Iodothyronine Metabolism in Rat Liver Homogenates

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ABSTRACT To investigate mechanisms of extrathyroidal thyroid hormone metabolism, conversion of thyroxine (T_4) to 3,5,3'-triiodothyronine (T_3) and degradation of 3,3',5'-triiodothyronine (rT_3) were studied in rat liver homogenates. Both reactions were enzymatic. For conversion of T_4 to T_3, the K_m of T_4 was 7.7 μM, and the V_{max} was 0.13 pmol T_3/min per mg protein. For rT_3 degradation, the K_m of rT_3 was 7.5 nM, and the V_{max} was 0.36 pmol rT_3/min per mg protein. Production of rT_3 or degradation of T_4 or T_3 was not detected under the conditions employed. rT_3 was a potent competitive inhibitor of T_4 to T_3 conversion with a K_i of 4.5 nM; 3,3'-diiodothyronine was a less potent inhibitor of this reaction. T_4 was a competitive inhibitor of rT_3 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-methylparatyrosine, Fe^{+2}, Fe^{+3}, reduced glutathione, β-hydroxybutyrate, or oleic acid.

Fasting resulted in inhibition of T_4 to T_3 conversion and of rT_3 degradation by rat liver homogenates which was reversible after refeeding. Serum T_4, T_3, and thyrotropin concentrations fell during fasting, with no decrease in serum protein binding as assessed by a T_3-charcoal uptake. There was no consistent change in serum rT_3 concentrations. Dexamethasone had no effect in vitro. In vivo dexamethasone administration resulted in elevated serum rT_3 concentrations after 1 day, and after 5 days, in inhibition of T_4 to T_3 conversion and rT_3 degradation without altering serum T_4, T_3, 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 T_4 to T_3 conversion or rT_3 production or degradation could be detected in other tissues.

These data suggest that one iodothyronine 5'-deiodinase is responsible for both T_4 to T_3 conversion and rT_3 degradation in liver and, perhaps, in kidney. Alterations in serum T_3 and rT_3 concentrations induced by drugs and disease states may result from decreases in both T_3 production and rT_3 degradation consequent to inhibition of a single reaction in the pathways of iodothyronine metabolism.

INTRODUCTION

Deiodination is a major mechanism of thyroxine (T_4)[1] disposal in the human and the rat (1, 2). The active thyroid hormone, 3,5,3'-triiodothyronine (T_3) is produced by 5'-deiodination of T_4, whereas a calorigenically inactive compound, 3,3',5'-triiodothyronine (reverse-T_3, rT_3), is produced by 5-deiodination. Rates of removal of the 5'- and 5-iodine of T_4 are approximately equal in humans and rats (3, 4), but these processes do not occur randomly. Decreased serum T_3 concentrations, resulting from decreased peripheral 5'-deiodination of T_4, are found in patients with a variety of illnesses, during fasting and in fetal life (4–21). In these situations, serum rT_3 concentrations are usually elevated, suggesting diversion of T_4 to the inactivating 5-deiodinating pathway (4, 7, 8, 10, 12, 15, 16, 18, 20, 21). However, elevated serum rT_3 concentrations in two such situations, hepatic cirrhosis and fasting, were recently shown (4, 22) to result from decreased rT_3 degradation rather than increased rT_3 production. In fetal sheep, the metabolic clearance rate of rT_3 is low, relative to adult sheep (23), and, although the production rate of rT_3 is elevated, it is not markedly different from the adult rate if expressed as a fraction of the daily T_4 production (0.32 for the fetal sheep vs. 0.25 for the adults).

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[1] *Abbreviations used in this paper*: PTU, 2-propyl-6-thiouracil; 3,5-T_2, 3,5-diiodothyronine; 3,3'-T_2, 3,3'-diiodothyronine; T_3, 3,5,3'-triiodothyronine; rT_3 (reverse T_3), 3,3',5'-triiodothyronine; T_4, thyroxine; TSH, thyrotropin.

Many tissues, including liver, kidney, heart, skeletal muscle, leukocytes, and fibroblasts, are capable of deiodinating T_4 in vitro (24–39). The techniques used to demonstrate T_4 deiodination in these studies included incubation of tissue homogenates, tissue slices, dispersed cells, and whole organ perfusion. T_3 has been identified as a deiodination product of T_4 (24, 26, 27, 29, 31, 35, 36). At the subcellular level, both T_4 deiodination and T_4 to T_3 conversion were localized to the microsomal fraction of liver homogenates (37–39), whereas production of rT_3 from T_4 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 T_4 metabolism in vitro in situations such as starvation or treatment with drugs which alter extrathyroidal T_4 or rT_3 metabolism in man.

The original aim of this study of T_4 metabolism in rat liver homogenates was to obtain information about T_3 and rT_3 production, inasmuch as the nature of these processes and their regulation are undefined. It soon became clear that, if rT_3 was being produced, it was also being destroyed too rapidly for its production to be observed. Further experiments showed that factors which inhibited T_4 to T_3 conversion also inhibited rT_3 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 rT_3 degradation, the experiments were extended to test the hypothesis that T_4 to T_3 conversion and rT_3 degradation reflect activities of the same enzyme. The results support this hypothesis. This report, therefore, describes biochemical characterization of T_4 to T_3 conversion and rT_3 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. rT_3 was a gift of Dr. R. Meltzer, Warner-Lambert Research Institute, Morris Plains, N. J.; 3,3'-diiodothyronine (3,3'-T_2) was a gift of Dr. H. Cahnmann, National Institutes of Health, Bethesda, Md.; 3,5-diiodothyronine (3,5-T_2) 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 ^{125}I-rT_3 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 (wt/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, microsomes, 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. Homogenates from individual rats were incubated separately in the in vivo studies but were pooled for kinetic and inhibition studies. In the latter, four to five rat livers were used, and the incubations were done in triplicate or quadruplicate. Iodothyronines were dissolved in 0.25% bovine serum albumin (BSA), 0.01 M PO_4, 0.15 M NaCl, pH 7.5 (BSA-phosphate-buffered saline). Other added compounds were dissolved in BSA-phosphate-buffered saline, water or ethanol. These substances were added to the homogenates in volumes of 10–60 μl/ml homogenate at the beginning of the incubation period; equal volumes of vehicle were added to control homogenates. 200–500-μl aliquots of the homogenate were removed after various time intervals and immediately mixed with 2 vol of 95% ethanol and stored at 4°C until assayed. Unincubated homogenate was also mixed with 2 vol 95% ethanol for use as a blank to which standards for the hormone radioimmunoassays were added. Slow formation of a fine precipitate occurred in the ethanol phase for about 24 h. This interfered in the assays, so the samples were stored at least 24 h before assay, and they were centrifuged just before assay to remove this precipitate. Recovery of iodothyronines added to prewarmed homogenates and immediately extracted and assayed as below was 82±7% for T_4, 62±2% for rT_3, and 75±5% for T_3. All measured hormone concentrations were corrected for recovery.

T_4, T_3, and rT_3 determinations in liver extracts. To duplicate tubes were added: (*a*) 100 μl of ^{125}I-T_4, ^{125}I-rT_3, or ^{125}I-T_3 dissolved in 0.4% BSA, 0.05 M K_2HPO_4, pH 7.4 (BSA-KPO_4); (*b*) 100 μl of anti-T_4, anti-T_3, or anti-rT_3 serum diluted in BSA-KPO_4 containing 0.05 M EDTA to give 35–50% binding of labeled hormone; (*c*) 50 μl of T_4, T_3, or rT_3 standards diluted in blank ethanol extract of liver or 50 μl of homogenate extract or dilution thereof; and (*d*) BSA-KPO_4 to make a final volume of 1 ml. Tubes were incubated for 18–24 h at 4°C and free and antibody-bound labeled hormone was separated with goat anti-rabbit IgG. The sensitivity of these assays was 60–80 pg T_4, 8–15 pg T_3, and 4–8 pg rT_3. In the T_3 and rT_3 assays, varying the volume of extract in the assay tubes from 10 to 75 μl resulted in a linear dose-response; with volumes $\geqslant$100 μl there was under-recovery.

Kinetic studies. In the calculation of reaction rates, the differences between initial measured T_3 or rT_3 concentrations and those at later times, specified below, were used. Addition of reagents, mixing, and sampling took $\cong$ 7 s. This was taken as zero in the T_4 to T_3 conversion experiments, but was taken as 7 s in the rT_3 degradation experiments, with initial concentrations calculated by extrapolation. Initial extracts taken after addition of T_4 had immunoreactivity for T_3 and rT_3 which increased in proportion to the added T_4. It cannot be determined how much of this activity was due to contamination of the T_4 preparation and how much was intrinsic T_4 cross-reactivity. In that the T_3 immunoreactivity did not change over 120 min when T_4 was incubated with buffer or heat inactivated homogenate, it was felt that subtracting the initial concentration from subsequent ones gave a valid measurement of T_3 production. The initial T_3 immunoreactivity limited the range of added T_4 concentrations which could be used, because, at high initial T_4 concentrations, the change in T_3 concentrations became a progressively smaller fraction of the initial T_3 immunoreactivity. For this reason, the concentration of T_4 used in the inhibition and in vivo studies had to be less than the K_m of T_4.

The rT_3 immunoreactivity after addition of T_4 to homogenates did not change over 120 min. In that rT_3 was degraded so rapidly (see below), this was considered to represent T_4 cross-reactivity in the rT_3 assay, and thus did not invalidate measurements of changes in rT_3 concentrations in the presence of T_4. A similar interaction of 3,3'-T_2 was noted in the rT_3 assay.

Endogenous T_4 concentrations in rat liver have been estimated to be 11.05±1.63 ng/g whole liver (41), which would be <5 nmol/liter of homogenate, <1% of the T_4 added in the present experiments. No information is available about endogenous hepatic rT_3 concentrations. They are probably very low; even with the unlikely assumption that the entire difference between blank liver extract and buffer in the rT_3 assay was due to endogenous rT_3, the endogenous rT_3 concentration would contribute <0.8 nmol/liter homogenate. Once again, this concentration is small compared to those used in these experiments. For the above reasons, endogenous T_4 and rT_3 were felt to be negligible in the kinetic calculations and were ignored.

The determination of K_m and K_i values for rT_3 was complicated by the substantial decrease in rT_3 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 rT_3 concentrations in the kinetic plots as suggested by Segel (42).

T_4, T_3, and rT_3 determinations in serum. Double antibody assays were performed as previously described (43–45). The anti-T_4 antibody was purchased from Endocrine Sciences, Tarzana, Calif. The rT_3 assay was modified from the assay for rT_3 in human serum described elsewhere (45), using 200 μl serum, 75 μl anti-rT_3 antiserum diluted 1:5000, ^{125}I-rT_3 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 T_4 was 0.04%, and cross-reactivity with T_3 was <0.01%. There was rT_3 immunoreactivity in all but one of 138 rat serum samples tested. In these 137, the measured rT_3 concentrations ranged from 1.6 to 7.2 ng/dl, with a mean of 4.0 ng/dl. Because 0.04% T_4 cross-reactivity combined with a serum T_4 concentration of 5 μg/dl accounts for 2 ng rT_3/dl, a substantial fraction of the total, the measured rT_3 concentrations were corrected by subtracting 0.04% of the T_4 concentration in each serum. The corrected mean normal rat serum rT_3 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 T_3 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 rT_3 concentrations in the range of 2 ng/dl are consistent with a recent report (48) of immeasurable rat serum rT_3 using an assay with a sensitivity of 6 ng/dl. The low values and the necessity of using a substantial correction for T_4 cross-reactivity require that the rT_3 concentrations be interpreted very cautiously.

To assess serum protein binding of T_3, a T_3-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 ^{125}I-T_3 in 50 μl 0.1% BSA, 0.075 M Na PO_4, 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 T_4 5.6±1.6 μg/dl SD) with serum from rats made hyperthyroid by injection of T_4, 10 μg/100 g/day s.c. for 12 days (total serum T_4 8.9±2.1 μg/dl) and with serum from thyroidectomized rats (total serum T_4 1.7 ±0.3 μg/dl). The mean T_3-charcoal uptakes were: normal, 39.1±3.5% SD; T_4-treated, 53±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 T_4, 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 rT_3, aliquots being taken at 0 and 3 min. With these incubation conditions rT_3 degradation was reliably measurable. The concentration of rT_3 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 T_3 as a function of time when varying

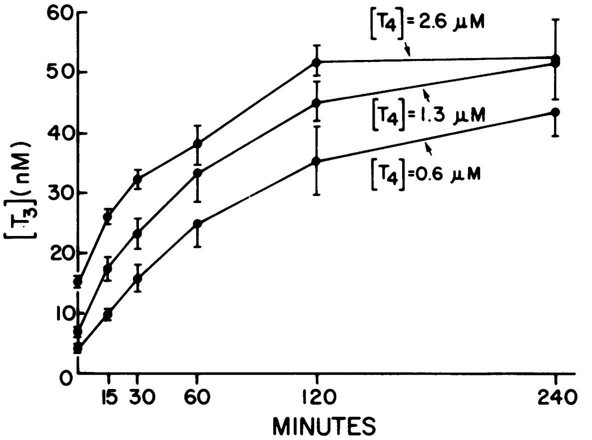

FIGURE 1 T_3 concentrations±SE in liver homogenate during 240-min incubations with initial T_4 concentrations of 0.6, 1.3, and 2.6 μM. No T_3 was added.

amounts of T_4 (0.6–2.6 μM) were incubated with liver homogenate. With each dose of T_4 the rate of T_3 production declined after 30–60 min. With initial T_4 concentrations of 0.65, 1.3, and 2.6 μmol/liter, the net production of T_3 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 T_4 added. No decline in directly measured T_4 concentrations was detectable in this period. With no added T_4, there was no increase in homogenate T_3 concentration over 120 min. No measurable production of rT_3 from T_4 occurred.

The rate of degradation of rT_3 (Fig. 2A) diminished after 2–3 min. At low initial concentrations (15.4 and 38.5 nM), most of the rT_3 disappeared in 10 min. At higher rT_3 concentrations (77 and 154 nM), degradation could be measured for up to 60 min (data not shown). When T_4 and T_3 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 T_3 production from T_4 or rT_3 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 Na_2HPO_4 – 0.15 M NaCl, or 0.05 M Na_2HPO_4 – 0.15 NaCl + 0.25 M glucose, all at a buffer pH of 7.4.

The rate of T_4 to T_3 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 rT_3 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 T_4 to T_3 conversion or rT_3 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 rT_3 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.

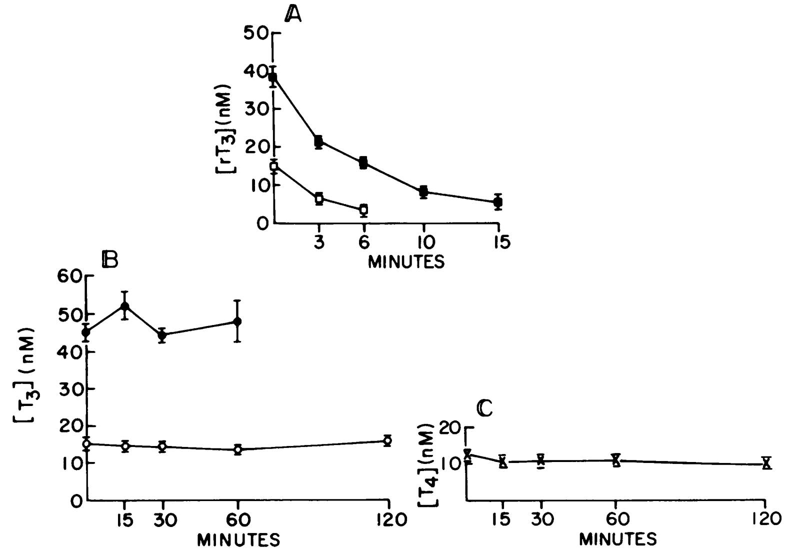

FIGURE 2 (A) rT_3 concentrations±SE in liver homogenate during 15-min incubations with initial rT_3 concentrations of 15.4 (□) and 30.8 (■) nM. (B) T_3 concentrations±SE in liver homogenate during 60- and 120-min incubations with initial T_3 concentrations of 15.4 (○) and 45 (●) nM. (C) T_4 concentrations±SE in liver homogenate during 120-min incubations with an initial T_4 concentration of 12.9 nM (x).

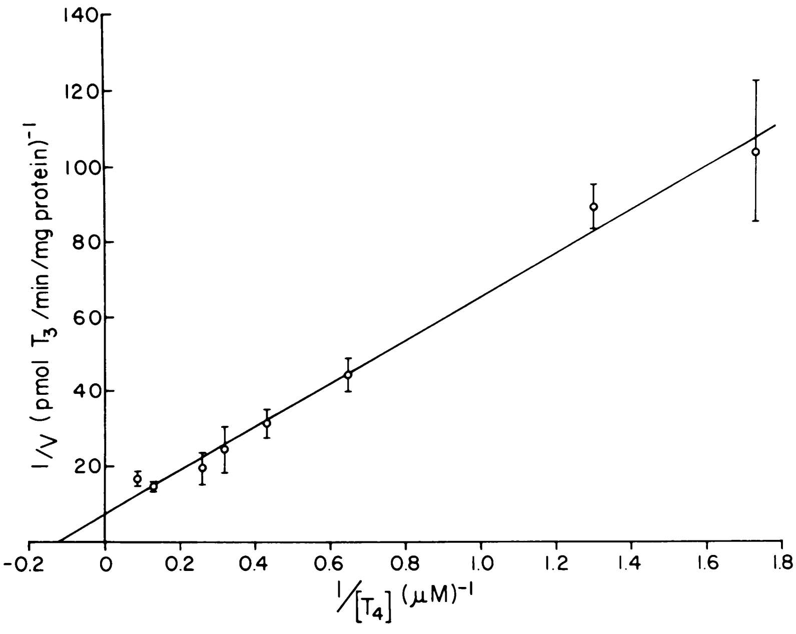

FIGURE 3 Lineweaver-Burk plot of T_4 to T_3 conversion. Values shown are mean±SE results from four incubation mixtures.

Reaction kinetics. Fig. 3 shows a Lineweaver-Burk plot for the conversion of T_4 to T_3. Values for V were calculated in picomoles T_3/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 K_m for T_4 of 7.7 μM and a V_{max} of 0.13 pmol T_3/min per mg protein. With an initial T_4 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 T_3/ min per mg protein. Fig. 4 shows a Lineweaver-Burk plot for rT_3 degradation. V was calculated in picomoles rT_3/minute per milligram protein from the 7-s, 0.5- and 1-min time points. The least squares line of best fit yielded a K_m for rT_3 of 7.5 nM and a V_{max} of 0.36 pmol rT_3/min per mg protein. The difference in the K_m values for T_4 and rT_3 implies that, if there is a single 5′-deiodinase, its affinity for rT_3 is about 1,000-fold greater than that for T_4.

Interactions of iodothyronines. Table I shows that conversion of T_4 to T_3 was not inhibited by addition of T_3 or of 3,5-T_2, but was inhibited in a dose-dependent manner by the addition of rT_3 and 3,3′-T_2. rT_3 was ≅four times as potent an inhibitor as 3,3′-T_2 on a molar basis. A Dixon plot of rT_3 inhibition of T_4 to T_3 conversion is shown in Fig. 5. As mentioned above, the mean of the initial and final rT_3 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 rT_3 is a competitive inhibitor of T_4 to T_3 conversion, with a K_i of 4.5 nM, close to the K_m of rT_3 (7.5 nM) in the rT_3 degradation reaction.

Table II shows that T_4 inhibited rT_3 degradation in a dose-dependent fashion, but that the other iodothyronines did not. In particular, 3,3′-T_2 at a 0.15 μM concentration had no effect in inhibiting rT_3 degradation, whereas the same 3,3′-T_2 concentration substantially inhibited T_4 to T_3 conversion (cf. Table I). Fig. 6 is a

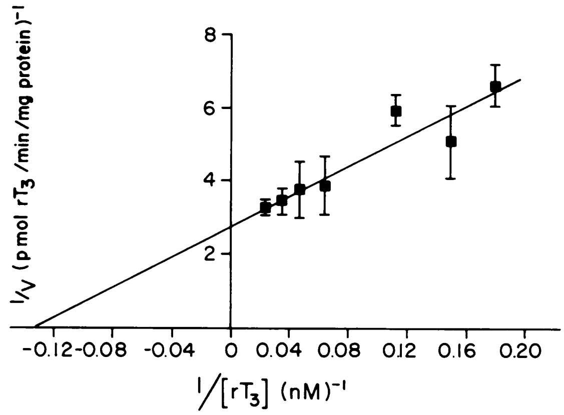

FIGURE 4 Lineweaver-Burk plot of rT_3 degradation. Each point represents the mean of five incubations of 1 min duration. The y axis shows 1/v±SE. The x axis shows the means of the reciprocals of the average [rT_3] (½ [added rT_3 + measured rT_3 after 1 min]) during the incubation. The standard errors of 1/[rT_3] (not shown) ranged from 0.001 to 0.005 nM^{-1}.

TABLE I
Inhibition of T_4 to T_3 Conversion by Iodothyronines

Iodothyronine	Concentration	(Inhibitor)/(Substrate)	T_3 production rate	P
	μM		% control mean±SE	
T_3	0.120	0.092	111±19	NS
rT_3	0.019	0.015	31±8	<0.001
	0.039	0.029	3±1	<0.001
	0.077	0.059	2±1	<0.001
3,3'-T_2	0.038	0.029	42±3	<0.001
	0.076	0.059	34±5	<0.001
	0.150	0.117	16±5	<0.001
3,5-T_2	0.150	0.117	87±6	NS

Initial T_4 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 T_4 alone.

TABLE II
Inhibition of rT_3 Degradation by Iodothyronines

Iodothyronine	Concentration	(Inhibitor)/(Substrate)	rT_3 degradation rate	P
	μM		% control mean±SE	
T_4	5.9	980	53±5	<0.01
	12	1940	54±9	<0.01
	24	3870	44±9	<0.01
T_3	15	1000	77±4	NS
3,3'-T_2	0.15	10	91±4	NS
3,5-T_2	0.15	10	113±9	NS

Initial rT_3 concentrations in the T_4 inhibition experiments were 6.2 nM, and they were 15 nM in the other experiments. Four to five identical incubations were used for each inhibitor concentration and compared to an equal number of control incubations with rT_3 alone.

Dixon plot of T_4 inhibition of rT_3 degradation. These data suggest that T_4 is a competitive inhibitor of rT_3 degradation, with a K_i of 10.2 μM, quite similar to the K_m of T_4 (7.7 μM) in the T_4 to T_3 conversion reaction.

Other inhibitors. Propylthiouracil (PTU) inhibited both T_4 to T_3 conversion and rT_3 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 T_3 production from T_4 from 70 to 46% of control, and a decrease in the rate of rT_3 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 T_4 to T_3 conversion suggests that PTU alters the affinity of the enzyme for T_4, i.e. is an allosteric inhibitor, rather

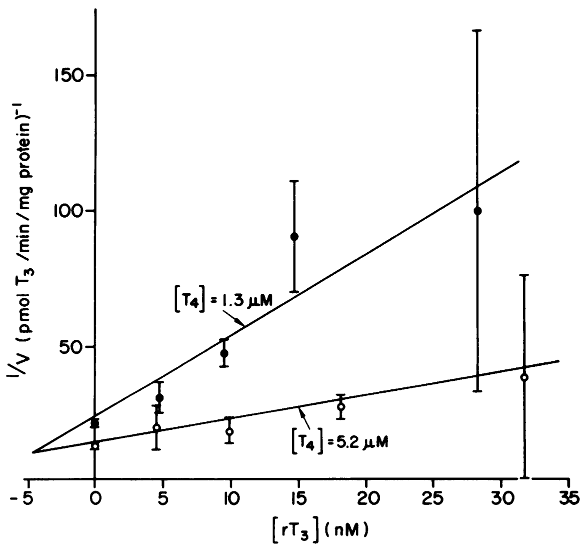

FIGURE 5 Dixon plot of rT_3 inhibition of T_4 to T_3 conversion. Each point represents the mean of five incubations of 5 min duration. The y axis shows 1/v±SE. The x axis shows the average [rT_3] (½ [added rT_3 + measured rT_3 after 5 min]) during the incubation. The standard errors of [rT_3] (not shown) ranged from 0.2 to 1.7 nM.

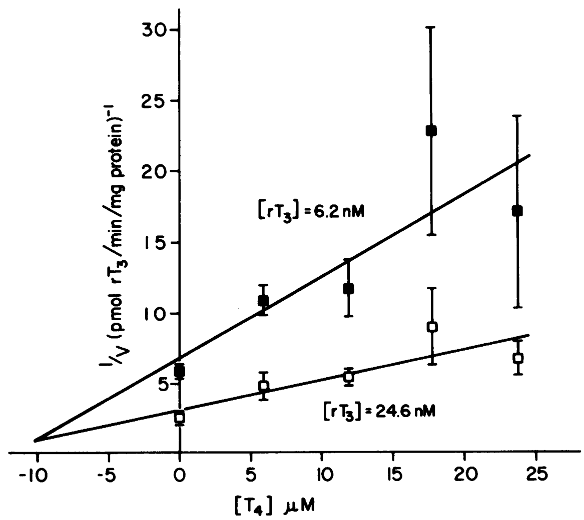

FIGURE 6 Dixon plot of T_4 inhibition of rT_3 degradation. Each point represents the mean of five incubations of 1 min duration. The y axis shows 1/v±SE. The x axis shows the concentration of added T_4.

TABLE III
Inhibition of T_4 to T_3 Conversion and rT_3 Degradation in Rat Liver Homogenate by Propylthiouracil

PTU concentration	n	T_4 to T_3 conversion	P*	n	rT_3 degradation	P*
μM		*% of control*‡			*% of control*‡	
1.76	5	70±2	<0.001	5	77±16	NS
5.9	5	57±3	<0.001	11	52±11	<0.001
59	5	46±3	<0.001	6	26±6	<0.001
352	5	49±8	<0.001	3	34±2	<0.001

* Incubations with PTU compared to an equal number (*n*) of simultaneous control incubations. The initial T_4 and rT_3 concentrations were 1.3 μM and 1.54 nM, respectively. T_4 incubations were carried out for 15 min and rT_3 incubations for 2 min.
‡ Mean±SE.

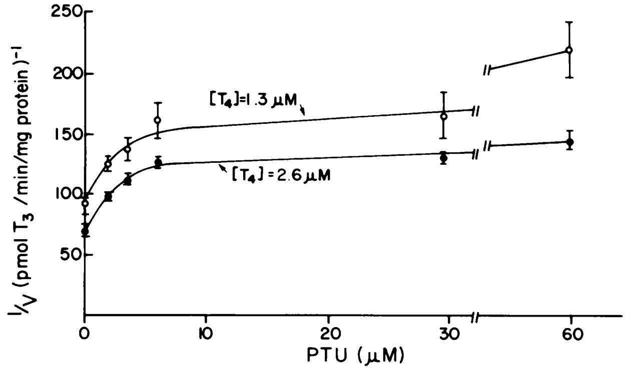

FIGURE 7 Dixon plot of inhibition of T_4 to T_3 conversion by PTU with initial T_4 concentrations of 1.3 (○) and 2.6 (●) μM. Values shown are the means±SE from four or five incubation mixtures.

than acting at the catalytic site. There was no measurable production of rT_3 from T_4 when rT_3 degradation was (partially) inhibited by PTU.

Table IV shows that 2,4-dinitrophenol and iopanoic acid were effective inhibitors both of T_4 to T_3 conversion and rT_3 degradation. There was no rT_3 production from T_4 when rT_3 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 rT_3 degradation. It appeared to inhibit T_4 to T_3 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 T_4 to T_3 conversion or rT_3 degradation included 13 μM dexamethasone, 8 μM NaI, 1 μM α-methylparatyrosine, 89 μM 1-methyl-2-mercaptoimidazole, 1 mM $FeCl_2$, 1 mM $FeCl_3$, 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 T_4 to T_3 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 rT_3 degradation.

Other tissues. Kidney homogenate was as active as liver in producing T_3 from T_4. At an initial T_4 concentration of 1.3 μM, the rate of T_3 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 T_3 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 T_4 to T_3 Conversion and rT_3 Degradation by 2,4-Dinitrophenol, Sodium Diatrizoate, and Iopanoic Acid

Inhibitor	Concentration	n	T_4 to T_3 conversion	P*	n	rT_3 degradation	P*
			% control‡			*% control*‡	
2,4-Dinitrophenol, *mM*	1	5	48±16	<0.05	5	21±2	<0.001
2,4-Dinitrophenol, *mM*	2	5	18±8	<0.001	5	6±2	<0.001
Sodium diatrizoate, *mM*	7.8	5	84±9	NS	5	81±3	<0.01
Iopanoic acid, *μM*	0.1	3	58±5	<0.01	3	73±7	<0.05
Iopanoic acid, *μM*	1	3	10±2	<0.001	3	43±6	<0.01
Iopanoic acid, *μM*	10	3	8±3	<0.001	3	19±4	<0.001

* Incubations with inhibitor compared to an equal number (*n*) of simultaneous control incubations. The initial T_4 and rT_3 concentrations were 1.3 μM and 1.54 nM, respectively. Incubations were carried out for 15 min (T_4) and 1 and 2 min (rT_3).
‡ Mean±SE.

quite like that observed in liver homogenate (cf. Table I). Kidney homogenate was likewise as active as liver homogenate in degrading rT_3; at an initial rT_3 concentration of 15.4 nM, the rate of disappearance of rT_3 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 T_3 or rT_3 (<0.5 fmol/min per mg protein) from T_4 or destruction of rT_3 (<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 T_4 to T_3 conversion in liver homogenate (initial T_4 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 T_4 to T_3 conversion by the liver homogenate to the control rate; there was still significant impairment of T_4 to T_3 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 rT_3 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 rT_3 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 T_4 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 T_4 concentration; it was normal after 72 h of refeeding. The mean serum T_3 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 T_3-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 T_3 charcoal uptake decreased to 31%, $P < 0.01$. These data suggest

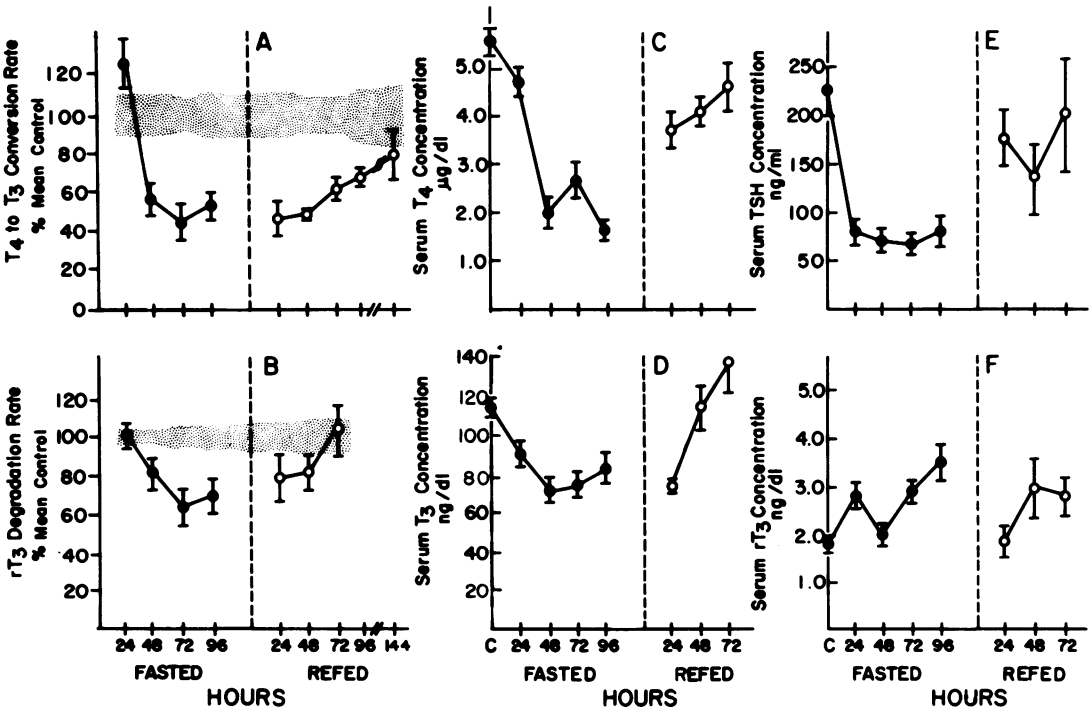

FIGURE 8 Liver homogenate T_4 to T_3 conversion and rT_3 degradation, serum TSH, and thyroid hormone concentrations in rats during starvation (●) and refeeding (○). The refeeding experiments were done in animals previously fasted for 72 h. Data from liver homogenate incubations are expressed as mean±SE 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 T_3 parallels the total T_3, and that fasting truly represents a low T_3 state in the rat.

Serum rT_3 concentrations, corrected for T_4 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 T_4 cross-reactivity, they must be interpreted with caution. With that reservation, there was no fall in serum rT_3 despite a 60% decrease in the mean serum T_4. 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 T_4 to T_3 conversion and rT_3 degradation than the controls. The mean serum concentrations of T_4, T_3, and TSH were also not significantly different in the treated animals and the controls. The corrected mean serum rT_3 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 T_4 to T_3 conversion in the liver homogenates was significantly diminished to 44±4% of control, $P < 0.05$, and the rate of rT_3 degradation was also significantly diminished to 35±6% of control, $P < 0.001$. There was no significant difference in mean serum concentrations of T_4, T_3, and TSH between the treated and the control rats, but the mean corrected serum rT_3 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 T_4 to T_3 or the rate of degradation of rT_3 in the liver homogenates from rats treated with endotoxin compared to controls and no change in the serum concentrations of T_4, T_3, rT_3, 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 T_4 to T_3 conversion or rT_3 degradation in liver and kidney homogenates as described in this paper, although nonenzymatic deiodination of T_4 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 K_m and K_i for T_4, and the K_m and K_i for rT_3 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

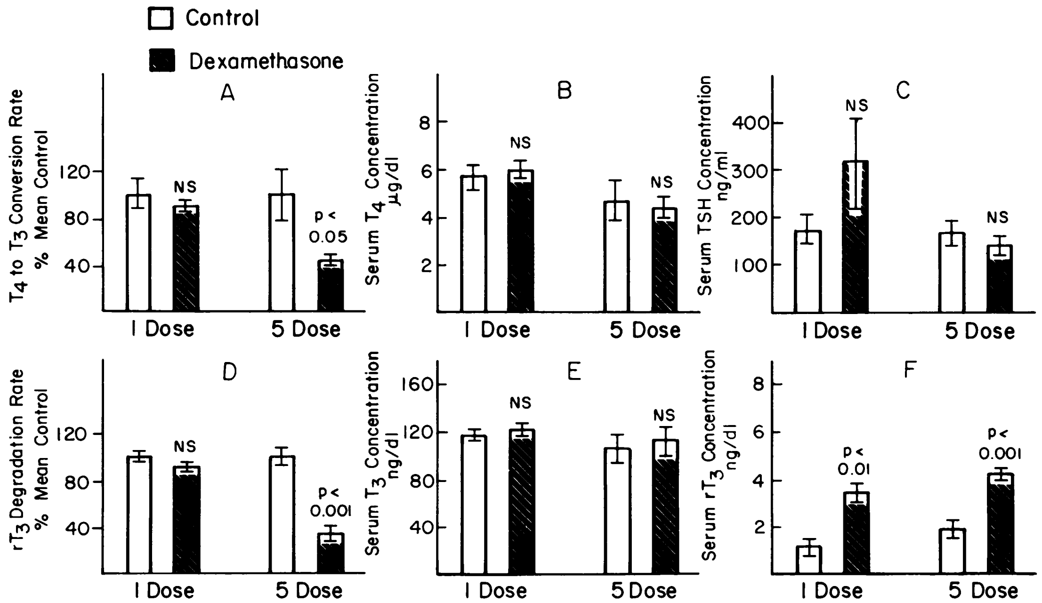

FIGURE 9 Liver homogenate T_4 to T_3 conversion and rT_3 degradation and serum TSH and thyroid hormone concentrations in rats treated with one and five daily doses of dexamethasone (1.5 mg/kg i.p.). Values are mean±SE results from five rats. Open bars represent data from control rats and hatched bars data from dexamethasone-treated rats.

T_4 and rT_3. Proof that rT_3 was degraded by 5′-deiodination to 3,3′-T_2 was not obtained in this study, but Chopra has reported rapid production of 3,3′-T_2 from rT_3 and inhibition of this reaction by PTU in a similar liver homogenate system (40). The similarity of the rates of T_4 to T_3 conversion in liver and kidney homogenates and of the rates of rT_3 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 T_4 and rT_3 were much less than in liver or kidney homogenates, and that rT_3 inhibits hepatic conversion of T_4 to T_3 (27). This latter phenomenon raises the possibility that rT_3 regulates T_3 production from T_4 in vivo in patients who have the diverse disorders associated with elevated serum rT_3 and decreased T_3 concentrations. Thus, the primary disturbance may be a decrease in rT_3 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 rT_3 concentrations in humans after short-term exogenous rT_3 administration have no effect on serum T_3 concentrations (50).

The decrease in serum T_3 and increase in serum rT_3 concentrations in humans (14, 16, 45) and decreased total T_4 to T_3 conversion in rats (47, 51) found after PTU administration in vivo are likely to be caused by decreased hepatic and renal T_3 production and decreased hepatic (and probably decreased renal) rT_3 degradation caused by the drug. The mechanism of PTU inhibition of T_4 to T_3 conversion and rT_3 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 T_4 to T_3 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 rT_3 metabolism.

Iopanoic acid, a widely used oral cholecystographic agent, when administered to humans, causes a reduction in serum T_3 concentrations and an increase in serum T_4, TSH, and rT_3 concentrations (16). Evidence is presented here that the effects of iopanoic acid may result from inhibition of T_3 production and rT_3 degradation in the liver (and perhaps elsewhere), with a compensatory increase in TSH secretion and a consequent increase in T_4 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 T_3 production and rT_3 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 T_4 or T_3 metabolism in other in vitro liver systems had effects in this one. A reduction in T_4 deiodination in adult rats (53), and prevention of the neonatal increase in serum T_3 in sheep (54), have been reported as consequences of in vivo administration of α-methylparatyrosine. This drug had no effect on T_4 to T_3 conversion or rT_3 degradation in liver homogenates. Hillier (55) found that 2,4-dinitrophenol inhibited T_4 deiodination by isolated perfused liver in a concentration range similar to that found here to inhibit both T_4 to T_3 conversion and rT_3 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 T_4 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 T_4, T_3, and TSH concentrations and decreased hepatic T_4 to T_3 conversion, whereas, in fasted humans, serum T_4 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 T_4 deiodination (58), and that liver slices from such rats have a reduced conversion rate of T_4 to T_3 (59). The fall in serum T_3 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 T_4 concentration fell only slightly, and the rate of hepatic T_4 to T_3 conversion did not change. Return of serum T_3 concentrations to normal with refeeding was also more rapid than recovery of hepatic T_4 to T_3 conversion as measured in vitro. Three possible mechanisms may thus contribute to the fall in serum T_3 in the fasted rat: decreased availability of the precursor, T_4, caused, in turn, by decreased TSH stimulation of T_4 secretion; decreased thyroidal secretion of T_3 itself; and a decreased capacity of the liver to convert T_4 to T_3. The present data suggest that all of those mechanisms are operative but do not allow an estimation of their relative importance. The failure of serum rT_3 to fall despite a 55% decrease in T_4 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 T_4 and rT_3 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 T_3 production from T_4 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 rT_3 degradation rate. The lack of fall in serum T_3 concentrations is unexplained; it could reflect either dexamethasone-mediated inhibition of T_3 degradation or increased T_4 to T_3 conversion at other sites. An elevation in serum rT_3 concentrations appeared to precede any substantial change in rT_3 metabolism by the liver homogenate. As in the case of fasting, the lack of information about rT_3 production in the rat makes comments about mechanisms of changes in serum concentrations speculative. These experiments did not show the expected fall in rat serum TSH and T_4 concentrations after dexamethasone treatment reported by several groups of investigators (60, 61). The elevation in rat serum rT_3 concentrations in $\leq$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 T_4 to T_3 conversion and rT_3 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 T_3 and elevated rT_3 concentrations (4, 7, 10, 12, 15, 16, 18, 21, 45). Moreover, decreases in both T_4 to T_3 conversion and rT_3 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 T_3 concentrations increase markedly within hours after birth in the human, but the high serum rT_3 concentrations remain elevated for several weeks (20). Also, reduced serum T_3 but normal, rather than elevated, rT_3 concentrations have been reported in some other clinical situations (7, 21, 62). These findings suggest that T_4 to T_3 converting (T_4-5′-deiodinase) and rT_3 degrading (rT_3-5′-deiodinase) activities are dissociable, 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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REFERENCES

1. Albert, A., and F. R. Keating, Jr. 1952. The role of the gastrointestinal tract, including the liver, in the metabolism of radiothyroxine. *Endocrinology.* **51:** 427–435.
2. Oppenheimer, J. H., H. L. Schwartz, H. C. Shapiro, G. Bernstein, and M. I. Surks. 1970. Differences in primary cellular factors influencing the metabolism and distribution of 3,5,3′-L-triiodothyronine and L-thyroxine. *J. Clin. Invest.* **49:** 1016–1024.
3. Surks, M. I., and J. H. Oppenheimer. 1971. Metabolism of phenolic- and tyrosylring labeled L-thyroxine in human beings and rats. *J. Clin. Endocrinol. Metab.* **33:** 612–618.
4. Chopra, I. J. 1976. An assessment of daily production and significance of thyroidal secretion of 3,3′,5′-triiodothyronine (reverse T_3) in man. *J. Clin. Invest.* **58:** 32–40.
5. Carter, J. N., C. J. Eastman, J. M. Corcoran, and L. Lazarus. 1974. Effect of severe chronic illness on thyroid function. *Lancet.* **II:** 971–974.
6. Bermudez, F., M. I. Surks, and J. H. Oppenheimer. 1975. High incidence of decreased serum triiodothyronine concentration in patients with non-thyroidal disease. *J. Clin. Endocrinol. Metab.* **41:** 27–40.
7. Chopra, I. J., U. Chopra, S. R. Smith, M. Reza, and D. H. Solomon. 1975. Reciprocal changes in serum concentrations of 3,3′,5′-triiodothyronine (reverse T_3) and 3,3′,5-triiodothyronine (T_3) in systemic illnesses. *J. Clin. Endocrinol. Metab.* **41:** 1043–1049.
8. Burger, A., P. Nicod, P. Suter, M. B. Valloton, A. Vagenakis, and L. Braverman. 1976. Reduced active thyroid hormone levels in acute illness. *Lancet.* **I:** 653–655.
9. Spector, D. A., P. J. Davis, J. H. Helderman, B. Bell, and R. D. Utiger. 1976. Thyroid function and metabolic

state in chronic renal failure. *Ann. Intern. Med.* **85:** 724–730.
10. Wartofsky, L., K. D. Burman, R. C. Dimond, G. L. Noel, A. G. Frantz, and J. M. Earll. 1977. Studies on the nature of thyroid suppression during acute falciparum malaria: integrity of pituitary response to TRH and alterations in serum T_3 and reverse T_3. *J. Clin. Endocrinol. Metab.* **44:** 85–90.
11. Duick, D. S., D. W. Warren, J. T. Nicoloff, C. L. Otis, and M. S. Croxon. 1974. Effect of single dose dexamethasone on the concentration of serum triiodothyronine in man. *J. Clin. Endocrinol. Metab.* **39:** 1151–1154.
12. Chopra, I. J., D. E. Williams, J. Orgiazzi, and D. H. Solomon. 1975. Opposite effects of dexamethasone on serum concentrations of 3,3′,5′-triiodothyronine (reverse T_3) and 3,3′-5-triiodothyronine (T_3). *J. Clin. Endocrinol. Metab.* **41:** 911–920.
13. Saberi, M., F. H. Sterling, and R. D. Utiger. 1975. Reduction in extrathyroidal triiodothyronine production by propylthiouracil in man. *J. Clin. Invest.* **55:** 218–223.
14. Geffner, D. L., M. Azukizawa, and J. M. Hershman. 1975. Propylthiouracil blocks extrathyroidal conversion of thyroxine to triiodothyronine and augments thyrotropin secretion in man. *J. Clin. Invest.* **55:** 224–229.
15. Burger, A., D. Dinichert, P. Nicod, M. Jenny, T. Lemarchand-Béraud, and M. B. Vallotton. 1976. Effect of amiodarone on serum triiodothyronine, reverse-triiodothyronine, thyroxine and thyrotropin. *J. Clin. Invest.* **58:** 255–259.
16. Bürgi, H., C. Wimpfheimer, A. Burger, W. Zaunbauer, H. Rösler, and T. Lemarchand-Béraud. 1976. Changes in circulating thyroxine, triiodothyronine and reverse triiodothyronine after radiographic contrast agents. *J. Clin. Endocrinol. Metab.* **43:** 1203–1210.
17. Portnay, G. I., J. T. O'Brien, J. Bush, A. G. Vagenakis, F. Azizi, R. A. Arky, S. H. Ingbar, and L. E. Braverman. 1974. The effect of starvation on the concentration and binding of thyroxine and triiodothyronine in serum and on the response to TRH. *J. Clin. Endocrinol. Metab.* **39:** 191–194.
18. Vagenakis, A. G., A. Burger, G. I. Portnay, M. Rudolf, J. T. O'Brian, F. Azizi, R. A. Arky, P. Nicod, S. H. Ingbar, and L. E. Braverman. 1975. Diversion of peripheral thyroxine metabolism from activating to inactivating pathways during complete fasting. *J. Clin. Endocrinol. Metab.* **41:** 191–194.
19. Moshang, T., Jr., J. S. Parks, L. Baker, V. Vaidya, R. D. Utiger, A. M. Bongiovanni, and P. J. Snyder. Low serum triiodothyronine in patients with anorexia nervosa. *J. Clin. Endocrinol. Metab.* **40:** 470–472.
20. Chopra, I. J., J. Sack, and D. A. Fisher. 1975. Circulating 3,3′,5′-triiodothyronine in the human newborn. *J. Clin. Invest.* **55:** 1137–1141.
21. Nicod, P., A. Burger, V. Staeheli, and M. B. Vallotton. 1976. A radioimmunoassay for 3,3′,5′-triiodo-L-thyronine in unextracted serum: Method and clinical results. *J. Clin. Endocrinol. Metab.* **42:** 823–829.
22. Eisenstein, A., S. Hagg, A. Vagenakis, S. Fang, L. Braverman, and S. Ingbar. 1977. Observations on the peripheral metabolism of 3,3′,5′-triiodothyronine (reverse T_3, rT_3) in fed and fasted patients. *Clin. Res.* **25:** 294A. (Abstr.)
23. Chopra, I. J., S. Sack, and D. A. Fisher. 1975. 3,3′,5′-triiodothyronine (reverse T_3) and, 3,3′,5-triiodothyronine (T_3) in fetal and adult sheep: Studies of metabolic clearance rates, production rates, serum binding, and thyroidal content relative to thyroxine. *Endocrinology.* **97:** 1080–1088.
24. Albright, E. C., and F. C. Larson. 1959. Metabolism of L-thyroxine by human tissue slices. *J. Clin. Invest.* **38:** 1899–1903.
25. Hillier, A. P. 1972. Deiodination of thyroid hormones by the perfused rat liver. *J. Physiol. (Lond.).* **222:** 475–485.
26. Visser, T. J., I. van der Does-Tobé, R. Docter, and G. Hennemann. 1975. Conversion of thyroxine into triiodothyronine by rat liver homogenate. *Biochem. J.* **150:** 489–93.
27. Chopra, I. J. 1977. A study of extrathyroidal conversion of thyroxine (T_4) to 3,3′,5-triiodothyronine (T_3) in vitro. *Endocrinology.* **101:** 453–463.
28. Nakagawa, S., and W. R. Ruegamer. 1967. Properties of a rat tissue iodothyronine deiodinase and its natural inhibitor. *Biochemistry.* **6:** 1249–1261.
29. Sterling, K., M. A. Brenner, and V. F. Saldanha. 1973. Conversion of thyroxine to triiodothyronine by cultured human cells. *Science (Wash. D. C.).* **179:** 1000–1001.
30. Chiraseveenuprapund, S., V. Buergi, A. Goswami, and I. N. Rosenberg. 1975. Formation of triiodothyronine from l-thyroxine in rat kidney homogenate. *In* Thyroid Research. Proceedings of the Seventh International Thyroid Conference. J. Robbins and L. E. Braverman, editors. American Elsevier Publishing Co., Inc., New York. 244–247.
31. Rabinowitz, J. L., and E. S. Hercker. 1971. Thyroxine: conversion to triiodothyronine by isolated perfused rat heart. *Science (Wash. D. C.).* **173:** 1242–1243.
32. Tata, J. R. 1957. Metabolism of L-thyroxine and 1-3:5:3′-triiodothyronine by homogenates of rat skeletal muscle. *Proc. Soc. Exp. Biol. Med.* **95:** 362–364.
33. Klebanoff, S. J., and W. L. Green. 1973. Degradation of thyroid hormones by phagocytosing human leukocytes. *J. Clin. Invest.* **52:** 60–72.
34. Woeber, K. A. 1976. A granule-associated L-thyroxine deiodinating system in the human leukocyte. *Endocrinology.* **98:** 802–806.
35. Refetoff, S., R. Matalon, and M. Bigazzi. 1972. Metabolism of L-thyroxine (T_4) and L-triiodothyronine (T_3) by human fibroblasts in tissue culture: evidence for cellular binding proteins and conversion of T_4 to T_3. *Endocrinology.* **91:** 934–947.
36. Cavalieri, R. R., L. A. Gavin, F. Mc Mahon, and M. Hammond. 1977. Thyroxine (T_4) deiodination in liver: subcellular localization of reverse T_3 (RT_3) forming and degrading systems. *Clin. Res.* **25:** 462A. (Abstr.)
37. Stanbury, J. B., M. L. Morris, H. J. Corrigan, and W. E. Lassiter. 1960. Thyroxine deiodination by a microsomal preparation requiring Fe^{++}, oxygen, and cysteine or glutathione. *Endocrinology.* **67:** 353–362.
38. Nakano, M., Y. Tsutsumi, and Y. Ushijima. 1971. Degradation of thyroxine by the microsomal particles from rat liver. *Biochim. Biophys. Acta.* **252:** 335–347.
39. Hesch, R. D., G. Brunner, and H. D. Söling. 1975. Conversion of thyroxine (T_4) and triiodothyronine (T_3) and the subcellular localisation of the converting enzyme. *Clin. Chim. Acta.* **59:** 209–213.
40. Chopra, I. J., S. Y. Wu, and D. H. Solomon. 1976. Extrathyroidal production of 3,3′-diiodothyronine (T_2) *in vitro*: a major pathway of rT_3 metabolism, a minor pathway for T_3. Program of the 58th Annual Meeting of the Endocrine Society. 102. (Abstr.)
41. Henninger, R. W., F. C. Larson, and E. C. Albright. 1968. Iodine-containing compounds of extrathyroidal tissues. *J. Clin. Invest.* **42:** 1761–1768.

42. Segel, I. 1975. Enzyme kinetics: behavior and analysis of rapid equilibrium and steady state enzyme systems. John Wiley and Sons, Inc., New York. 57.
43. Lieblich, J. M., and R. D. Utiger. 1972. Triiodothyronine radioimmunoassay. *J. Clin. Invest.* **51**: 157–166.
44. Chopra, I. J. 1972. A radioimmunoassay for measurement of thyroxine in unextracted serum. *J. Clin. Endocrinol. Metab.* **34**: 938–947.
45. Kaplan, M. M., M. Schimmel, and R. D. Utiger. 1977. Changes in serum 3,3′,5′-triiodothyronine (reverse T_3) concentrations with altered thyroid hormone secretion and metabolism. *J. Clin. Endocrinol. Metab.* **47**: 447–456.
46. Lowry, O. H., N. J. Rosebrough, A. L. Farr, and J. Randall. 1951. Protein measurement with Folin phenol reagent. *J. Biol. Chem.* **193**: 265–275.
47. Frumess, R. D., and P. R. Larsen. 1975. Correlation of serum triiodothyronine (T_3) and thyroxine (T_4) with biologic effects of thyroid hormone replacement in propylthiouracil-treated rats. *Metab. Clin. Exp.* **24**: 547–554.
48. Hüfner, M., and M. Knöpfle. 1976. Pharmacological influences on T_4 to T_3 conversion in rat liver. *Clin. Chim. Acta.* **72**: 337–341.
49. Kobayashi, I., T. Yamada, and K. Shichijo. 1966. Nonenzymatic deiodination of thyroxine in vitro. *Metab. Clin. Exp.* **15**: 1140–1148.
50. Nicod, P., A. Burger, G. Strauch, A. G. Vagenakis, and L. E. Braverman. 1976. The failure of physiologic doses of reverse T_3 to effect thyroid-pituitary function in man. *J. Clin. Endocrinol. Metab.* **43**: 478–481.
51. Oppenheimer, J. H., H. I. Schwartz, and M. I. Surks. 1972. Propylthiouracil inhibits the conversion of L-thyroxine to L-triiodothyronine. *J. Clin. Invest.* **51**: 2493–2497.
52. Taurog, A. 1976. The mechanism of action of the thioureylene antithyroid drugs. *Endocrinology.* **98**: 1031–1046.
53. Dratman, M. B., F. L. Crutchfield, E. Marsh, J. Axelrod, and F. H. Sterling. 1975. Iodothyronine metabolism and catecholamine enzymes: effect of alpha-methyl-paratyrosine in thyroidectomized euthyroid rats. *In* Thyroid Research. Proceedings of the Seventh International Thyroid Conference. J. Robbins and L. E. Braverman, editors. American Elsevier Publishing Co., Inc., New York. 248–250.
54. Fisher, D. A., and J. Sack. 1975. Thyroid function in the neonate and possible approaches to newborn screening for hypothyroidism. *In* Perinatal Thyroid Physiology and Disease. D. A. Fisher and G. N. Burrow, editors. Raven Press, New York. 197–209.
55. Hillier, A. P. 1974. Antagonistic effects of dinitrophenol and cyanide on hepatic thyroxine deiodination. *Acta Endocrinol.* **77**: 122–127.
56. Campbell, G. A., M. Kurcz, S. Marshall, and J. Meites. 1977. Effects of starvation in rats on serum levels of follicle stimulating hormone, luteinizing hormone, thyrotropin, growth hormone and prolactin, response to LH-releasing hormone and thyrotropin-releasing hormone. *Endocrinology.* **100**: 580–587.
57. Schussler, G. C. 1966. Paradoxical effects of starvation and cortisone administration on free thyroxine concentration and thyroxine metabolism. *J. Clin. Invest.* **45**: 1072. (Abstr.)
58. Ingbar, D. H., and V. A. Galton. 1975. The effect of food deprivation on the peripheral metabolism of thyroxine in rats. *Endocrinology.* **96**: 1525–1532.
59. Balsam, A., F. Sexton, and S. H. Ingbar. 1977. Independent inhibitory effects of hypothyroidism and fasting on hepatic conversion of thyroxine (T_4) to triiodothyronine (T_3) in the rat. Program of the 59th Annual Meeting of the Endocrine Society. 239. (Abstr.)
60. Wilber, J. F., and R. D. Utiger. 1969. The effect of glucocorticoids on thyrotropin secretion. *J. Clin. Invest.* **48**: 2096–2103.
61. Koch, B., M. Jobin, S. Dulac, and C. Fortier. 1972. Thyrotropin (TSH) response to synthetic TSH-releasing factor following pharmacologic blockade of adrenocorticotropin secretion. *Can. J. Physiol. Pharmacol.* **50**: 360–363.
62. Spaulding, S. W., I. J. Chopra, R. S. Sherwin, and S. S. Lyall. 1976. Effect of caloric restriction and dietary composition on serum T_3 and reverse T_3 in man. *J. Clin. Endocrinol. Metab.* **42**: 197–200.