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Research Article

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Fasting resulted in a progressive decrease in hepatic T_4 uptake to 42% of control levels by the 3rd d of fasting; this was accompanied by a proportionate decrease in T_3 production. The rate of conversion of T_4 to T_3 did not change during fasting. When T_4 uptake in 2-d-fasted rat livers was raised to levels found in fed rats by increasing the perfusate T_4 concentration from 10 to 30 μg/dl, T_3 production returned to normal. Again, no change in the rate of conversion of T_4 to T_3 was observed.

These results indicate that the decreased hepatic T_3 production during fasting primarily results from decreased hepatic uptake of T_4, rather than from changes in T_4-5'-deiodinase activity. Thus, these studies have delineated a new mechanism that functions independently of enzyme quantity or activity whereby production of T_3 from T_4 is regulated.

INTRODUCTION

Many tissues are known to deiodinate thyroxine (T_4)[1] with the resultant production of 3,5,3'-triiodothyroxine (T_3) (1–9), but only recently has it been appreciated that most of the circulating T_3 is derived from extrathyroidal 5'-monodeiodination of T_4 rather than from thyroidal secretion (10–13). This deiodination increases the metabolic effect of T_4 because T_3 is more potent than T_4. Additional studies have shown that T_4 can also undergo 5-monodeiodination in extrathyroidal tissues with the formation of 3,3',5'-triiodothyronine (rT_3) (14, 15); this mechanism accounts for nearly all of the daily rT_3 production. Unlike T_3, rT_3 has little intrinsic metabolic activity, though it inhibits the conversion of T_4 to T_3 in vitro (16, 17). These alternate pathways of T_4 metabolism provide a mechanism whereby the metabolic effects of T_4 can be regulated by decreasing the availability of T_3 to tissues. That such regulation occurs is suggested by the findings of decreased serum T_3 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 T_4 to T_3 (15, 20). However, the elevated serum rT_3 concentrations were found to result primarily from decreased rT_3 degradation rather than from increased rT_3 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; MMI, methimazole; PTU, propylthiouracil; rT_3, 3,3',5'-triiodothyronine; T_4, thyroxine; T_3, 3,5,3'-triiodothyronine.

ciated with decreased generation of T_3 from T_4 (17, 21, 22) and decreased rT_3 degradation (17) and have suggested that decreased T_4-5'-deiodinase activity was responsible for these changes. Although it is clear that hepatic T_4-5'-deiodinase activity is decreased in fasted rats, it is not clear whether the liver produces less T_3 from T_4 during fasting or, if it does, that decreased deiodinase activity is responsible for decreased T_3 production under physiologic conditions in vivo. This study was undertaken to determine what factors control the production of T_3 from T_4 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. T_3, T_4, bovine serum albumin (BSA), propyl-thiouracil (PTU), and methimazole (MMI) were purchased from Sigma Chemical Co., St. Louis, Mo. The T_3 and T_4 used in perfusions were dissolved in 0.01 N NaOH (100 μg/ml) and stored at −20°C. [^{125}I]T_3 and [^{125}I]T_4 were prepared by iodination of 3,5-T_2 and T_3, respectively, using pre-viously described techniques (23). Iobead resin was pur-chased 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 ship-ment 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. Fast-ing was begun at 9 a.m. and the perfusions performed be-tween 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 bi-carbonate 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 pre-pared 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 T_4 to T_3 and rT_3 (27), the leukocyte layer was carefully aspirated with each washing. Unless otherwise stated, the T_4 and glucose concentrations were 10 μg/dl and 100 mg/dl, re-spectively. The medium (95 ml) was equilibrated in the apparatus with 95% O_2–5% CO_2 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 com-pleted within 4–6 min after opening the abdomen, and at no time was the liver unperfused. For the duration of the per-fusion, livers from fed rats appeared grossly normal and main-tained 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 preweighted grinding vessel containing 1 ml 0.01 M PO₄, 0.15 M NaCl, pH 7.4, at 4°C. The grinding vessel was quickly reweighed and then stored at −70°C until further processing. The per-fusion medium was then pumped out of the apparatus and its volume determined. The sum of this volume, the volume of the samples removed during the perfusion, and the residual vol-ume, 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, recentrifuged, the final volume recorded, and an aliquot stored at −20°C for assay. Using this method, [^{125}I]T_3 was extracted with an efficiency that was inversely proportional to the liver weight according to the following equation: extraction efficiency (%) = −21.6 × liver weight (g) + 91.9 (r = 0.96, P < 0.001).

The final T_3 concentration in the liver extracts was cor-rected for the extraction efficiency obtained using this formula. The extraction efficiency of T_4 was not influenced by the weight of the liver sample and averaged 58.93±0.53%. The efficiency of extraction of T_3 and T_4 was unaffected by fasting.

T_3 and T_4 determinations in perfusion media. Samples were assayed in quadruplicate using double antibody radio-

immunoassay methods previously described for serum (28, 29). The T_3 assay conditions were identical to the serum assay with the following exceptions: (a) use of $[^{125}I]T_3$ of a high specific activity (23); (b) dilution of anti-T_3 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 T_3/tube. The perfusate T_4 assay was identical to the serum assay except the standards were prepared with KHBB and BSA. Assay sensitivity was 20 pg/tube. Deiodination of $[^{125}I]T_3$ was determined using Iobeads, an ion exchange resin that removes inorganic iodide from aqueous solutions.

T_3 and T_4 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 T_3 and 20–40 pg/tube for T_4. Varying the volume of liver extract from 10 to 100 μl resulted in linear dose-response curves.

Calculations. The uptake of T_4 by the liver was calculated by the following formulas: (a) T_4 uptake = (initial T_4 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 T_3 in the perfusion medium was calculated as follows: (c) T_3 appearance (perfusate) = (final T_3 concentration – initial T_3 concentration) × mean corrected perfusion volume.

The appearance of T_3 in the liver was calculated by subtracting, from the observed liver T_3 concentration, the mean liver T_3 concentration in a comparable group of rats following perfusion with medium containing no T_4 and multiplying this difference by the total liver weight. Perfusion with medium containing no T_4 did not alter the endogenous liver T_3 concentration in either fed or fasted rats. Thus, T_3 production was calculated as the sum of the perfusate and liver productions. This does not take into account any biliary secretion of T_3 and its conjugates or deiodination of T_3 by liver (see Results). The conversion of T_4 to T_3 was calculated by dividing the T_3 production by the T_4 taken up by the liver.

Expression of results. The uptake of T_4 and the production of T_3 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, T_4 uptake and T_3 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 T_4 concentration of 10 μg/dl, a BSA concentration of 4%, and a flow rate of 1.5 ml/g liver per min, the rat liver readily extracted T_4 from the media as evidenced by a significant decline in the perfusion medium T_4 concentration by 30 min ($P < 0.001$). Though the T_4 concentration continued to
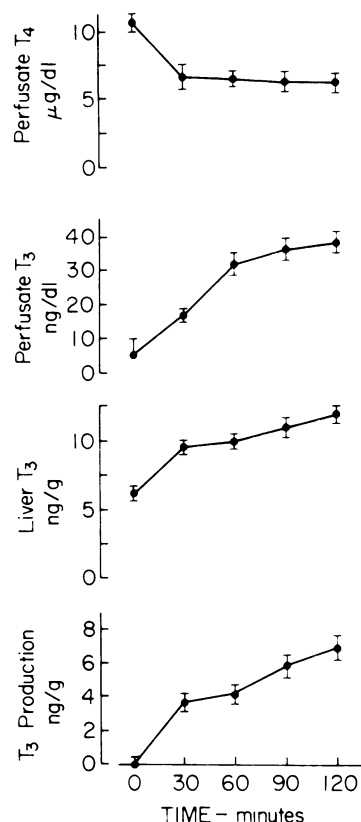

FIGURE 1 Changes in perfusate T_4 and T_3 concentration, liver T_3 concentration, and T_3 production during perfusion. The perfusion medium contained 4 g/dl BSA and 10 μg/dl T_4. The perfusate data in the upper two panels represent points (mean±SEM) obtained from eight 2-h perfusions. Corresponding points in the lower two panels represent the mean±SEM of eight perfusions of the indicated duration.

fall during the course of the perfusion, this decline was minimal and subsequent T_4 concentrations were not significantly lower than the 30-min value. The extraction of T_4 by the liver was associated with a prompt, significant increase in both the perfusate T_3 concentration ($P < 0.001$) and the liver T_3 concentration ($P < 0.001$) by 30 min. The perfusate T_3 concentration increased in a linear fashion for 60–90 min before reaching a plateau, whereas the liver T_3 concentration increased most dramatically in the first 30 min of perfusion, followed by a more gradual but linear increase thereafter. Total T_3 production (perfusate + liver) increased the most in the first 30 min of perfusion, coincident with the period of rapid T_4 uptake by the liver. T_3 production proceeded in a linear fashion thereafter, though at a slower rate. The T_3 in the perfusate generally accounted for 20–30% of the total T_3 production. Approximately 2–4% of the T_4 extracted by the liver was converted to T_3 in 1–2 h of perfusion. When livers were perfused with media containing no

T_4, neither T_3 nor T_4 could be detected in the perfusion media. Likewise, when media containing T_4 was circulated in the system without a liver, no T_3 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, T_3 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, T_3 production was significantly lower ($P < 0.05$). The hepatic extraction of T_4 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 T_3 production at an initial perfusate T_4 concentration of either 10 or 30 µg/dl. Similarly, T_3 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 T_3 production by livers from either fed or fasted rats.

Effect of perfusion with T_3. When perfused for 1 h with T_3, 60 ng/dl, and tracer amounts of $[^{125}I]T_3$, $68.5 \pm 1.0\%$ ($n = 4$) of the $[^{125}I]T_3$ was taken up by the liver in fed rats, and $54.6 \pm 2.1\%$ ($n = 4$) in fasted rats. Biliary excretion of $[^{125}I]T_3$ represented $12.4 \pm 2.4\%$ ($n = 4$) of the total $[^{125}I]T_3$ in the system in fed rats and $14.7 \pm 0.6\%$ in fasted animals. Deiodination of $[^{125}I]T_3$ was $12.0 \pm 1.3\%$ ($n = 4$) in fed rats and $11.7 \pm 0.8\%$ ($n = 4$) in fasted rats. Neither the biliary excretion nor the deiodination of T_3 were used in calculating T_3 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 T_4 concentration of 10 µg/dl and a BSA concentration of 3 g/dl, large rat livers (12.65 ± 0.75 g) took up more T_4 ($P < 0.02$) and produced more T_3 ($P < 0.05$) than did small rat livers (7.65 ± 0.52 g). Both T_4 uptake and T_3 production were

TABLE I
Effect of Perfusion Flow Rate on T_3 Production and T_4 Uptake

Flow rate	T_3 production	T_4 uptake
ml/g/min	*ng/g/2 h*	*µg/g/2 h*
1.0	5.99±0.87	0.18±0.02
1.5	6.87±0.82	0.15±0.02
3.0	2.76±0.64	0.16±0.01

Perfusion medium contained 4 g/dl BSA and 10 µg/dl T_4. Perfusions were of 2 h duration.

TABLE II
T_3 Production (ng/g/90 min) with Varying Concentrations of Glucose

	Glucose concentration		
	0	100	300
		mg/dl	
T_4—30 µg/dl	16.25±2.21	14.04±1.82	14.96±0.80
T_4—10 µg/dl		3.66±0.25	4.44±0.82

Perfusion medium contained 4.0 g/dl BSA. Perfusions were of 1.5 h duration.

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 T_4 to T_3.

Effects of the perfusate T_4 concentration (Fig. 3). Increasing the perfusate T_4 concentration from 3 to 60 µg/dl, at a BSA concentration of 4 g/dl, resulted in a progressive increase in T_4 uptake by the liver ($P < 0.001$ for 30 and 60 µg/dl vs. 3 and 10 µg/dl). This increase in T_4 uptake was associated with an increased T_3 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 T_4 to T_3 were found as the T_4 concentration was increased, again indicating that a relatively constant fraction of the extracted T_4 was converted to T_3. Even with a perfusate T_4 concentration of 60 µg/dl, the maximal response may not have been obtained. It is important to note that at T_4 concentrations
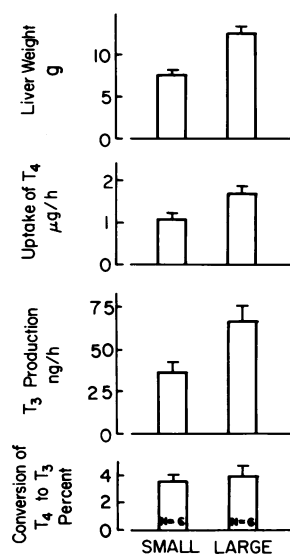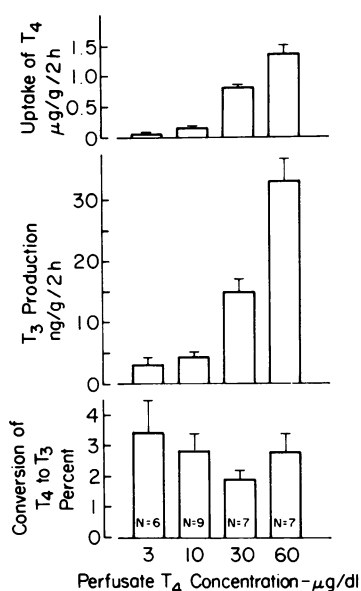

FIGURE 2 Effect of liver weight on the uptake of T_4, T_3 production, and conversion of T_4 to T_3 in fed rats. Perfusion medium contained 3 g/dl BSA and 10 µg/dl T_4. Perfusions were 1 h in duration. Values shown are mean±SEM.

FIGURE 3 Effect of perfusate T_4 concentration on the uptake of T_4, T_3 production, and conversion of T_4 to T_3. 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 T_4 concentration was not associated by a proportionate increase in either T_4 uptake or T_3 production.
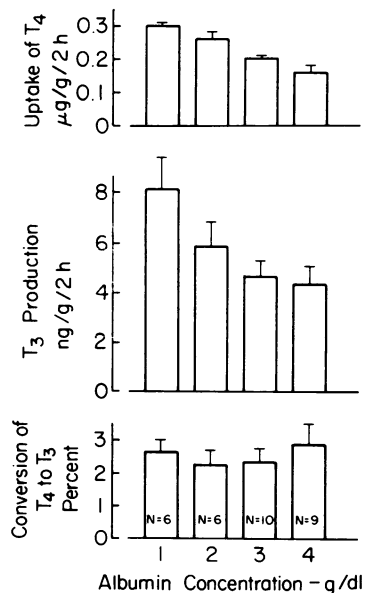

FIGURE 4 Effect of perfusate albumin concentration on the uptake of T_4, T_3 production, and conversion of T_4 to T_3. The perfusate T_4 concentration was 10 μg/dl. Perfusions were 2 h in duration. Values shown are mean±SEM.

Effects of the perfusate BSA concentration (Fig. 4). Increasing the perfusate BSA concentration from 1 to 4 g/dl, at a T_4 concentration of 10 μg/dl, resulted in a progressive stepwise reduction in the uptake of T_4 by the liver ($P < 0.001$ for 3 and 4 g/dl vs. 1 g/dl). This reduction in T_4 uptake was associated with a concomitant reduction in T_3 production ($P < 0.05$ for 3 and 4 g/dl vs. 1 g/dl). No difference in the percent conversion of T_4 to T_3 was observed as the BSA concentration was increased, indicating that the quantity of T_3 produced was directly proportional to the quantity of T_4 taken up by the liver.

Effects of PTU and MMI (Fig. 5). Because PTU is an inhibitor of T_4-5'-deiodinase activity in homogenate systems (16, 17), perfusion with PTU should inhibit T_3 production by decreasing the percent conversion of T_4 to T_3. When livers of fed rats were perfused with media containing PTU (58.7 μM), a significant reduction in both T_3 production ($P < 0.001$) and the percent conversion of T_4 to T_3 ($P < 0.001$) was found. Likewise, when PTU was given to rats as a 0.05% solution in 25% glucose, a significant reduction in both T_3 production ($P < 0.001$) and percent conversion of T_4 to T_3 ($P < 0.001$) was found when compared with rats given only 25% glucose. Addition of MMI (87.7 μM) to perfusion media had no effect on T_3 production.
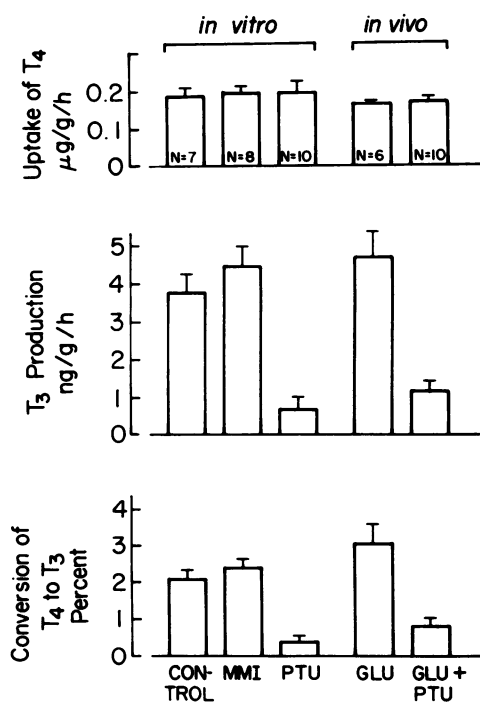
Effects of fasting (Fig. 6). Fasting for 1–3 d was

FIGURE 5 Effect of MMI and PTU on the uptake of T_4, T_3 production, and T_4 conversion to T_3. The perfusion medium contained 3 g/dl BSA and 10 μg/dl T_4. 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 T_4 on a per gram basis did not change during fasting (0.17 ± 0.01 μg/g per h), total uptake of T_4 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 T_4 was associated with a concomitant fall in total T_3 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 T_3 production during fasting was the result of decreased hepatic extraction of T_4, as there was no significant change in the rate of conversion of T_4 to T_3 during fasting. Because the conversion of T_4 to T_3 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 T_3 production.

Effect of perfusate T_4 concentration on T_4 uptake and T_3 production in fasting (Fig. 7). If decreased uptake of T_4 is the primary defect responsible for decreased hepatic T_3 production in fasting, then in-
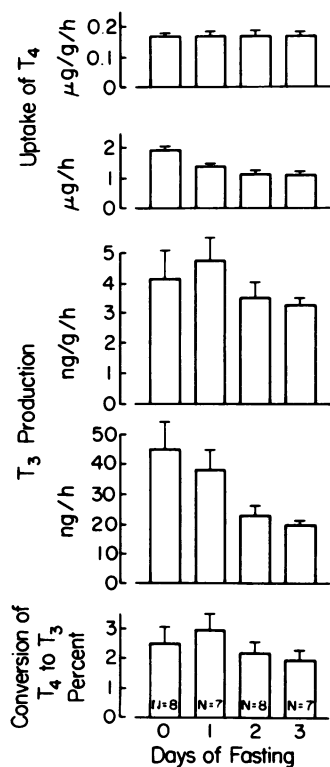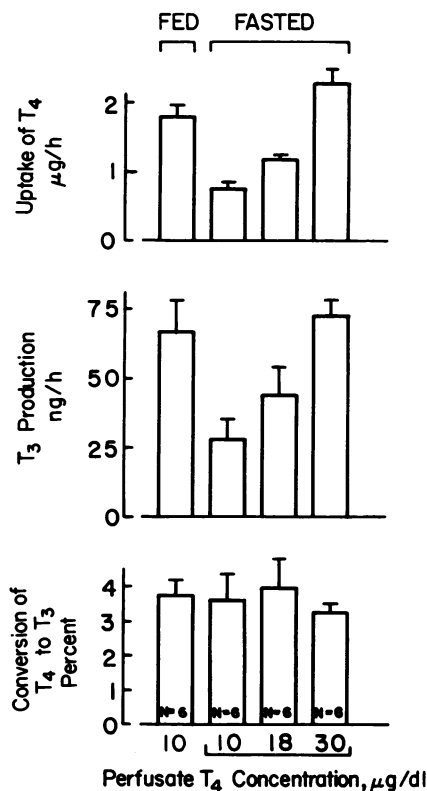

FIGURE 7. Effect of perfusate T_4 concentration on the uptake of T_4, T_3 production, and conversion of T_4 to T_3 in 2-d-fasted rats. The perfusate BSA concentration was 3.0 g/dl. Perfusions were 1 h in duration. Values shown are mean$\pm$SEM.

creasing T_4 uptake to levels found in fed rats should return T_3 production to normal. When the perfusate T_4 concentration was increased from 10 to 30 μg/dl in 2-d fasted rats (3 g/dl BSA), T_4 uptake increased progressively to levels found in fed rats. T_3 production was restored to normal when the perfusate T_4 concentration was 30 μg/dl. The rate of conversion of T_4 to T_3 did not change over this range of T_4 concentrations.

However, when livers of 2-d fasted rats were perfused at 100 μg/dl, the conversion of T_4 to T_3 fell from 3.93 ± 0.66 to $2.19\pm0.25\%$ ($P < 0.05$). These data indicate that the decline in conversion of T_4 to T_3 observed in homogenate preparations in fasted rats can be reproduced in the perfused liver at high, unphysiologic T_4 concentrations. However, at more physiologic T_4 concentrations, this phenomenon is not observed.

Effects of fasting on the endogenous liver T_3 and T_4 content (Fig. 8). Fasting was associated with a decrease in the hepatic content of both T_3 and T_4 which was statistically significant after 1 d of fasting ($P < 0.001$ for both T_3 and T_4). The ratio of the content of T_3 to T_4 remained relatively constant, between 0.25 to 0.32, during the course of the fast. This sup-
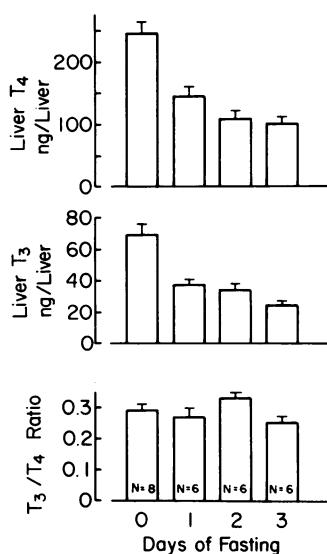

FIGURE 6 Effect of fasting on the uptake of T_4, T_3 production, and conversion of T_4 to T_3. The perfusion medium contained 3 g/dl BSA and 10 μg/dl T_4. Perfusions were 1 h in duration. Values shown are mean$\pm$SEM.

FIGURE 8 Endogenous liver content of T_4 and T_3 and the hepatic T_3/T_4 ratio during fasting. Values shown are mean±SEM.

ports the contention that the fall in hepatic deiodinase activity observed during fasting is not the primary regulatory mechanism for decreasing the hepatic production of T_3 from T_4 because if it were, a decrease in the hepatic ratio of T_3 to T_4 should have been found.

DISCUSSION

The present studies demonstrate the utility of the perfused rat liver for studying hepatic T_3 production. The results show that three factors are important in determining hepatic T_3 production: liver size; T_4 uptake by the liver; and hepatic T_4-5'-deiodinase activity. The liver was found to have a large capacity for both T_4 uptake and T_4-5'-deiodination, which was not saturated at high, unphysiologic T_4 concentrations. Finally, the results show that hepatic production of T_3 from T_4 was decreased during fasting and provide evidence that at physiologic concentrations of T_4, the decline in T_3 production resulted primarily from decreased hepatic T_4 uptake rather than from changes in the rate of conversion of T_4 to T_3, i.e., in T_4-5'-deiodinase activity.

Previous studies using rat liver homogenates and slices have clearly demonstrated a decrease in T_4-5'-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 T_4-5'-deiodinase activity to normal levels in liver homogenates from fasted rats. In contrast, agents that oxidize sulfhydryl groups, such as diamide, oxidized gluthathione, 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 noncatalytic 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 T_4-5'-deiodination, it is not clear whether the decrease in deiodinase activity is the primary regulatory mechanism mediating the decreased conversion of T_4 to T_3 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 T_4 (50–500 μg/dl). In such preparations homogenate deiodinase activity had a K_m for T_4 of 1.5–7.7 μM (116.5–592.9 μg/dl) (17, 34), a priori suggesting lack of a regulatory role because the enzymatic capacity greatly exceeds the quantities of endogenous T_4 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 T_4 to T_3 at physiologic T_4 concentrations. Finally, homogenate preparations, although allowing study of the enzymatic and biochemical aspects of deiodination, cannot be used to evaluate the possibility that T_3 neogenesis, like gluconeogenesis, might be regulated by the availability of substrate, i.e., T_4, 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 T_4 concentration (10 μg/dl) used was close to that found in the normal rat (3–5 μg/dl), although the percentage of protein-bound T_4 would be lower. The isolated perfused liver readily extracted T_4 from the perfusion media and 5'-deiodinated it with the production of T_3, in accord with previous studies with this preparation (40). The data indicating that T_3 production at near physiologic con-

centrations of T_4 is in some instances regulated by alterations in T_4 uptake rather than changes in deiodinase activity require elaboration of the distinctions between these processes. Total production of T_3 is a function of liver size, T_4 uptake, and T_4-5'-deiodinase activity. In contrast, the percent conversion of T_4 to T_3 reflects the fraction of intracellular T_4 converted to T_3 and is a function of T_4-5'-deiodinase activity. Only this latter component of T_3 production can be measured in homogenate systems. In the perfused liver, increasing liver weight resulted in proportionate increases in T_4 uptake and T_3 production; the percentage of T_4 to T_3 conversion was unchanged. Similarly, T_3 production increased proportionately when T_4 uptake was increased by increasing perfusate T_4 or decreasing perfusate albumin concentrations. Again, the percentage of T_4 converted to T_3 was unchanged, even at a perfusate T_4 concentration of 60 $\mu g/dl$, far exceeding that ever found in vivo. In contrast, PTU decreased T_3 production by reducing the percent conversion of T_4 to T_3, as would be expected of an inhibitor of T_4-5'-deiodinase, without altering T_4 uptake.

These perfusion results indicate that the primary factor responsible for the decreased hepatic T_3 production observed during fasting was decreased T_4 uptake. When rats were fasted for 1-3 d, hepatic T_4 uptake decreased in proportion to the fall in liver weight. Decreased T_4 uptake was accompanied by a proportionate decrease in T_3 production. There was no change in the conversion of T_4 to T_3. Furthermore, when T_4 uptake by livers from fasted rats was increased to levels found in livers from fed rats, by increasing the perfusate T_4 concentration from 10 to 30 $\mu g/dl$, total T_3 production was restored to normal. Again, there was no change in the rate of conversion of T_4 to T_3. Only at a very high T_4 concentration (100 $\mu g/dl$) was the conversion of T_4 to T_3 decreased during fasting. Therefore, at physiologic T_4 concentrations, the 50-60% decrease in T_4-5'-deiodinase activity in liver homogenates from fasted rats probably does not play a major regulatory role in decreasing hepatic T_3 production during fasting, consistent with the kinetic measurements in homogenates indicating a high K_m (high capacity) enzyme. The concomitant and proportionate fall in the endogenous liver T_4 and T_3 content during fasting further supports the validity of the in vitro findings.

It is important to appreciate that the perfusate-free T_4 concentration was higher than that found in normal rat serum because the only T_4-binding protein present was albumin, a high capacity, low affinity T_4-binding protein (T_3-resin uptake 35.9% for rat serum, 47.5% for 4.0% BSA) and because the T_4 concentration (10 $\mu g/dl$) was two- to three-fold greater than that in normal rat serum (3-5 $\mu g/dl$). This was evidenced by the finding that perfusion with a T_4 concentration of 10

$\mu g/dl$ and BSA concentration of 4 g/dl increased the liver T_4 concentration to 8-10 times above the endogenous T_4 concentration. The perfused liver was therefore driven to produce more T_3 than usually occurs in vivo because of the increased hepatic uptake of T_4. In the presence of additional and more potent T_4-binding proteins, as are present in serum, T_4 uptake by the liver would be less, limiting to a greater extent the T_4 available for conversion of T_3. Therefore, under in vivo conditions, the uptake of T_4 into the liver would be even more rate limiting for the conversion of T_4 to T_3 than was apparent in the perfused liver.

Although these studies underscore the importance of the T_4 uptake into the liver as a regulatory mechanism for T_3 production, altered conversion of T_4 to T_3 (altered enzyme activity) is also important. This is the mechanism by which PTU inhibits T_3 production. Preliminary studies in situations of extreme metabolic abnormality, i.e., untreated streptozotocin-induced diabetes, indicate that decreased T_3 production is a result of both decreased T_4 uptake and decreased conversion of T_4 to T_3 (deiodinase activity). Furthermore, the methods used in the present study may not be sufficiently sensitive to detect small changes in conversion of T_4 to T_3.

Decreased hepatic uptake of T_4 during fasting could result from either decreased activity of a specific transport system for T_4, decreased quantities of tissue T_4-binding proteins, or increased serum concentration of a compound(s) that competed with T_4 for either transport sites or T_4-binding proteins. Although there is little evidence to support the existence of a specific T_4 transport system, there is considerable evidence that hepatic T_4 uptake is influenced by the quantity and the affinity of T_4-binding proteins on each side of the plasma membrane (41-45). The studies reported here demonstrating decreased T_4 uptake into the liver with increasing perfusate albumin concentrations provide further validation for this concept. It seems likely that the decreased T_4 uptake in fasted rat livers resulted from a decrease in T_4-binding proteins in the liver. A number of cellular proteins have binding sites for T_4. 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 T_4-binding proteins is most important in regulating the uptake of T_4 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 T_4 metabolism. The results demonstrate the large capacity of the liver for T_4 uptake and its 5'-deiodination to T_3. Hepatic

production of T_3 from T_4 was decreased by fasting; this decrease primarily resulted from decreased T_4 uptake by the liver. The data further suggest, though they do not prove, that the uptake of T_4 into the liver is determined by the relative abundance of cellular and extracellular T_4 binding sites and that the decreased hepatic uptake of T_4 during fasting resulted from loss of cellular T_4-binding proteins.

ACKNOWLEDGMENTS

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