Regulation of the Conversion of Thyroxine to Triiodothyronine in the Perfused Rat Liver

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ABSTRACT This study was undertaken to determine what factors control the conversion of thyroxine (T₄) to triiodothyronine (T₃) in rat liver under conditions approximating those found in vivo. Conversion of T₄ to T₃ was studied in the isolated perfused rat liver, a preparation in which the cellular and structural integrity is maintained and that can perform most of the physiologic functions of the liver. The perfused liver readily extracted T₄ from perfusion medium and converted it to T₃. Production of T₃ by the perfused liver was a function of the size of the liver, the uptake of T₄ by the liver, and the presence of T₄-5'-deiodinase activity. Production of T₃ was increased by increasing the uptake of T₄ by liver, which could be accomplished by increasing the liver size, by increasing the perfusate T₄ concentration, or by decreasing the perfusate albumin concentration. These changes occurred without altering the conversion of T₄ to T₃. The liver had a large capacity for extracting T₄ and for T₄-5'-deiodination to T₃, which was not saturated at a T₄ concentration of 60 µg/dl. Production of T₃ was decreased by inhibiting hepatic T₄-5'-deiodinase with propylthiouracil, which decreased T₃ production by decreasing the conversion of T₄ to T₃. Propylthiouracil did not alter hepatic T₄ uptake.

Fasting resulted in a progressive decrease in hepatic T₄ uptake to 42% of control levels by the 3rd d of fasting; this was accompanied by a proportionate decrease in T₃ production. The rate of conversion of T₄ to T₃ did not change during fasting. When T₄ uptake in 2-d-fasted rat livers was raised to levels found in fed rats by increasing the perfusate T₄ concentration from 10 to 30 µg/dl, T₃ production returned to normal. Again, no change in the rate of conversion of T₄ to T₃ was observed.

These results indicate that the decreased hepatic T₃ production during fasting primarily results from decreased hepatic uptake of T₄, rather than from changes in T₄-5'-deiodinase activity. Thus, these studies have delineated a new mechanism that functions independently of enzyme quantity or activity whereby production of T₃ from T₄ is regulated.

INTRODUCTION

Many tissues are known to deiodinate thyroxine (T₄) with the resultant production of 3,5,3'-triiodothyronine (T₃) (1–9), but only recently has it been appreciated that most of the circulating T₃ is derived from extrathyroidal 5'-monodeiodination of T₄ rather than from thyroidal secretion (10–13). This deiodination increases the metabolic effect of T₄ because T₃ is more potent than T₄. Additional studies have shown that T₄ can also undergo 5-monodeiodination in extrathyroidal tissues with the formation of 3,3',5'-triiodothyronine (rT₃) (14, 15); this mechanism accounts for nearly all of the daily rT₃ production. Unlike T₄, rT₃ has little intrinsic metabolic activity, though it inhibits the conversion of T₄ to T₃ in vitro (16, 17). These alternate pathways of T₄ metabolism provide a mechanism whereby the metabolic effects of T₄ can be regulated by decreasing the availability of T₃ to tissues. That such regulation occurs is suggested by the findings of decreased serum T₃ concentrations in humans during starvation, acute and chronic illness, in the fetus, and in response to several pharmacologic agents (18, 19). Kinetic studies in fasted or cirrhotic patients directly demonstrated decreased peripheral conversion of T₄ to T₃ (15, 20). However, the elevated serum rT₃ concentrations were found to result primarily from decreased rT₃ degradation rather than from increased rT₃ production. In vitro studies using rat liver homogenates and slices have shown that fasting is asso-

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1 Abbreviations used in this paper: BSA, bovine serum albumin; KHBB, Krebs-Henseleit bicarbonate buffer; M1, methimazole; PTU, propylthiouracil; rT₃, 3,3',5'-triiodothyronine; T₃, thyroxine; T₄, 3,5,3'-triiodothyronine.
cialed with decreased generation of T₃ from T₄ (17, 21, 22) and decreased rT₃ degradation (17) and have suggested that decreased T₂-5'-deiodinase activity was responsible for these changes. Although it is clear that hepatic T₂-5'-deiodinase activity is decreased in fasted rats, it is not clear whether the liver produces less T₃ from T₄ during fasting or, if it does, that decreased deiodinase activity is responsible for decreased T₃ production under physiologic conditions in vivo. This study was undertaken to determine what factors control the production of T₃ from T₄ using the isolated perfused liver, a preparation in which cellular and structural integrity are preserved, and that is capable of performing most of the physiologic functions of the liver.

METHODS

Materials. T₄, T₃, bovine serum albumin (BSA), propylthiouracil (PTU), and methimazole (MMI) were purchased from Sigma Chemical Co., St. Louis, Mo. The T₄ and T₃ used in perfusions were dissolved in 0.01 N NaOH (100 µg/ml) and stored at −20°C. [¹²⁵I]T₃ and [¹²³I]T₂ were prepared by iodination of 3.5-T₄ and T₃, respectively, using previously described techniques (23). Iobead resin was purchased from Technicon Instruments Corp., Tarrytown, N. Y.

Animals. Male Sprague-Dawley rats weighing 250–300 g were used in all experiments. The animals were fed Wayne Lab-Blox rat feed (Allied Mills, Inc., Chicago, Ill.) and had free access to tap water. In each study, rats from one shipment were used and the mean animal weight for each group was comparable. In the fasting studies, each group of rats was matched for weight before the initiation of fasting. Fasting was begun at 9 a.m. and the perfusions performed between 9 a.m. and 1 p.m. To study the effect of PTU in vivo, rats were given PTU as a 0.05%-solution in 25% glucose (to enhance its palatability) for 3 d in place of drinking water. As a control, another group of rats was given 25% glucose for 3 d in place of drinking water.

Technique of rat liver perfusion. A modification of the method of Exton and Park (24, 25) was used for isolation and perfusion of the rat liver in situ. After induction of anesthesia with intraperitoneal sodium pentobarbital (60 mg/kg) (Abbott Laboratories, North Chicago, Ill.), the abdomen was opened and ligatures placed around the inferior vena cava above the right renal vein, the superior mesenteric and celiac arteries, and the portal vein. A cannula was rapidly inserted into the portal vein and the perfusion pump started. The vena cava below the right renal vein was then cut, allowing perfusion medium to flow through the liver and escape; the ligatures around the portal vein and the arteries were tied. The thorax was then opened and an outflow cannula inserted through the right atrium into the thoracic vena cava. Finally, the ligature around the abdominal inferior vena cava was tied, thus closing the circuit. The perfusate leaving the liver flowed by gravity to a reservoir 15–20 cm below the liver and was subsequently recirculated. The livers contained little endogenous blood by the time the circuit was completed, because, when perfused with buffer containing no BSA or erythrocytes, the livers lost all color and the perfusate leaving the liver contained few erythrocytes (<5,000/mm³) and little protein (<0.1 mg/ml). Livers were perfused for 1–2 h and in any study the perfusion duration was identical in all animals (see figure legends for exact perfusion times).

The perfusion medium consisted of Krebs-Henseleit bicarbonate buffer (KHBB) containing BSA (3 g/dl except when otherwise stated) and sufficient outdated human erythrocytes to give a hematocrit of 12–16%. The BSA was initially prepared as a 25 g/dl solution in KHBB, which was dialyzed for 48 h at 4°C against three to four changes of KHBB. The erythrocytes were washed twice with 2–3 vol of both 0.9% NaCl and KHBB before use. Because leukocytes are reported to concentrate amino acids (26) and to convert T₄ to T₃ and rT₃ (27), the leukocyte layer was carefully aspirated with each washing. Unless otherwise stated, the T₄ and glucose concentrations were 10 µg/dl and 100 mg/dl, respectively. The medium (95 ml) was equilibrated in the apparatus with 95% O₂–5% CO₂ before perfusion to achieve a temperature of 37°C and a pH of 7.4. The final volume in the system when the circuit was complete was 56–58 ml, and care was taken to keep the final volume as constant as possible from perfusion to perfusion.

Using this technique the preparation was routinely completed within 4–6 min after opening the abdomen, and at no time was the liver unperfused. For the duration of the perfusion, livers from fed rats appeared grossly normal and maintained a normal oxygen consumption and bile production (2.31±0.20 [SEM] µmol/min per g liver, n = 8; 49.3±1.1 µl/h per g liver, n = 8). No liver swelling occurred during the perfusion as evidenced by an unchanged wet to dry weight ratio (perfused, 2.25±0.05, n = 9; unperfused, 2.23±0.04, n = 8) and liver weight to body weight ratio (perfused, 3.92±0.09 g/100 g, n = 8; unperfused, 3.98±0.06 g/100 g, n = 8). The metabolic normalcy of the preparation was evidenced by attainment of maximal rates of gluconeogenesis from lactate (20 mM), 96±5 µmol/g per h, n = 7, during the first 15 min of perfusion in 48-h-fasted rats (24).

Samples (1 ml) were taken from the reservoir every 30 min and kept on ice until separation of the erythrocytes. The medium was stored at −20°C until assayed. At the end of the perfusion, the pump was stopped, and a 0.6–0.9-g portion of liver was rapidly placed in a preweighed grinding vessel containing 1 ml 0.01 M PO₄, 0.15 M NaCl, pH 7.4, at 4°C. The grinding vessel was quickly weighed and then stored at −70°C until further processing. The perfusion medium was then pumped out of the apparatus and its volume determined. The sum of this volume, the volume of the sample removed during the perfusion, and the recirculating volume, i.e., the volume remaining in the apparatus after evacuation, −0.5 ml, was taken as the initial volume in the recirculating system. The remaining liver was removed and weighed, thus allowing calculation of the total liver weight.

Processing of liver samples. The liver samples were thawed to 4°C and then homogenized with a motor driven Teflon (DuPont Instrument Corp., Wilmington, Del.) pestle. The homogenate was twice extracted with 4 ml 100% ethanol and the extracts pooled. The pooled extract was stored at −20°C for 24–36 h to allow further precipitation of liver tissue. The crude extracts were then evaporated to dryness and diluted to a final volume of 1 ml of 0.01 M PO₄, pH 7.4, and stored at −20°C for assay. Using this method, [¹²³I]T₂ was extracted with an efficiency that was inversely proportional to the liver weight according to the following equation: extraction efficiency (% on total liver weight) = x −21.6 × liver weight (g) + 91.9 (r = 0.96, P < 0.001).

The final T₃ concentration in the liver extracts was corrected for the extraction efficiency obtained using this formula. The extraction efficiency of T₄ was not influenced by the weight of the liver sample and averaged 58.93±0.53%. The efficiency of extraction of T₃ and T₄ was unaffected by fasting.

T₄ and T₃ determinations in perfusion media. Samples were assayed in quadruplicate using double antibody radio-
immunoassay methods previously described for serum (28, 29). The T4 assay conditions were identical to the serum assay with the following exceptions: (a) use of [125I]T4 of a high specific activity (23); (b) dilution of anti-T3 antibody to a final dilution of 1:15,000; and (c) preparation of the standard curve with KHBB and the appropriate concentration of BSA. The sensitivity of this assay was 4–8 pg T4/tube. The perfusate T4 assay was identical to the serum assay except the standards were prepared with KHBB and BSA. Assay sensitivity was 20 pg/tube. Deiodination of [125I]T3 was determined using Ibeads, an ion exchange resin that removes inorganic iodide from aqueous solutions.

T4 and T3 determinations in liver extracts. These determinations were carried out in quadruplicate using double antibody radioimmunoassay techniques as previously described (17). The sensitivity of the assays was 4–8 pg/tube for T4 and 20–40 pg/tube for T3. Varying the volume of liver extract from 10 to 100 µl resulted in linear dose-response curves.

Calculations. The uptake of T4 by the liver was calculated by the following formulas: (a) T4 uptake = (initial T4 concentration – final concentration) × mean corrected perfusion volume; (b) mean corrected perfusion volume = final volume/[erythrocyte volume + 0.5 (sample volume removed during perfusion)].

The appearance of T3 in the perfusion medium was calculated as follows: (c) T3 appearance (perfusion) = (final T3 concentration – initial T3 concentration) × mean corrected perfusion volume.

The appearance of T3 in the liver was calculated by subtracting, from the observed liver T3 concentration, the mean liver T3 concentration in a comparable group of rats following perfusion with medium containing no T4 and multiplying this difference by the total liver weight. Perfusion with medium containing no T4 did not alter the endogenous liver T3 concentration in either fed or fasted rats. Thus, T3 production was calculated as the sum of the perfusate and liver productions. This does not take into account any biliary secretion of T3 and its conjugates or deiodination of T3 by liver (see Results). The conversion of T4 to T3 was calculated by dividing the T3 production by the T3 taken up by the liver.

Expression of results. The uptake of T4 and the production of T3 are expressed relative to the wet weight of the liver in grams except when an experimental manipulation resulted in a change in liver size between groups, i.e., fed vs. fasted animals (see Results, Fig. 6). When such comparisons were made, T4 uptake and T3 production were then expressed as the absolute quantity in micrograms or nanograms, respectively.

Statistical evaluation of data. In experiments with two groups of animals, statistical analysis was performed using Student’s t test. When three or more treatment groups were studied, a one-way analysis of variance was performed and Duncan’s multiple range test was then applied to identify significant differences among specific groups (30). Data are presented as the mean ± SEM.

RESULTS

General characteristics of the perfusion system (Fig. 1). When perfused at a T4 concentration of 10 µg/dl, a BSA concentration of 4%, and a flow rate of 1.5 ml/g per min, the rat liver readily extracted T4 from the media as evidenced by a significant decline in the perfusion medium T4 concentration by 30 min (P < 0.001). Though the T4 concentration continued to fall during the course of the perfusion, this decline was minimal and subsequent T4 concentrations were not significantly lower than the 30-min value. The extraction of T4 by the liver was associated with a prompt, significant increase in both the perfusate T3 concentration (P < 0.001) and the liver T3 concentration (P < 0.001) by 30 min. The perfusate T3 concentration increased in a linear fashion for 60–90 min before reaching a plateau, whereas the liver T3 concentration increased most dramatically in the first 30 min of perfusion, followed by a more gradual but linear increase thereafter. Total T3 production (perfusion + liver) increased the most in the first 30 min of perfusion, coincident with the period of rapid T4 uptake by the liver. T3 production proceeded in a linear fashion thereafter, though at a slower rate. The T3 in the perfusate generally accounted for 20–30% of the total T3 production. Approximately 2–4% of the T4 extracted by the liver was converted to T3 in 1–2 h of perfusion. When livers were perfused with media containing no
T4, neither T3 nor T4 could be detected in the perfusion media. Likewise, when media containing T4 was circulated in the system without a liver, no T3 production was demonstrable.

**Effect of perfusion flow rate (Table I).** When livers were perfused at different flow rates based on the average liver wt/100 g body wt, T3 production was maximal at flow rates of 1.0–1.5 ml/g liver per min. When perfused at 3 ml/g liver per minute, T3 production was significantly lower (P < 0.05). The hepatic extraction of T4 was unaffected by these variations in flow rate. All subsequent studies were performed using a flow rate of 1.5 ml/g liver per min.

**Effect of perfusate glucose concentration (Table II).** Varying the glucose concentration from 0 to 300 mg/dl had no effect on T3 production at an initial perfusate T4 concentration of either 10 or 30 μg/dl. Similarly, T3 production by livers from fasted rats was the same at medium glucose concentrations of 40, 100, and 300 mg/dl. The addition of insulin to perfusion medium containing 100 or 300 mg/dl glucose had no effect on T3 production by livers from either fed or fasted rats.

**Effect of perfusion with T3.** When perfused for 1 h with T3, 60 ng/dl, and tracer amounts of [125I]T3, 68.5 ± 1.0% (n = 4) of the [125I]T3 was taken up by the liver in fed rats, and 54.6 ± 2.1% (n = 4) in fasted rats. Biliary excretion of [125I]T3 represented 12.4 ± 2.4% (n = 4) of the total [125I]T3 in the system in fed rats and 14.7 ± 0.6% in fasted animals. Deiodination of [125I]T3 was 12.0 ± 1.3% (n = 4) in fed rats and 11.7 ± 0.8% (n = 4) in fasted rats. Neither the biliary excretion nor the deiodination of T3 were used in calculating T3 production in the system and therefore the reported values underestimate the true production. However, it is clear that differences in biliary excretion or deiodination cannot explain any difference between fed and fasted rats.

**Effect of liver weight (Fig. 2).** When livers of fed rats were perfused at a T4 concentration of 10 μg/dl and a BSA concentration of 3 g/dl, large rat livers (12.65 ± 0.75 g) took up more T4 (P < 0.02) and produced more T3 (P < 0.05) than did small rat livers (7.65 ± 0.52 g). Both T4 uptake and T3 production were increased in proportion to the increased liver weight for the reason that values were not different when expressed on a per gram basis. There was no difference in the conversion of T4 to T3.

**Effects of the perfusate T4 concentration (Fig. 3).** Increasing the perfusate T4 concentration from 3 to 60 μg/dl, at a BSA concentration of 4 g/dl, resulted in a progressive increase in T3 uptake by the liver (P < 0.001 for 30 and 60 μg/dl vs. 3 and 10 μg/dl). This increase in T3 uptake was associated with an increased T3 production (P < 0.005 and < 0.001 for 30 and 60 μg/dl vs. 3 and 10 μg/dl). No differences in the percent conversion of T4 to T3 were found as the T4 concentration was increased, again indicating that a relatively constant fraction of the extracted T4 was converted to T3. Even with a perfusate T4 concentration of 60 μg/dl, the maximal response may not have been obtained. It is important to note that at T4 concentrations

**TABLE II**

<table>
<thead>
<tr>
<th>Glucose concentration (mg/dl)</th>
<th>T4 Production (ng/g/10 min) with Varying Concentrations of Glucose</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>100</td>
</tr>
<tr>
<td>T4—30 μg/dl</td>
<td>16.25±2.21</td>
</tr>
<tr>
<td>T4—10 μg/dl</td>
<td>3.66±0.25</td>
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</tbody>
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Perfusion medium contained 4.0 g/dl BSA. Perfusions were of 1.5 h duration.

**FIGURE 2** Effect of liver weight on the uptake of T4, T3 production, and conversion of T4 to T3 in fed rats. Perfusion medium contained 3 g/dl BSA and 10 μg/dl T4. Perfusions were 1 h in duration. Values shown are mean±SEM.
Effects of the perfusate BSA concentration (Fig. 4). Increasing the perfusate BSA concentration from 1 to 4 g/dl, at a T4 concentration of 10 μg/dl, resulted in a progressive stepwise reduction in the uptake of T4 by the liver \( (P < 0.001) \) for 3 and 4 g/dl vs. 1 g/dl. This reduction in T4 uptake was associated with a concomitant reduction in T3 production \( (P < 0.05) \) for 3 and 4 g/dl vs. 1 g/dl. No difference in the percent conversion of T4 to T3 was observed as the BSA concentration was increased, indicating that the quantity of T3 produced was directly proportional to the quantity of T4 taken up by the liver.

Effects of PTU and MMI (Fig. 5). Because PTU is an inhibitor of T4-5′-deiodinase activity in homogenate systems \( (16, 17) \), perfusion with PTU should inhibit T3 production by decreasing the percent conversion of T4 to T3. When livers of fed rats were perfused with media containing PTU (58.7 μM), a significant reduction in both T4 production \( (P < 0.001) \) and percent conversion of T4 to T3 \( (P < 0.001) \) was found. Likewise, when PTU was given to rats as a 0.05% solution in 25% glucose, a significant reduction in both T4 production \( (P < 0.001) \) and percent conversion of T4 to T3 \( (P < 0.001) \) was found when compared with rats given only 25% glucose. Addition of MMI (87.7 μM) to perfusion media had no effect on T3 production.

Effects of fasting (Fig. 6). Fasting for 1–3 d was

\[
\text{Uptake of } T_4 \quad \mu g/g/2h
\]

\[
\text{T_4 Production} \quad ng/g/2h
\]

\[
\text{Conversion of } T_4 \text{ to } T_3 \quad \%
\]

\[
\text{Albumin Concentration} \quad g/dl
\]

FIGURE 3 Effect of perfusate T4 concentration on the uptake of T4, T3 production, and conversion of T4 to T3. The perfusate BSA concentration was 4 g/dl. Perfusions were 2 h in duration. Values shown are mean±SEM.

similar to those found in the rat, i.e., 3–10 μg/dl, the slope of the dose response was shallow and an increase in the perfusate T4 concentration was not associated by a proportionate increase in either T4 uptake or T3 production.

\[
\text{Uptake of } T_4 \quad \mu g/g/2h
\]

\[
\text{T_3 Production} \quad ng/g/2h
\]

\[
\text{Conversion of } T_4 \text{ to } T_3 \quad \%
\]

\[
\text{Albumin Concentration} \quad g/dl
\]

FIGURE 4 Effect of perfusate albumin concentration on the uptake of T4, T3 production, and conversion of T4 to T3. The perfusate T4 concentration was 10 μg/dl. Perfusions were 2 h in duration. Values shown are mean±SEM.

FIGURE 5 Effect of MMI and PTU on the uptake of T4, T3 production, and T4 conversion to T3. The perfusion medium contained 3 g/dl BSA and 10 μg/dl T4. Perfusions were 1 h in duration. Values shown are mean±SEM.
associated with a significant decline in the total liver weight (fed, 11.28±0.38 g; 1-d fast, 7.91±0.29 g \(P < 0.001\); 2-d fast, 6.78±0.23 g \(P < 0.001\); 3-d fast, 6.08±0.40 g \(P < 0.001\). Although the hepatic uptake of T₃ on a per gram basis did not change during fasting (0.17±0.01 µg/g per h), total uptake of T₄ decreased from 1.91±0.14 µg/h in the fed rat to 1.35±0.15 µg/h after a 1-d fast \((P < 0.01)\), 1.13±0.11 µg/h after 2 d \((P < 0.001)\), and 1.11±0.10 µg/h after 3 d \((P < 0.001)\). This decrease in the uptake of T₄ was associated with a concomitant fall in total T₃ production from 39.7±7.9 to 19.7±2.9 ng/h by day 2 \((P < 0.005)\) and to 16.8±1.4 ng/h \((P < 0.005)\) on day 3. This fall in T₃ production during fasting was the result of decreased hepatic extraction of T₃, as there was no significant change in the rate of conversion of T₄ to T₃ during fasting. Because the conversion of T₄ to T₃ is an index of the deiodinase enzyme activity, these data indicate that the fall in hepatic deiodinase activity reported to occur in fasting did not appreciably contribute to the fall in T₃ production.

**Effect of perfusate T₄ concentration on T₄ uptake and T₃ production in fasting (Fig. 7).** If decreased uptake of T₄ is the primary defect responsible for decreased hepatic T₃ production in fasting, then increasing T₄ uptake to levels found in fed rats should return T₃ production to normal. When the perfusate T₄ concentration was increased from 10 to 30 µg/dl in 2-d fasted rats (3 g/dl BSA), T₄ uptake increased progressively to levels found in fed rats. T₃ production was restored to normal when the perfusate T₄ concentration was 30 µg/dl. The rate of conversion of T₄ to T₃ did not change over this range of T₄ concentrations.

However, when livers of 2-d fasted rats were perfused at 100 µg/dl, the conversion of T₄ to T₃ fell from 3.93±0.66 to 2.19±0.25% \((P < 0.05)\). These data indicate that the decline in conversion of T₄ to T₃ observed in homogenate preparations in fasted rats can be reproduced in the perfused liver at high, unphysiologic T₄ concentrations. However, at more physiologic T₄ concentrations, this phenomenon is not observed.

**Effects of fasting on the endogenous liver T₃ and T₄ content (Fig. 8).** Fasting was associated with a decrease in the hepatic content of both T₃ and T₄, which was statistically significant after 1 d of fasting \((P < 0.001\) for both T₃ and T₄). The ratio of the content of T₃ to T₄ remained relatively constant, between 0.25 to 0.32, during the course of the fast. This sup-

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**FIGURE 7.** Effect of perfusate T₄ concentration on the uptake of T₄, T₃ production, and conversion of T₄ to T₃ in 2-d-fasted rats. The perfusate BSA concentration was 3.0 g/dl. Perfusions were 1 h in duration. Values shown are mean±SEM.

**FIGURE 6.** Effect of fasting on the uptake of T₄, T₃ production, and conversion of T₄ to T₃. The perfusion medium contained 3 g/dl BSA and 10 µg/dl T₄. Perfusions were 1 h in duration. Values shown are mean±SEM.

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The present studies demonstrate that the fall in hepatic deiodinase activity observed during fasting is not the primary regulatory mechanism for decreasing the hepatic production of T₃ from T₄ because if it were, a decrease in the hepatic ratio of T₃ to T₄ should have been found.

DISCUSSION

The present studies demonstrate the utility of the perfused rat liver for studying hepatic T₃ production. The results show that three factors are important in determining hepatic T₃ production: liver size; T₄ uptake by the liver; and hepatic T₄-5'-deiodinase activity. The liver was found to have a large capacity for both T₄ uptake and T₄-5'-deiodination, which was not saturated at high, unphysiologic T₄ concentrations. Finally, the results show that hepatic production of T₃ from T₄ was decreased during fasting and provide evidence that at physiologic concentrations of T₄, the decline in T₃ production resulted primarily from decreased hepatic T₄ uptake rather than from changes in the rate of conversion of T₄ to T₃, i.e., in T₄-5'-deiodinase activity.

Previous studies using rat liver homogenates and slices have clearly demonstrated a decrease in T₄-5'-deiodinase activity during fasting (17, 21, 22), untreated streptozotocin-induced diabetes (21), hypothyroidism (22, 31-33), and in the fetus (34, 35). This decrease in 5'-deiodinase activity in fasting has been attributed wholly or in part to deficiency of a cytosol cofactor rather than to a quantitative decrease in the enzyme itself (36-38). Substances that maintain sulfhydryl groups in the reduced state, such as reduced glutathione, NADPH, and dithiothreitol were shown to restore T₄-5'-deiodinase activity to normal levels in liver homogenates from fasted rats. In contrast, agents that oxidize sulfhydryl groups, such as diamide, oxidized glutathione, mercuric chloride, and N-ethylmaleimide, produced a decrease in homogenate deiodinase activity. More recently, PTU, an allosteric inhibitor of deiodinase activity (16, 17), was shown to compete with reduced glutathione for a non-catalytic site on the deiodinase enzyme (39). Taken together, these data have suggested that hepatic 5'-deiodinase activity is modulated by altering the availability of reduced sulfhydryl groups, presumably glutathione.

Although these studies have shed considerable light on the biochemistry of T₄-5'-deiodination, it is not clear whether the decrease in deiodinase activity is the primary regulatory mechanism mediating the decreased conversion of T₄ to T₃ under physiologic conditions. The data cited above were obtained primarily from in vitro studies using rat liver homogenates. Such broken cell preparations lack both the control systems associated with intact cellular and organ structure and the ability to respond to metabolic and hormonal perturbations. The incubations were performed in buffer solutions containing little or no binding proteins, with inadequate or absent oxygenation, and using high concentrations of T₄ (50-500 mg/dl). In such preparations homogenate deiodinase activity had a Km for T₄ of 1.5-7.7 μM (116-592.9 μg/dl) (17, 34), a priori suggesting lack of a regulatory role because the enzymatic capacity greatly exceeds the quantities of endogenous T₄ available for deiodination. Thus, the 50% reduction in deiodinase activity found in liver homogenates from fasted rats should have little measurable effect on the conversion of T₄ to T₃ at physiologic T₄ concentrations. Finally, homogenate preparations, although allowing study of the enzymatic and biochemical aspects of deiodination, cannot be used to evaluate the possibility that T₃ neogenesis, like gluconeogenesis, might be regulated by the availability of substrate, i.e., T₄, as well as by the prevailing enzyme activity.

Use of the isolated perfused liver obviates some of the above-mentioned problems. The cellular and structural integrity of the liver is maintained in a milieu similar to that of the intact rat. The ability to respond to both substrate and hormonal signals was maintained, and the T₄ concentration (10 mg/dl) used was close to that found in the normal rat (3-5 mg/dl), although the percentage of protein-bound T₄ would be lower. The isolated perfused liver readily extracted T₄ from the perfusion media and 5'-deiodinated it with the production of T₃, in accord with previous studies with this preparation (40). The data indicating that T₃ production at near physiologic con-
centrations of T₄ is in some instances regulated by alterations in T₄ uptake rather than changes in deiodinase activity require elaboration of the distinctions between these processes. Total production of T₃ is a function of liver size, T₄ uptake, and T₄-5′-deiodinase activity. In contrast, the percent conversion of T₄ to T₃ reflects the fraction of intracellular T₄ converted to T₃ and is a function of T₄-5′-deiodinase activity. Only this latter component of T₃ production can be measured in homogenate systems. In the perfused liver, increasing liver weight resulted in proportionate increases in T₄ uptake and T₃ production; the percentage of T₄ to T₃ conversion was unchanged. Similarly, T₃ production increased proportionately when T₄ uptake was increased by increasing perfusate T₄ or decreasing perfusate albumin concentrations. Again, the percentage of T₄ converted to T₃ was unchanged, even at a perfusate T₄ concentration of 60 µg/dl, far exceeding that ever found in vivo. In contrast, PTU decreased T₃ production by reducing the percent conversion of T₄ to T₃, as would be expected of an inhibitor of T₄-5′-deiodinase, without altering T₄ uptake.

These perfusion results indicate that the primary factor responsible for the decreased hepatic T₃ production observed during fasting was decreased T₄ uptake. When rats were fasted for 1-3 d, hepatic T₄ uptake decreased in proportion to the fall in liver weight. Decreased T₄ uptake was accompanied by a proportionate decrease in T₃ production. There was no change in the conversion of T₄ to T₃. Furthermore, when T₄ uptake by livers from fasted rats was increased to levels found in livers from fed rats, by increasing the perfusate T₄ concentration from 10 to 30 µg/dl, total T₃ production was restored to normal. Again, there was no change in the rate of conversion of T₄ to T₃. Only at a very high T₄ concentration (100 µg/dl) was the conversion of T₄ to T₃ decreased during fasting. Therefore, at physiologic T₄ concentrations, the 50-60% decrease in T₄-5′-deiodinase activity in liver homogenates from fasted rats probably does not play a major regulatory role in decreasing hepatic T₃ production during fasting, consistent with the kinetic measurements in homogenates indicating a high Kₘ (high capacity) enzyme. The concomitant and proportionate fall in the endogenous liver T₄ and T₃ content during fasting further supports the validity of the in vitro findings.

It is important to appreciate that the perfusate-free T₄ concentration was higher than that found in normal rat serum because the only T₄-binding protein present was albumin, a high capacity, low affinity T₄-binding protein (T₄-resin uptake 35.9% for rat serum, 47.5% for 4.0% BSA) and because the T₄ concentration (10 µg/dl) was two- to three-fold greater than that in normal rat serum (3-5 µg/dl). This was evidenced by the finding that perfusion with a T₄ concentration of 10 µg/dl and BSA concentration of 4 g/dl increased the liver T₄ concentration to 8-10 times above the endogenous T₄ concentration. The perfused liver was therefore driven to produce more T₃ than usually occurs in vivo because of the increased hepatic uptake of T₄. In the presence of additional and more potent T₄-binding proteins, as are present in serum, T₄ uptake by the liver would be less, limiting to a greater extent the T₄ available for conversion of T₃. Therefore, under in vivo conditions, the uptake of T₄ into the liver would be even more rate limiting for the conversion of T₄ to T₃ than was apparent in the perfused liver.

Although these studies underscore the importance of the T₄ uptake into the liver as a regulatory mechanism for T₃ production, altered conversion of T₄ to T₃ (altered enzyme activity) is also important. This is the mechanism by which PTU inhibits T₃ production. Preliminary studies in situations of extreme metabolic abnormality, i.e., untreated streptozocin-induced diabetes, indicate that decreased T₃ production is a result of both decreased T₄ uptake and decreased conversion of T₄ to T₃ (deiodinase activity). Furthermore, the methods used in the present study may not be sufficiently sensitive to detect small changes in conversion of T₄ to T₃.

Decreased hepatic uptake of T₄ during fasting could result from either decreased activity of a specific transport system for T₄, decreased quantities of tissue T₄-binding proteins, or increased serum concentration of a compound(s) that competed with T₄ for either transport sites or T₄-binding proteins. Although there is little evidence to support the existence of a specific T₄ transport system, there is considerable evidence that hepatic T₄ uptake is influenced by the quantity and the affinity of T₄-binding proteins on each side of the plasma membrane (41-45). The studies reported here demonstrating decreased T₄ uptake into the liver with increasing perfusate albumin concentrations provide further validation for this concept. It seems likely that the decreased T₄ uptake in fasted rat livers resulted from a decrease in T₄-binding proteins in the liver. A number of cellular proteins have binding sites for T₄. These proteins are located in a variety of cell fractions including the nucleus (46, 47), mitochondria (48, 49), microsomes (50), cytosol (51, 52), and plasma membranes (53, 54). Recent evidence suggests that the binding capacity of both the hepatocyte nucleus (55-57) and cytosol (58) for thyroid hormones is reduced by fasting. Which of the cellular T₄-binding proteins is most important in regulating the uptake of T₄ into the cell and the mechanisms through which these binding sites are regulated remain to be determined.

In summary, the data demonstrate the utility of the isolated perfused liver in the study of T₄ metabolism. The results demonstrate the large capacity of the liver for T₄ uptake and its 5′-deiodination to T₃. Hepatic
production of T₃ from T₄ was decreased by fasting; this decrease primarily resulted from decreased T₄ uptake by the liver. The data further suggest, though they do not prove, that the uptake of T₃ into the liver is determined by the relative abundance of cellular and extracellular T₄ binding sites and that the decreased hepatic uptake of T₄ during fasting resulted from loss of cellular T₄-binding proteins.

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