor)</th>
<th>T₃ production rate</th>
<th>P</th>
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<tbody>
<tr>
<td>T₃</td>
<td>0.120</td>
<td>0.092</td>
<td>111±19 NS</td>
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<tr>
<td>rT₃</td>
<td>0.019</td>
<td>0.015</td>
<td>31±8 &lt;0.001</td>
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</tr>
<tr>
<td></td>
<td>0.039</td>
<td>0.029</td>
<td>3±1 &lt;0.001</td>
<td></td>
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<tr>
<td></td>
<td>0.077</td>
<td>0.059</td>
<td>2±1 &lt;0.001</td>
<td></td>
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<tr>
<td>3,3'-T₂</td>
<td>0.038</td>
<td>0.029</td>
<td>42±3 &lt;0.001</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.076</td>
<td>0.059</td>
<td>34±5 &lt;0.001</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.150</td>
<td>0.117</td>
<td>16±5 &lt;0.001</td>
<td></td>
</tr>
<tr>
<td>3,5-T₂</td>
<td>0.150</td>
<td>0.117</td>
<td>87±6 NS</td>
<td></td>
</tr>
</tbody>
</table>

Initial T₄ concentration was 1.3 μM. Three to five identical incubations were used for each inhibitor concentration and compared to an equal number of simultaneous control incubations with T₄ alone.

Dixon plot of T₄ inhibition of rT₃ degradation. These data suggest that T₄ is a competitive inhibitor of rT₃ degradation, with a Kᵣ of 10.2 μM, quite similar to the Kᵣ of T₄ (7.7 μM) in the T₄ to T₃ conversion reaction.

Other inhibitors. Propylthiouracil (PTU) inhibited both T₄ to T₃ conversion and rT₃ degradation (Table III). The PTU dose-response relationships were similar for both reactions, but inhibition was incomplete even with very large quantities of PTU. Increasing the concentration of PTU from 1.76 μM, the lowest concentration at which inhibition was consistently observed, to 59 μM, a 33-fold increase, resulted in a decrease in the rate of T₃ production from T₄ to 70 to 46% of control, and a decrease in the rate of rT₃ degradation from 77 to 26% of control. A further six-fold increase in PTU concentration had little further effect. The hyperbolic shape of the Dixon plot (Fig. 7) of the PTU inhibition of T₄ to T₃ conversion suggests that PTU alters the affinity of the enzyme for T₄, i.e. is an allosteric inhibitor, rather

![Figure 5](image)

**Figure 5** Dixon plot of rT₃ inhibition of T₄ to T₃ conversion. Each point represents the mean of five incubations of 5 min duration. The y axis shows 1/v±SE. The x axis shows the average [rT₃] (½ [added rT₃ + measured rT₃ after 5 min]) during the incubation. The standard errors of [rT₃] (not shown) ranged from 0.2 to 1.7 nM.

![Figure 6](image)

**Figure 6** Dixon plot of T₄ inhibition of rT₃ degradation. Each point represents the mean of five incubations of 1 min duration. The y axis shows 1/v±SE. The x axis shows the concentration of added T₄.

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than acting at the catalytic site. There was no measurable production of rT3 from T4 when rT3 degradation was (partially) inhibited by PTU.

Table IV shows that 2,4-dinitrophenol and iopanoic acid were effective inhibitors both of T4 to T3 conversion and rT3 degradation. There was no rT3 production from T4 when rT3 degradation was almost completely inhibited by 10 μM iopanoic acid. Sodium diatrizoate, an iodinated contrast agent like iopanoic acid, had a modest inhibitory effect of rT3 degradation. It appeared to inhibit T4 to T3 conversion to the same degree, but this inhibition was not statistically significant. Other agents tested and found to have neither stimulatory nor inhibitory effects on T4 to T3 conversion or rT3 degradation included 13 μM dexamethasone, 8 μM NaI, 1 μM α-methylparatyrosine, 89 μM 1-methyl-2-mercaptopimidazole, 1 mM FeCl2, 1 mM FeCl3, 1 mM reduced glutathione, 1 mM ascorbic acid, and 5 mM 1-β-hydroxybutyrate (10 mM Na salt of d,1-β-hydroxybutyrate). 1.5 mM oleic acid resulted in a rate of T4 to T3 conversion of 135±6% SE of control (P < 0.05), but with 2.5 mM oleic acid concentration the rate was not different from control (98±19%). 1.5 mM oleic acid did not alter rT3 degradation.

Other tissues. Kidney homogenate was as active as liver in producing T3 from T4. At an initial T4 concentration of 1.3 μM, the rate of T3 production was 0.021±0.004 pmol/min per mg protein, compared to 0.025±0.003 pmol/min per mg protein for liver homogenate. PTU at a concentration of 3.5 μM in kidney homogenate reduced T3 production to 80±3% SE of control, at 7 μM the rate was 43±8% of control and at 59 μM PTU, 45±15% of control, a pattern

### TABLE IV

**Inhibition of T4 to T3 Conversion and rT3 Degradation by 2,4-Dinitrophenol, Sodium Diatrizoate, and Iopanoic Acid**

<table>
<thead>
<tr>
<th>Inhibitor</th>
<th>Concentration</th>
<th>n</th>
<th>T4 to T3 conversion</th>
<th>P</th>
<th>n</th>
<th>rT3 degradation</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>2,4-Dinitrophenol, mM</td>
<td>1</td>
<td>5</td>
<td>48±16</td>
<td>&lt;0.05</td>
<td>5</td>
<td>21±2</td>
<td>&lt;0.001</td>
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<td>2,4-Dinitrophenol, mM</td>
<td>2</td>
<td>5</td>
<td>18±8</td>
<td>&lt;0.001</td>
<td>5</td>
<td>6±2</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Sodium diatrizoate, mM</td>
<td>7.8</td>
<td>5</td>
<td>84±9</td>
<td>NS</td>
<td>5</td>
<td>81±3</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Iopanoic acid, μM</td>
<td>0.1</td>
<td>3</td>
<td>58±5</td>
<td>&lt;0.01</td>
<td>3</td>
<td>73±7</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Iopanoic acid, μM</td>
<td>1</td>
<td>3</td>
<td>10±2</td>
<td>&lt;0.001</td>
<td>3</td>
<td>43±6</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Iopanoic acid, μM</td>
<td>10</td>
<td>3</td>
<td>8±3</td>
<td>&lt;0.001</td>
<td>3</td>
<td>19±4</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

* Incubations with inhibitor compared to an equal number (n) of simultaneous control incubations. The initial T4 and rT3 concentrations were 1.3 μM and 1.54 nM, respectively. Incubations were carried out for 15 min (T4) and 1 and 2 min (rT3).

† Mean±SE.
quite like that observed in liver homogenate (cf. Table I). Kidney homogenate was likewise as active as liver homogenate in degrading rT₃; at an initial rT₃ concentration of 15.4 nM, the rate of disappearance of rT₃ was 0.083±0.011 pmol/min per mg protein in kidney homogenate, compared to 0.070±0.011 pmol/min per mg protein in liver homogenate. There was no measurable production of T₃ or rT₃ (<0.5 fmol/min per mg protein) from T₄ or destruction of rT₃ (<1.2 fmol/min per mg protein) in homogenates of brain, lung, heart muscle, spleen, or intestine.

In vivo studies

Fasting experiments. Results of these studies are shown in Fig. 8. The rate of T₄ to T₃ conversion in liver homogenate (initial T₄ concentration 1.3 μM) was not significantly different from control at 24 h. It fell to 57±8% of control after 48 h (P < 0.02) and remained at this level subsequently. The rates at 48, 72, and 96 h were not significantly different from one another. After refeeding there was slow return of T₄ to T₃ conversion by the liver homogenate to the control rate; there was still significant impairment of T₄ to T₃ conversion after 96 h of refeeding (68±4% of control, P < 0.05), but no significant difference from control after 144 h (6 days) of refeeding.

The degradation rate of rT₃ in the liver homogenate was normal after 24 h of fasting. It was reduced to 82±8% of control (P < 0.05) after 48 h, and was further reduced after 72 and 96 h (P < 0.01). After refeeding, the rT₃ degradation rate returned to normal by 24 h. There was no change in the protein content of the liver homogenates from fasting animals.

The mean serum T₄ concentration after 24 h of fasting was slightly but significantly lower (P < 0.02) than the control value. It fell further after 48 h (P < 0.001) and remained in the same range thereafter. During refeeding, there was a progressive increase in the mean serum T₄ concentration; it was normal after 72 h of refeeding. The mean serum T₃ concentration after 24 h of fasting was significantly lower than control (P < 0.01), was lower still after 48 h and remained in the same range thereafter. In the fasted rats, the mean T₃-charcoal uptakes were 38% at 24 h, 36% at 48 h, and 35% at 72 h, not significantly different from control (39%). After 96 h the T₃ charcoal uptake decreased to 31%, P < 0.01. These data suggest

**FIGURE 8** Liver homogenate T₄ to T₃ conversion and rT₃ degradation, serum TSH, and thyroid hormone concentrations in rats during starvation (•) and refeeding (○). The refeeding experiments were done in animals previously fasted for 72 h. Data from liver homogenate incubations are expressed as mean±SE values compared to simultaneously studied fed controls. Serum hormone results are mean±SE concentrations. Each point represents data from 5 to 15 rats. The shaded area in panels A and B represents ±SE for the control rats at each time point.
that the serum free T₃ parallels the total T₃, and that fasting truly represents a low T₂ state in the rat.

Serum rT₃ concentrations, corrected for T₄ cross-reactivity (see above), showed no consistent change, although the mean values on several days during fasting and refeeding were higher than control. Because these values are so low and involve a substantial correction for T₄ cross-reactivity, they must be interpreted with caution. With that reservation, there was no fall in serum rT₃ despite a 60% decrease in the mean serum T₄. Serum TSH concentrations fell significantly after 24 h of fasting (P < 0.001) and remained at the same level thereafter. Serum TSH concentrations returned to normal after 72 h of refeeding.

Dexamethasone. Results are shown in Fig. 9. The liver homogenates from rats treated with a single dose of dexamethasone did not have significantly lower mean rates of T₄ to T₃ conversion and rT₃ degradation than the controls. The mean serum concentrations of T₄, T₃, and TSH were also not significantly different in the treated animals and the controls. The corrected mean serum rT₃ concentration, 3.5±0.4 ng/dl, was significantly greater than the mean control value of 1.1±0.3 ng/dl, P < 0.01.

In the animals given daily injections of dexamethasone for 5 days, the mean rate of T₄ to T₃ conversion in the liver homogenates was significantly diminished to 44±4% of control, P < 0.05, and the rate of rT₃ degradation was also significantly diminished to 35±6% of control, P < 0.001. There was no significant difference in mean serum concentrations of T₄, T₃, and TSH between the treated and the control rats, but the mean corrected serum rT₃ concentration was significantly higher in the dexamethasone treated rats, 4.4±0.2 ng/dl, than in controls, 2.0±0.3 ng/dl, P < 0.001.

Endotoxin. There was no significant alteration in the rate of conversion of T₄ to T₃ or the rate of degradation of rT₃ in the liver homogenates from rats treated with endotoxin compared to controls and no change in the serum concentrations of T₄, T₃, rT₃, and TSH.

DISCUSSION

Several inferences can be made about the nature of the reactions that were studied. There is little doubt of the enzymatic nature of T₄ to T₃ conversion or rT₃ degradation in liver and kidney homogenates as described in this paper, although nonenzymatic deiodination of T₄ has been described (49). Typical features of enzymatic catalysis demonstrated for these reactions include temperature and pH dependence, abolition of activity by heating the liver homogenate to 56°C, and tissue specificity. The similarity of the Kₘ and Kᵢ for T₄, and the Kₘ and Kᵢ for rT₃ and the similarity of effects of inhibitors (e.g. PTU and iopanoic acid) suggest, but do not prove, that a single hepatic enzyme catalyzes 5'-mono-deiodination of both

![Figure 9](image-url)

**Figure 9** Liver homogenate T₄ to T₃ conversion and rT₃ degradation and serum TSH and thyroid hormone concentrations in rats treated with one and five daily doses of dexamethasone (1.5 mg/kg i.p.). Values are mean±SE results from five rats. Open bars represent data from control rats and hatched bars data from dexamethasone-treated rats.
T₄ and rT₃. Proof that rT₃ was degraded by 5'-deiodination to 3,3'-T₂ was not obtained in this study, but Chopra has reported rapid production of 3,3'-T₂ from rT₃ and inhibition of this reaction by PTU in a similar liver homogenate system (40). The similarity of the rates of T₄ to T₃ conversion in liver and kidney homogenates and of the rates of rT₃ degradation in liver and kidney shown above are also in general agreement with the findings of Chopra, who reported that the activities of heart, lung, intestine, spleen, and brain homogenates in deiodinating T₄ and rT₃ were much less than in liver or kidney homogenates, and that rT₃ inhibits hepatic conversion of T₄ to T₃ (27). This latter phenomenon raises the possibility that rT₃ regulates T₃ production from T₄ in vivo in patients who have the diverse disorders associated with elevated serum rT₃ and decreased T₃ concentrations. Thus, the primary disturbance may be a decrease in rT₃ degradation. However, it is not possible to make quantitative estimates of in vivo effects from these data, and there is some evidence against such a mechanism because moderate elevations in serum rT₃ concentrations in humans after short-term exogenous rT₃ administration have no effect on serum T₃ concentrations (50).

The decrease in serum T₃ and increase in serum rT₃ concentrations in humans (14, 16, 45) and decreased total T₄ to T₃ conversion in rats (47, 51) found after PTU administration in vivo are likely to be caused by decreased hepatic and renal T₃ production and decreased hepatic (and probably decreased renal) rT₃ degradation caused by the drug. The mechanism of PTU inhibition of T₄ to T₃ conversion and rT₃ degradation suggested by these experiments, allosteric alteration of the enzyme, differs from the mechanism by which PTU inhibits thyroid hormone synthesis in the thyroid. Taurog has shown that both PTU and 1-methyl-2-mercaptoimidazole are metabolized by thyroperoxidase as they inhibit organification of iodide (52). Inasmuch as 1-methyl-2-mercaptoimidazole had no effect in the present system, it is not surprising that the mechanism of PTU inhibition in the liver and kidney differs from its thyroidal mechanism of action. The PTU response characteristics for T₄ to T₃ conversion are similar to those reported by Visser et al. (26), whose method formed the basis of that used here, but who did not investigate rT₃ metabolism.

Iopanoic acid, a widely used oral cholecystographic agent, when administered to humans, causes a reduction in serum T₃ concentrations and an increase in serum T₄, TSH, and rT₃ concentrations (16). Evidence is presented here that the effects of iopanoic acid may result from inhibition of T₃ production and rT₃ degradation in the liver (and perhaps elsewhere), with a compensatory increase in TSH secretion and a consequent increase in T₄ production. It is likely that inhibition by iopanoic acid is a consequence of its molecular structure, and not of iodide derived from it, because diatrizoate, which has an iodide content similar to that of iopanoic acid, is a much less potent inhibitor of T₃ production and rT₃ degradation, and because iodide itself, in a concentration similar to that present in the contrast dyes, had no effect.

Several experiments were performed to assess whether compounds effective as stimulators or inhibitors of T₄ or T₃ metabolism in other in vitro liver systems had effects in this one. A reduction in T₄ deiodination in adult rats (53), and prevention of the neonatal increase in serum T₃ in sheep (54), have been reported as consequences of in vivo administration of α-methylparathyroprotein. This drug had no effect on T₄ to T₃ conversion or rT₃ degradation in liver homogenates. Hillier (55) found that 2,4-dinitrophenol inhibited T₃ deiodination by isolated perfused liver in a concentration range similar to that found here to inhibit both T₄ to T₃ conversion and rT₃ degradation in liver homogenate. Nakagawa and Ruegamer (28), using a more dilute liver homogenate, and Stanbury et al. (37), using a liver microsomal preparation, both of whom used tracer techniques, found stimulation of T₄ deiodination by ferrous ion and reduced glutathione, neither of which had a measurable effect in the present system. The time-course of deiodination and the stimulatory effects of dialysis and preheating reported by those workers also contrast to the time-course of the reactions and the inhibitory effect of dialysis (data not shown) and preheating found here. The substantial differences in methods used in those studies and the present one prevent direct comparisons and analysis of discrepancies.

Fasting and administration of dexamethasone and endotoxin were tested as models of the disease states in man characterized by altered extrathyroidal thyroid hormone metabolism (8, 10–12, 17, 18). They were not entirely satisfactory models, however, because serum thyroid hormone and TSH concentrations did not always change as they do in humans in similar situations. Endotoxin, in fact, had no effect on any of the measurements.

In the fasted rats there were decreased serum T₄, T₃, and TSH concentrations and decreased hepatic T₄ to T₃ conversion, whereas, in fasted humans, serum T₄ and TSH do not change markedly (17). Other workers have reported that fasted rats have decreased serum TSH (56), lowered serum protein bound iodine (57), and a decrease in the rate of whole-body T₄ deiodination (58), and that liver slices from such rats have a reduced conversion rate of T₄ to T₃ (59). The fall in serum T₃ concentrations corresponded most closely in time to the fall in serum TSH concentrations, inasmuch as both fell substantially after
24 h of starvation, whereas the mean serum T₄ concentration fell only slightly, and the rate of hepatic T₄ to T₃ conversion did not change. Return of serum T₃ concentrations to normal with refeeding was also more rapid than recovery of hepatic T₄ to T₃ conversion as measured in vitro. Three possible mechanisms may thus contribute to the fall in serum T₃ in the fasted rat: decreased availability of the precursor, T₄, caused, in turn, by decreased TSH stimulation of T₄ secretion; decreased thyroidal secretion of T₃ itself; and a decreased capacity of the liver to convert T₄ to T₃. The present data suggest that all of these mechanisms are operative but do not allow an estimation of their relative importance. The failure of serum rT₃ to fall despite a 55% decrease in T₄ is also likely due to a combination of several processes, including reduced hepatic degradation, but kinetic studies would be needed to verify this. The exact events responsible for reduced hepatic activity in metabolizing T₄ and rT₃ are not clear, but simple enzyme inhibition by two substances, β-hydroxybutyrate and oleic acid, known to rise during fasting, was not evident when concentrations achieved endogenously were tested in vitro.

A reduction in T₃ production from T₄ in rat liver homogenate after in vivo dexamethasone treatment has been reported recently (48); the results given above confirm that finding and extend it to include a reduction in rT₃ degradation rate. The lack of fall in serum T₃ concentrations is unexplained; it could reflect either dexamethasone-mediated inhibition of T₃ degradation or increased T₄ to T₃ conversion at other sites. An elevation in serum rT₃ concentrations appeared to precede any substantial change in rT₃ metabolism by the liver homogenate. As in the case of fasting, the lack of information about rT₃ production in the rat makes comments about mechanisms of changes in serum concentrations speculative. These changes did not show the expected fall in rat serum TSH and T₄ concentrations after dexamethasone treatment reported by several groups of investigators (60, 61). The elevation in rat serum rT₃ concentrations in ≥24 h and lack of change for several days thereafter is the same pattern seen in humans given pharmacological doses of dexamethasone (11, 12). Whether dexamethasone was ineffective in vitro because of limited exposure of liver tissue to it or because the hepatic effect is a result of an extrahepatic steroid action is not known.

The parallel alterations in hepatic T₄ to T₃ conversion and rT₃ degradation in the in vivo experiments provide further evidence, in addition to the in vitro kinetic and inhibitor data, that both processes are catalyzed by the same enzyme. These results are consistent with the changes generally observed in various states in humans, namely decreased serum T₃ and elevated rT₃ concentrations (4, 7, 10, 12, 15, 16, 18, 21, 45). Moreover, decreases in both T₄ to T₃ conversion and rT₃ degradation rates in patients with cirrhosis were reported by Chopra (4). Other observations, however, are not in accord with this hypothesis. For example, low serum T₃ concentrations increase markedly within hours after birth in the human, but the high serum rT₃ concentrations remain elevated for several weeks (20). Also, reduced serum T₄ but normal, rather than elevated, rT₃ concentrations have been reported in some other clinical situations (7, 21, 62). These findings suggest that T₄ to T₃ converting (T₄-5'-deiodinase) and rT₃ degrading (rT₃-5'-deiodinase) activities are dissociated, and thus may be separate enzymes. The question can ultimately be resolved only by subcellular localization and purification of the enzyme(s).

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