Sources and Quantity of 3,5,3′-Triiodothyronine in Several Tissues of the Rat

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ABSTRACT The local conversion of thyroxine (T_4), which is an important source of intracellular 3,5,3′-triiodothyronine (T_3) in several rat tissues, has been subject of recent investigations. In the present study the regulation of this phenomenon in vivo was investigated in various peripheral tissues of the rat.

Intact euthyroid and radiothyroidectomized (Tx) rats received a continuous intravenous infusion of [^{125}I]T_4 and [^{131}I]T_3 until isotope equilibrium was attained. In addition to the labeled iodothyronines, Tx rats received a continuous intravenous infusion of 0.2 or 1.0 μg carrier T_4/100 g body wt per d, to create hypothyroid or slightly hypothyroid conditions, respectively. After the animals were bled and perfused the contribution of T_3 derived from local conversion of T_4 to T_3 [Lc $T_3(T_4)$] to the total T_3 in homogenates from several tissues and subcellular fractions from the liver, kidney, and anterior pituitary gland could be calculated. In all experiments T_3 in muscle was derived exclusively from the plasma. In the cerebral cortex and cerebellum, however, most of the intracellular T_3 was derived from the intracellular conversion of T_4 to T_3. It is demonstrated that for hypothyroid rats an increased relative contribution of Lc $T_3(T_4)$ reduced the loss of total T_3 in the brain. This phenomenon was also encountered for the anterior pituitary gland, although in this tissue the proportion of the total tissue T_3, contributed by locally produced T_3 was considerably lower than the values found for the cerebral cortex and cerebellum in all experiments.

The present findings, regarding the source and quantity of pituitary nuclear T_3 strongly suggest that both plasma T_3 and T_4 (through its local conversion into T_3) play a role in the regulation of thyrotropin secretion. The contribution of Lc $T_3(T_4)$ to the total pituitary nuclear T_3 was of minor importance in euthyroid rats (~20%), compared with that found for both groups in T_4-supplemented athyreotic rats (~40%).

The total T_3 concentration in the liver decreased from euthyroid to hypothyroid rats and was associated with a decrease in the tissue/plasma T_3 concentration gradient. A minor proportion of hepatic T_3 was contributed by Lc $T_3(T_4)$, which in fact decreased significantly from the euthyroid to the hypothyroid state. In contrast to other subcellular fractions from the liver, no Lc $T_3(T_4)$ could be demonstrated in the nuclear fraction. It is suggested that the liver plays an important role with respect to regulation of the circulating T_3 concentration.

In the kidney, a very small proportion of the total T_3 was derived from locally produced T_3 in all experiments (4–7%). As found in the liver, all nuclear T_3 appeared to be derived from the plasma. In contrast to the liver, subcellular T_3 pools in the kidney seemed to be exchangeable.

INTRODUCTION

Under normal conditions thyroxine (T_4)[1] is the main secretory product of the thyroid gland. More than two-thirds of 3,5,3′-triiodothyronine (T_3) in man and in rat is produced by 5′-monodeiodination of T_4 in peripheral tissues (1, 2). The quantitative contribution of different tissues to the total production of T_3 from T_4 is unknown, but T_4 to T_3 conversion can be measured in liver, kidney, pituitary gland, and central nervous system tissues in vitro (3). T_3, probably the main metabolically active substance, becomes effective by binding to a specific nuclear receptor. Specific nuclear T_3

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[1] *Abbreviations used in this paper:* α-GPD, α-glycerophosphate dehydrogenase; Lc $T_3(T_4)$, local conversion of T_4 to T_3; MCR, metabolic clearance rate; PTU, propylthiouracil; T_3, 3,5,3′-triiodothyronine; T_4, thyroxine; TSH, thyrotropin; Tx, radiothyroidectomized, radiothyroidectomy.

binding sites have been identified in numerous tissues (4, 5). It seems likely that, at the intracellular level, the biological effects of T_4 can be attributed solely to the T_3 derived from it.

For years it has been widely accepted that the T_3 derived from T_4 was rapidly and totally exchangeable, within the volume of distribution, with the T_3 secreted by the thyroid into the bloodstream. Usually a positive correlation can be found between the concentration of tissue T_3 (but not of tissue T_4) and the endpoint chosen for measurement of hormonal activity. On the other hand, in a variety of studies that compare hormonal activity with only the circulating T_3 and T_4 levels, the biological effect appears to correlate with T_4 rather than with T_3. These apparent contradictions, previously reviewed by others (3, 6), cannot be explained if the T_3 derived from T_4 and the circulating (plasma) T_3 pool are totally and rapidly exchangeable. Indeed, various studies on both hypothyroid and euthyroid rats have demonstrated the reverse (7–13). It has been found that local conversion of T_4 to T_3 in the anterior pituitary gland is important for the negative feedback for thyrotropin (TSH) secretion (8, 10, 14, 15). A single injection of tracer T_4 and T_3 demonstrated that in the cerebral cortex and cerebellum, as in the pituitary gland, a substantial proportion of the nuclear T_3 appears to be derived from intracellular T_4 monodeiodination rather than T_3 in the plasma (12, 13). However, in vivo data on the source and quantity of T_3 in various peripheral tissues of rats equilibrated with labeled hormones are still scarce and incomplete. Because the conversion of local T_4 to T_3 [Lc $T_3(T_4)$] contributes to the level of intracellular T_3 in various target tissues, it is of interest to evaluate this conversion at different levels of circulating T_4 and T_3.

Therefore, in this study both source and quantity of T_3 were determined in several tissue preparations from rats equilibrated simultaneously with $[^{125}I]T_4$ and $[^{131}I]T_3$ under hypothyroid, slightly hypothyroid, and euthyroid conditions.

METHODS

Animals and diet. Adult male Wistar rats bred in our laboratory and weighing ~300 g were used in all experiments. Two groups of six animals each were rendered hypothyroid by ^{131}I thyroid ablation (a single intraperitoneal injection of 0.75 mCi $Na^{131}I$) 2 mo before the experiments began. Hypothyroidism was defined as the lack of detectable plasma T_4 (<47 ng/100 ml) and plasma T_3 (<2 ng/100 ml) concentrations. Plasma TSH concentrations were between 12 and 14 μg/ml. Radiothyroidectomized (Tx) and intact, euthyroid rats were maintained on commercial pellet food (Hope Farms, Linschoten, The Netherlands) ad lib. 1 wk before and during the continuous infusion, the food consisted of a modified AIN 76™ diet (16) in the form of dry food mixed with distilled water (60% dry weight, 40% water). To prevent reutilization of tracer I^- by eventual thyroid remnants in Tx rats and by the thyroid of intact rats, KI was added to the food the week before infusion. During the infusion period KI was administered exclusively via the infusion (100 μg/100 g body wt per d). During the continuous infusion the feeding period extended from 20.00–08.00 h (dark phase), while drinking water was available continuously.

Experimental protocol. 2 mo after Tx and 1 wk before the continuous infusion was started, one group of Tx animals received 0.2 μg T_4/100 g body wt per d (experiment A) and another group 1.0 μg T_4/100 g body wt per d (experiment B) in small aliquots of saline containing 1% (vol/vol) hypothyroid rat serum administered as subcutaneous injections. Subsequently, T_4 (0.2 μg or 1.0 μg/100 g body wt per d, experiment A or B, respectively) and KI were administered via the continuous intravenous infusion. 5 d after the start of the infusion, $[^{125}I]T_4$ (experiment A: 3.5 μCi/100 g body wt per d; experiment B: 4 μCi/100 g body wt per d) was added to the infusion fluid without interruption for the next 10–12 d. Starting either 4 or 5 d after the start of the $[^{125}I]T_4$ infusion, the rats received $[^{131}I]T_3$ (experiment A, 3 μCi/100 g body wt per d; experiment B, 2 μCi/100 g body wt per d) simultaneously for the remaining period (6 or 7 d).

In a third experiment (C), infusions were administered to intact euthyroid rats according to the above protocol ($[^{125}I]T_4$, 5 μCi/100 g body wt per d; $[^{131}I]T_3$, 3.3 μCi/100 g body wt per d) but without carrier T_4.

Throughout the infusion period 24-h samples of feces and urine were collected. The ^{125}I and ^{131}I contents were counted and expressed as the proportion of the daily infused radioactivity. When the individual urinary and fecal excretion curves reached a plateau and approximately all of the infused dose was recovered in the excreta, the animals were considered to be in isotope equilibrium as far as the major pools of T_3, T_4, and their metabolites were concerned (11, 17).

To investigate whether the infused dose of KI prevented significant reutilization of tracer I^- by the thyroid (experiment C) or eventual thyroid remnants (experiments A and B) a control experiment was performed. Control intact euthyroid rats received a continuous intravenous infusion of 100 μg KI/100 g body wt per d for the same period. In addition to stable I^-, $^{125}I^-$ (5 μCi/100 g body wt per d) was administered during the last 10 d.

During continuous infusion the behavior of the animals was normal (by observation). The mean increase in body weight for both groups of intact euthyroid rats and the Tx rats infused with 1.0 μg T_4/100 g body wt per d (experiment B) was ~22 g/wk. Tx rats given 0.2 μg T_4/100 g body wt per d (experiment A) exhibited slower growth (16 g/wk), probably due to metabolic hypothyroidism.

Continuous intravenous infusion. The continuous intravenous infusion was administered via a cannula inserted in the neck and extended under the skin to the right atrium, according to the method described by Roelfsema et al. (18). The rats were unrestrained and could drink and eat normally. The infusion fluid was administered by means of a roller pump, one for each animal (HR flow inducer, type MRHE 2, Watson Marlow Ltd., London). The rate of flow (10 ml/d) was checked daily with the aid of a calibrated pipette in the infusion system. The animals were housed in metabolic cages (Acme Research Products, Cincinnati, OH) to allow separation of urine and feces. The experiments were performed in a temperature-controlled room (23°±1°C).

Iodothyronines. High-specific activity $[^{125}I]T_4$ (~2,500 μCi/μg sp act) and $[^{131}I]T_3$ (~3,500 μCi/μg sp act) were prepared 1 d before the start of infusion, according to the method of Weeke and Ørskov (19) with modifications by

Kjeld et al. (20). $Na^{125}I$ and ^{131}I preparations from the Radiochemical Center at Amersham (Amersham Corp., Arlington Heights, IL) with 3,5,3′-L-T_3 (Sigma Chemical Co., St. Louis, MO) were used as the respective substrate. As assessed on the last day of the infusion period by thin-layer chromatography, the infused $[^{125}I]T_4$ contained a maximum of 0.7% $[^{125}I]T_3$, while no other labeled iodothyronines were detectable in the $[^{131}I]T_3$. T_3 contamination of stable T_4 was 0.8%, as estimated by the method described by Zimmerman et al. (21). All infusion solutions were prepared in a sterile 0.9% NaCl solution containing 0.2 μg/ml carbenicillin (Pyopen) and 0.3 U/ml heparin. The stock infusion solutions were stored at 4°C in the dark. The infusion flasks were refilled every 2 d and protected from light to minimize artifactual deiodinations of the iodothyronines.

Preparation of tissue homogenates and subcellular fractions. At the end of the infusion period the rats were lightly anaesthetized with ether. Blood samples from the tail were collected in heparinized tubes. The plasma was separated and propylthiouracil (PTU) was added to a final concentration of 2×10^{-3} M. The plasma samples were stored at −20°C. To reduce plasma contamination of the tissues the rats were perfused with 40–50 ml phosphate-buffered saline (containing 3 U heparin/ml and 10^{-4} M PTU) via the cannula; outflow was obtained by puncturing the inferior vena cava. Tissues were then immediately excised and kept on ice. Aliquots of liver, kidney, pooled whole anterior pituitary glands, (in groups of two) and pieces of thigh muscle, cerebral cortex, and cerebellum were finely minced and carefully homogenized in 5–10 vol of saline containing 10^{-4} M PTU. Suspensions, corresponding to 0.50 g liver, 0.12 g kidney, 0.01 g anterior pituitary, 0.44 g thigh muscle, 0.12 g cerebral cortex, and 0.17 g cerebellum, were stored at −20°C until analysis.

Subcellular fractions from the liver, kidney, and anterior pituitary gland were obtained by differential centrifugation, according to the separation scheme shown in Fig. 1. Pooled whole anterior pituitary glands (in groups of two or three) and aliquots of liver (5 g) and kidney (1.5 g) were minced

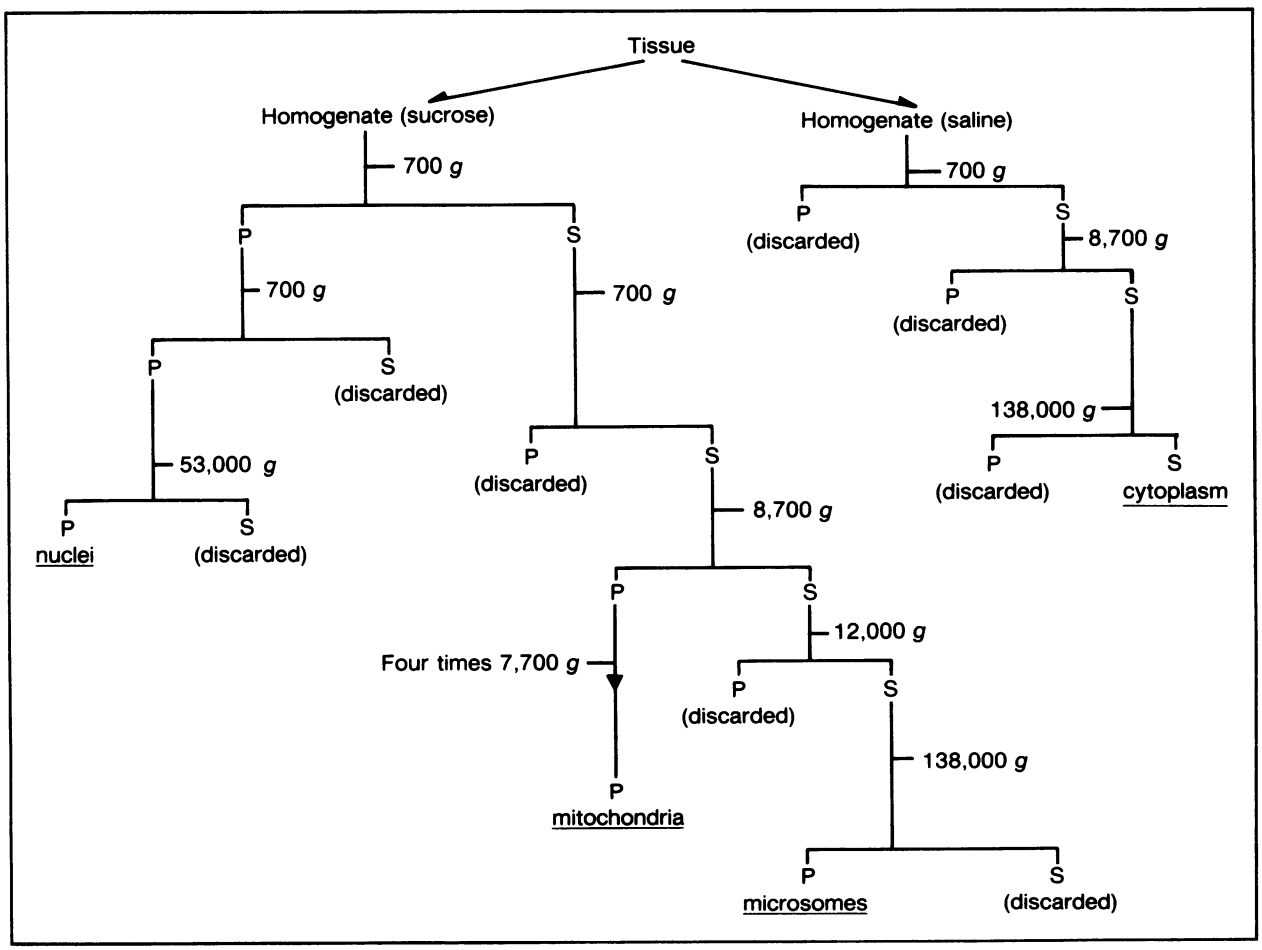

FIGURE 1 Separation scheme for subcellular fractions from liver, kidney (nuclei, mitochondria, microsomes, cytoplasm), and anterior pituitary (nuclei, cytoplasm). Subcellular fractions were obtained by differential centrifugation. The various steps are indicated. P, pellet (sediment); S, supernatant.

and homogenized in 4 vol of ice-cold 0.32 M sucrose, 10^{-3} M $MgCl_2$ and 10^{-4} M PTU by 12 passes through a teflon-glass homogenizer rotating at 1,200 rpm. The homogenates were diluted with distilled water and 0.25 M sucrose to obtain a 10% wt/vol suspension, in which the final concentrations were sucrose, 0.25 M; $MgCl_2$, 10^{-3} M; and PTU, 10^{-4} M. The 10% homogenate was centrifuged at 700 *g* for 10 min. The pellet was resuspended in 0.32 M sucrose, 10^{-3} M $MgCl_2$, 10^{-4} M PTU, and 0.25% wt/vol Triton X-100 and spun for 10 min at 700 *g*. The nuclei in the sediment were separated by centrifugation through 2.3 M sucrose, 10^{-3} M $MgCl_2$, and 10^{-4} M PTU, according to the method used by DeGroot and Strausser (22).

To prepare a mitochondrial fraction (liver and kidney) the supernatant from the first 700 *g* run was centrifuged (700 *g*). The supernatant was carefully decanted and spun for 10 min at 8,700 *g* and 4°C. The fluffy layer was discarded and the mitochondrial pellet was washed four times by resuspension in 0.25 M sucrose and 10^{-4} M PTU, followed by centrifugation for 10 min (7,700 *g*, 4°C) to reduce microsomal contamination (23). In addition, the first (8,700 *g*) supernatant was centrifuged at 12,000 *g* for 10 min at 4°C. The pellet was discarded and the supernatant centrifuged at 138,000 *g* (4°C) for 1 h to obtain the microsomal fraction. Nuclear, mitochondrial, and microsomal pellets were finally suspended in saline.

When the supernatant from the 138,000-*g* run was used as the cytoplasmic fraction the high concentration of sucrose in the sample did not allow a good separation of the iodothyronines by thin-layer chromatography. Therefore, cytoplasm was obtained from the remaining tissue homogenates in saline (see above) as follows (Fig. 1): the homogenates were centrifuged for 100 min at 700 *g* to remove cell debris and nuclei. Subsequently the supernatant was spun at 8,700 *g* (10 min, 4°C). The 8,700-*g* supernatant was recentrifuged at 138,000 *g* for 1 h. The final supernatant was taken as the cytoplasmic fraction. Measured aliquots of the subcellular preparations (1–2 ml) were stored at −20°C until processing. The purity of the various hepatic and renal subcellular fractions was verified by determination of the distribution patterns of the specific activities of various marker enzymes, as described previously (9).

Identification of radioactivity in the plasma and tissue preparations. Measured aliquots of plasma, tissue homogenates, and subcellular fractions were counted accurately to determine their ^{125}I and ^{131}I contents and then extracted twice in ethanol/ammonia (99:1, vol/vol) containing 10^{-4} M PTU. Subsequently the bulked extracts were counted and processed for thin-layer chromatography (9). The locations on the chromatogram of the labeled iodothyronines were determined by staining unlabeled iodocompounds (which had been added to the samples) with diazotized sulfanilic acid. The T_3 band and adjacent areas were cut into strips, 0.5 cm wide, and the ^{125}I and ^{131}I radioactivity was counted. The $[^{131}I]T_3$ peak on the chromatograms of the extracts of the various tissue preparations coincided with the ^{125}I peak, and usually all radioactivity was concentrated within three strips. However, the $[^{125}I]T_3$ peak obtained by thin-layer chromatography of plasma probably also contained another ^{125}I-containing metabolite. This was indicated by the asymmetric patterns of distribution of ^{125}I and ^{131}I radioactivity within the T_3 peak. To avoid this difficulty plasma T_3 was first rapidly semi-purified by column chromatography. A dried ethanol extract of 2 ml of plasma dissolved in 0.05 M phosphate buffer, pH 11.9 and 10^{-4} M PTU was applied on a small column (7 × 0.8 cm) of Sephadex G-25 (fine). Subsequently the sample was eluted with 0.05 M phosphate buffer, pH 11.9 and 10^{-4} M PTU; 0.5-ml fractions were collected. Five peak fractions of $[^{131}I]T_3$ were pooled and extracted with ethanol/ammonia for thin-layer chromatography. On the basis of distribution of ^{125}I and ^{131}I within the T_3 peak on the chromatogram no lack of agreement between $[^{125}I]T_3$ and $[^{131}I]T_3$ could then be observed. For all plasma and tissue (subcellular) extracts, the center of the $[^{131}I]T_3$ peak (one strip) on the chromatogram was used for the determination of the ratio between $[^{125}I]T_3$ and $[^{131}I]T_3$. For individual plasma samples from the intact rats of experiment C, the T_4 region on the chromatogram was also identified and the ^{125}I radioactivity was counted to estimate the specific activity of plasma $[^{125}I]T_4$. The $[^{131}I]T_3$ recovery (that percentage of the total ^{131}I radioactivity that represents T_3), together with the $[^{125}I]T_3$ counts of the T_3 strips, were used to calculate the total $[^{125}I]T_3$ initially present in the various plasma and tissue preparations. All $[^{125}I]T_3$ and $[^{131}I]T_3$ data are expressed as the percentage of the daily infused $[^{125}I]T_4$ and $[^{131}I]T_3$, respectively. An aliquot of the latter was always counted on the same day, using the same geometry as for the samples. The counting error was ≤3% and the counting rates were at least five times background, even in the tissue preparations with the lowest radioactivities. Appropriate corrections for crossover of ^{131}I into the ^{125}I spectrophotometer channel were made.

A methodological study was undertaken to assess the impact of spontaneous (i.e., artifactual) conversion of $[^{125}I]T_4$ to $[^{125}I]T_3$ during thin-layer chromatography. Samples of the infused $[^{125}I]T_4$ tracer were subjected to thin-layer chromatography. The silica gel was removed from the chromatogram at the location of $[^{125}I]T_4$, and extracted with ethanol/ammonia (10^{-4} M PTU). Then the extract was evaporated and the purified $[^{125}I]T_4$ (10^6 cpm plus 10^{-4} M PTU) rechromatographed. $[^{125}I]T_3$ could not be detected on the chromatogram. Another series of experiments was carried out, taking purified $[^{125}I]T_4$ (2×10^{-6} cpm, 10^{-4} M PTU) in plasma through the entire procedure, i.e., column chromatography followed by thin-layer chromatography, as described above, with $[^{131}I]T_3$ present to correct for losses during the process. No significant amounts of $[^{125}I]T_3$ could be demonstrated (i.e, $[^{125}I]T_4$ to $[^{125}I]T_3$ conversion was ≤0.01%).

Other determinations. Plasma TSH content was measured by the specific radioimmunoassay (RIA) developed for the rat by the National Institute of Arthritis, Metabolic, and Digestive Diseases of the National Institutes of Health.

Plasma T_4 and T_3 concentrations were assessed by RIA. After decay of the ^{131}I initially present in the samples, the T_4 and T_3 levels were determined using ^{131}I-labeled T_4 and T_3, respectively.

DNA was measured by the method of Karsten and Wollenberger (24) and protein was determined according to Lowry et al. (25) with bovine serum albumin as standard.

Liver and kidney mitochondrial α-glycerophosphate dehydrogenase (α-GPD) was measured by the method of Lee and Hardy (26). Suitable dilutions of the mitochondrial fractions in 0.25 M sucrose were prepared. Results were expressed as Δ OD_{500}/min per mg of protein.

Calculations. For calculation of the molar $[^{125}I]T_3/[^{131}I]T_3$ ratios in the plasma and various tissue preparations, $[^{125}I]T_3$ was multiplied by 2 to correct for loss of ^{125}I from the distal ring of T_4. Using the individual plasma $[^{125}I]T_3/[^{131}I]T_3$ ratio and a given tissue (subcellular) $[^{125}I]T_3/[^{131}I]T_3$ ratio, as determined at isotope equilibrium, the contribution of Lc $T_3(T_4)$ to tissue $[^{125}I]T_3$ could be determined directly, in principle according to the calculation procedure used by Silva and Larsen (8):

Let

x = % tissue [^{125}I]T_3 from plasma

$$= \frac{\text{plasma } [^{125}\text{I}]T_3 \text{ (\% dose)}/\text{plasma } [^{131}\text{I}]T_3 \text{ (\% dose)}}{\text{tissue } [^{125}\text{I}]T_3 \text{ (\% dose)}/\text{tissue } [^{131}\text{I}]T_3 \text{ (\% dose)}} \times 100\%$$

$$= \frac{\text{tissue } [^{131}\text{I}]T_3 \text{ (\% dose)}}{\text{plasma } [^{131}\text{I}]T_3 \text{ (\% dose)}} \times \text{plasma } [^{125}\text{I}]T_3 \text{ (\% dose)} \times \frac{1}{\text{tissue } [^{125}\text{I}]T_3 \text{ (\% dose)}} \times 100\%.$$

y = % tissue [^{125}I]T_3 from Lc $T_3(T_4)$ = 100 − x.

Then the percentage contribution of Lc $T_3(T_4)$ to the total (stable) T_3 level in a given tissue (or subcellular fraction) from a euthyroid intact animal could be calculated using the following formula:

$$\% \text{ contribution of Lc } T_3(T_4) = \frac{y}{y + (100/Z)x} \times 100\% = L,$$

where Z is the percentage of the total plasma T_3 (RIA) derived solely from extrathyroidal T_3 production, as determined from the known molar-specific activity of plasma [^{125}I]T_4 and the previously determined concentration of circulating [^{125}I]T_3. The calculations for the Tx rats given T_4 (experiments A and B) become special cases, where Z = 100.

For the euthyroid rats of experiment C the quantity of T_3 in a given tissue (per gram wet weight) was calculated using the equation:

$$\text{Tissue } T_3 \text{ (ng/g)} = \frac{\text{tissue } [^{131}\text{I}]T_3 \text{ (\% dose/g)}}{\text{plasma } [^{131}\text{I}]T_3 \text{ (\% dose/g)}} \times \text{plasma } T_3 \text{ (ng/ml)} \times \frac{100}{100 - L}.$$

For the rats of experiment A or B the quantities of T_3 in the plasma or various tissues was obtained from the [^{125}I]T_3 contents:

$$\text{Tissue } T_3 \text{ (ng/g)} = \frac{\text{tissue } [^{125}\text{I}]T_3 \text{ (\% dose/g)}}{100} \times \text{daily } T_4 \text{ dose (ng)} \times \frac{651}{777},$$

where 651 and 777 represent the molecular weights of T_3 and T_4, respectively. In all cases the quantity of T_3 in the subcellular fractions was expressed as picograms T_3/milligram protein or DNA.

The [^{125}I]T_3 level in plasma is composed of that generated in the tissues and returning to plasma and the small amount contaminating the infused [^{125}I]T_4. The contaminating [^{125}I]T_3 and the infused [^{131}I]T_3 are distributed over the various tissues and the plasma in a similar fashion, i.e., when expressed as a percentage of the daily quantities of infused contaminating [^{125}I]T_3 and [^{131}I]T_3, respectively, the concentrations of both in a particular tissue or plasma sample would be the same. Because the percentage of [^{125}I]T_3 contaminating each [^{125}I]T_4 tracer is known, it is possible to determine the relative contribution from this source to plasma [^{125}I]T_3. Contaminating [^{125}I]T_3 accounted for 5% or less of the total plasma [^{125}I]T_3. Since the maximum molar T_3 contamination of the infused stable T_4 (experiments A and B) is on the same order of magnitude, it is reasonable to expect that the stable contaminating T_3 will not affect the results either.

The infusion rate of [^{125}I]T_4 (stable T_4 in experiments A and B) or [^{131}I]T_3 and their respective blood levels were used to calculate the metabolic clearance rate (MCR) of T_4 or T_3 (27). If the plasma concentrations are expressed as a percentage of the infused dose (in 1 h/100 g body wt) the computation is:

$$\text{MCR ([ml/h]/100 g body wt)} = \frac{100}{\text{\% dose (h/100 g body wt)/ml}}.$$

Statistical analysis. For statistical analysis of the differences between mean values, the unpaired Student's t test was used. The significance of the differences between various tissue (subcellular) preparations from animals of the same experiment was determined by the paired t test. Since anterior pituitary glands from several rats were pooled, all statistical comparisons for this tissue (or subcellular fractions) were made with the unpaired t test.

RESULTS

The infusion of each rat was continued until a steady state had been attained, i.e., when the radioactivity in urine and feces became constant and the total excretion of ^{125}I and ^{131}I equalled the daily input for at least 3 d. For intact euthyroid rats (experiment C) isotope equilibrium was reached after 5 d of infusion of [^{125}I]T_4 (Fig. 2 *a*) and after 3 d of infusion of [^{131}I]T_3 (Fig. 2 *b*). At this stage 55–60% of the total amount of ^{125}I excreted daily, and ~60% of the total amount of ^{131}I excreted daily was present in the urine. Tx rats supplemented with 0.2 or 1.0 μg T_4/100 g body wt per d (experiment A or B) excreted maximum and constant amounts of labeled metabolites into the urine and feces after the 7th d of [^{125}I]T_4 infusion and after the 4th d of [^{131}I]T_3 infusion. Urinary ^{125}I and ^{131}I always accounted for 60–70% of the total radioactivity excreted daily (excretion curves not shown).

At the end of the infusion period, plasma T_3, T_4, and TSH concentrations were determined for each rat by RIA. In addition, mitochondrial α-GPD-specific activities were measured for the liver and kidney. According to these parameters (listed in Table I), Tx rats receiving a continuous infusion of 0.2 μg T_4/100 g body wt per d (experiment A) were clearly hypothyroid at the end of the infusion period. Infusion of Tx rats with 1.0 μg T_4/100 g body wt per d (experiment B) produced a slightly hypothyroid state when the parameters are compared with those for the intact euthyroid rats of experiment C.

The MCR of T_3 decreased from euthyroid to hypothyroid rats (Table I). The MCR of T_4 for hypothyroid rats was lower than that for slightly hypothyroid or euthyroid animals.

Blockage of the recycling of tracer I^- in intact euthyroid rats. To assess efficiency of the blockage of

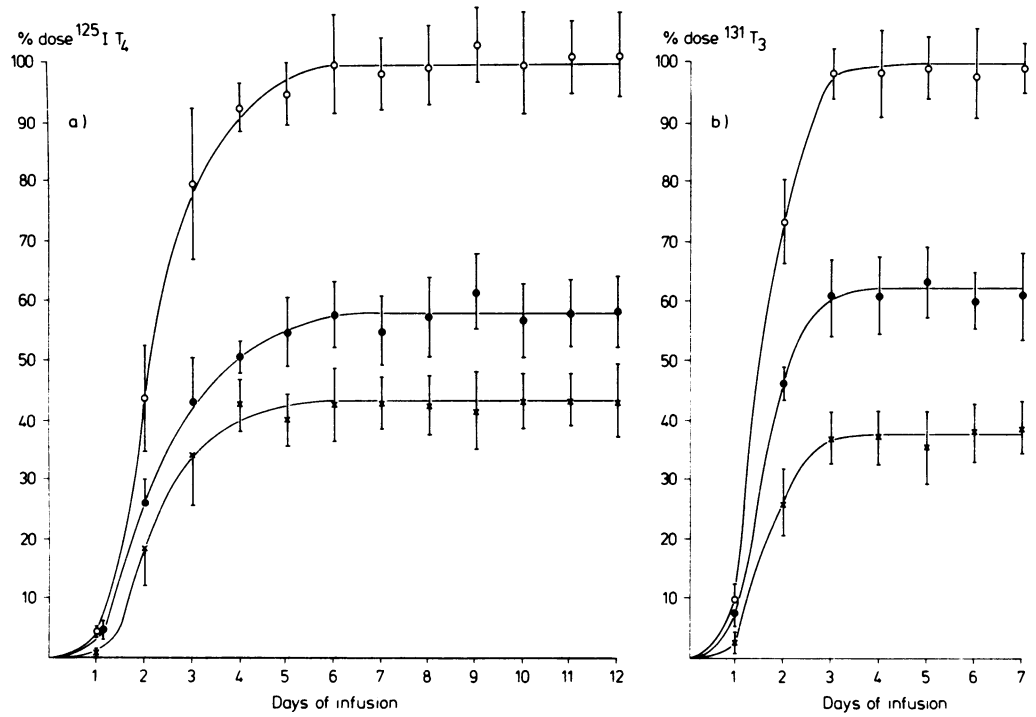

FIGURE 2 The radioactivity in the excreta of intact euthyroid rats (experiment C) during the [^{125}I]T_4 infusion (*a*) and the simultaneous infusion of [^{131}I]T_3, which was started either 4 or 5 d after the start of the [^{125}I]T_4 infusion (*b*). (○) total radioactivity; (●) urinary radioactivity; (X) fecal radioactivity. Data represent the mean±SD for eight rats, except for day 11 (mean±SD for four rats) and 12 (mean±SD for two rats) during infusion of [^{125}I]T_4 (*a*) and day 7 (mean±SD for four rats) during infusion of [^{131}I]T_3 (*b*).

recycling by excess KI in euthyroid rats, intact control animals were equilibrated with $^{125}I^-$ and the same daily dose of KI as used in the other experiments. Equilibrium was reached after 5 d, when ~98% of the daily excreted radioactivity was present in the urine. Plasma T_3, T_4, and TSH levels (mean±SD, n = 4), measured

TABLE I

Concentration of T_3, T_4, and TSH in Plasma, the MCR of T_3 and T_4, and the Specific Activity of Liver and Kidney Mitochondrial α-GPD in Tx Rats Receiving Supplements of T_4 and Intact Euthyroid Rats, after Simultaneous [^{125}I]T_4 and [^{131}I]T_3 Infusion until Isotope Equilibrium Was Achieved*

		Experiment A (n = 6)	Experiment B (n = 6)	Experiment C (n = 8)	*P* value (unpaired *t* test) A vs. B	B vs. C	A vs. C
T_3 (*ng/100 ml*)		10±4	38±10	53±13	<0.005	<0.05	<0.001
T_4 (*μg/100 ml*)		1.5±0.8	5.4±1.2	5.1±1.0	<0.001	NS	<0.001
TSH (*ng/ml*)		7700±1441	470±135	289±121	<0.001	<0.05	<0.001
MCR (*ml/h*)/100 g body wt	T_3	16.4±3.6	23.1±1.9	30.2±5.4	<0.005	<0.01	<0.001
	T_4	0.56±0.12	0.77±0.16	0.86±0.17	<0.05	NS	<0.005
α-GPD (Δ OD_{500}/min/mg protein)	liver	0.102±0.032	0.198±0.062	0.272±0.017	<0.01	<0.01	<0.001
	kidney	0.130±0.024	0.244±0.026	0.311±0.049	<0.001	<0.02	<0.001

Data represent mean±SD. T_3, T_4, and TSH concentrations were determined by RIA.

* Experiment A, 0.2 μg/100 g body wt per d; experiment B, 1.0 μg/100 g body wt per d.

at the end of the infusion period, did not differ significantly from levels found for rats of experiment C (T_3, 50±13 ng/100 ml; T_4, 4.9±1.2 μg/100 ml; TSH, 270±80 ng/ml). In the ethanol extracts of the plasma samples and several tissue homogenates (cerebral cortex, cerebellum, pituitary gland, thigh muscle, liver, and kidney) taken from the control animals, [^{125}I]T_3 and [^{125}I]T_4 were not detectable by thin-layer chromatography. For the rats of experiment C, virtually no [^{131}I]T_4 radioactivity appeared on the chromatograms of the tissue and plasma samples investigated. Therefore, it was concluded that for rats infused with the labeled iodothyronines any recycled tracer I^- would add only a negligibly small fraction to the [^{125}I]T_3 or [^{131}I]T_3 concentrations.

^{125}I T_3/^{131}I T_3 ratios in plasma and various tissue (subcellular) fractions. As can be seen from the ratios of [^{125}I]T_3 (as derived from [^{125}I]T_4) to [^{131}I]T_3 (infused), shown in Table II, the individual values for a given tissue were similar for different animals from the same experiment. Except for the thigh muscle the values for the tissue homogenates were always higher than those determined for plasma, indicating that circulating [^{125}I]T_3 and [^{125}I]T_3 in tissue are not totally exchangeable.

Data for the subcellular fractions from the liver, kidney, and anterior pituitary gland are shown in Table III. In contrast to the pituitary nuclear fraction, the nuclear [^{125}I]T_3/[^{131}I]T_3 ratios for both the liver and the kidney did not differ significantly from the plasma values in all three experiments.

Source of circulating T_3 in intact, euthyroid animals. For each rat used in experiment C, plasma T_3 derived from peripheral 5'-monodeiodination was estimated by using the plasma [^{125}I]T_3 concentration (assessed by thin-layer chromatography) and the corresponding specific activity of plasma [^{125}I]T_4. The concentration of T_3 (generated peripherally from T_4) in the plasma amounted to 34±8 ng/100 ml (mean±SD). When expressed as a percentage of the total plasma T_3 concentration (measured by RIA, see Table I), 65±9% (mean±SD) of the total circulating T_3 was derived from peripheral conversion of T_4, the remaining being contributed directly by thyroidal secretion of T_3.

Contribution of T_3 generated locally from T_4 to the total tissue (subcellular) T_3 concentration. In experiments A and B, infused T_4 was without a doubt the main source of T_3 in both plasma and tissues. Hence, the percentage contributed by Lc $T_3(T_4)$ to the total tissue (subcellular) T_3 concentration could be computed directly from the ratio of [^{125}I]T_3 to [^{131}I]T_3 found for a given tissue preparation and for the corresponding plasma (Methods). However, for euthyroid rats (experiment C), the proportion of circulating T_3 being secreted directly by the thyroid gland (see above) had to be taken into account using the calculation procedures described in the Methods section. The data for the tissue homogenates are shown in Table IV. The percentage contributed by Lc $T_3(T_4)$ to the total tissue T_3 concentration was substantial but different for brain cortex and cerebellum ($P < 0.001$) in all experiments.

TABLE II

[^{125}I]T_3/[^{131}I]T_3 Ratios for Plasma and Tissue Homogenates from Various Organs of Hypothyroid (Experiment A), Slightly Hypothyroid (Experiment B), and Euthyroid (Experiment C) Rats Equilibrated with [^{125}I]T_4 and [^{131}I]T_3

	[^{125}I]T_3 (% daily dose [^{125}I]T_4) / [^{131}I]T_3 (% daily dose [^{131}I]T_3)			*P* value (paired *t* test) Tissue vs. plasma		
Tissue	Exp. A	Exp. B	Exp. C	Exp. A	Exp. B	Exp. C
Liver	0.477±0.056	0.321±0.021	0.667±0.091	<0.05	<0.005	<0.001
Kidney	0.412±0.017	0.279±0.037	0.323±0.021	<0.05	<0.05	<0.01
Thigh muscle	0.403±0.027	0.235±0.021	0.288±0.020	NS	NS	NS
Pituitary	0.791±0.009	0.346±0.004	0.380±0.005	<0.025	<0.05	<0.05
Cerebral cortex	1.572±0.135	0.677±0.047	1.211±0.286	<0.001	<0.001	<0.001
Cerebellum	1.021±0.063	0.517±0.029	0.732±0.078	<0.001	<0.001	<0.001
Plasma	0.397±0.024	0.245±0.020	0.287±0.026	—	—	—

Intact euthyroid rats (experiment C) and Tx rats received continuous intravenous infusion of [^{125}I]T_4, [^{131}I]T_3, and KI. In addition, Tx rats received 0.2 (experiment A) or 1.0 (experiment B) μg T_4/100 g body wt/d. Data represent the mean±SD. In each experiment pituitary glands from two rats were pooled; n = 2. For the other tissues and plasma: n = 5 in experiment A; n = 6 in experiment B; and n = 7 in experiment C.

TABLE III

$[^{125}I]T_3/[^{131}I]T_3$ Ratios for Subcellular Fractions from Liver, Kidney, and Pituitary of Hypothyroid (Experiment A), Slightly Hypothyroid (Experiment B), and Euthyroid (Experiment C) Rats Equilibrated with $[^{125}I]T_4$ and $[^{131}I]T_3$

Tissue	Subcellular fraction	$\frac{[^{125}I]T_3 \text{ (\% daily dose } [^{125}I]T_4)}{[^{131}I]T_3 \text{ (\% daily dose } [^{131}I]T_3)}$			*P* value (paired *t* test) (Subcellular fraction vs. plasma)		
		Exp. A	Exp. B	Exp. C	Exp. A	Exp. B	Exp. C
Liver	Nuclear	0.411±0.027	0.254±0.023	0.300±0.030	NS	NS	NS
	Mitochondrial	0.424±0.054	0.315±0.021	0.464±0.037	NS	<0.001	<0.001
	Microsomal	0.444±0.023	0.343±0.025	0.535±0.070	<0.005	<0.001	<0.001
	Cytoplasmic	0.666±0.110	0.370±0.048	0.597±0.083	<0.01	<0.005	<0.001
Kidney	Nuclear	0.414±0.024	0.249±0.023	0.309±0.054	NS	NS	NS
	Mitochondrial	0.432±0.029	0.255±0.026	0.311±0.038	<0.02	NS	<0.005
	Microsomal	0.412±0.025	0.276±0.034	0.346±0.031	NS	NS	<0.01
	Cytoplasmic	0.414±0.036	0.277±0.031	0.308±0.049	NS	<0.05	NS
Pituitary	Nuclear	0.707±0.124	0.435±0.040	0.375±0.007	<0.025	<0.025	<0.05
	Cytoplasmic	—	0.336±0.007	0.378±0.007	—	<0.05	<0.05
Plasma		0.397±0.024	0.245±0.020	0.287±0.026			

Data represent the mean±SD. In each experiment pituitary subcellular fractions were prepared from pooled glands of two or three rats; $n = 2$. For all hepatic and renal subcellular fractions: $n = 5$ in experiment A, $n = 6$ in experiment B, and $n = 7$ in experiment C.

The Lc $T_3(T_4)$ contribution to total hepatic cellular T_3 was greater in the euthyroid then in the hypothyroid state, whereas the reverse was found for the anterior pituitary gland. In the kidney, Lc $T_3(T_4)$ contributed only a minor amount to the total intracellular T_3.

At the subcellular level, for the pituitary gland the relative contribution of Lc $T_3(T_4)$ to the total nuclear T_3 under hypothyroid and slightly hypothyroid conditions was increased about twofold when compared with the euthyroid state (euthyroid, 19.7±1.1%; mildly hypothyroid, 43.9±7.1%; hypothyroid, 39.9±6.6%). In the pituitary gland of euthyroid rats the percentage contributed by Lc $T_3(T_4)$ to nuclear and cytoplasmic T_3 (19.9±1.1%) was identical, whereas this was clearly not the case for slightly hypothyroid animals (Lc $T_3[T_4]$ cytoplasmic fraction: 23.3±7.6%). Unfortunately the cytoplasmic fractions of hypothyroid rats were lost.

Virtually no Lc $T_3(T_4)$ was contributed to the nuclear T_3 level in both the liver and kidney (Table III) in all groups of animals. In the liver of hypothyroid rats Lc $T_3(T_4)$ contributed predominantly to the cytoplasmic T_3 pool (mitochondrial fraction, 8.9±2.9%;

TABLE IV

Contribution of Lc T_3 (T_4) to the Total T_3 in Tissue Homogenates from Several Organs of Hypothyroid (Experiment A), Slightly Hypothyroid (Experiment B), and Euthyroid (Experiment C) Rats

Tissue	Contribution of Lc T_3 (T_4)			P value (unpaired *t* tests)		
	Exp. A	Exp. B	Exp. C	A vs. B	B vs. C	A vs. C
Liver	15.0±7.3	22.1±5.3	45.9±6.2	<0.05	<0.01	<0.001
Kidney	3.6±3.6	7.7±6.2	7.4±5.1	NS	NS	NS
Thigh muscle	1.5±3.7	0.0±8.3	0.0±4.0	NS	NS	NS
Pituitary gland	49.8±0.8	33.7±3.0	23.5±1.3	<0.02	<0.05	<0.005
Cerebral cortex	74.7±1.7	63.5±5.0	67.3±4.1	<0.005	NS	<0.01
Cerebellum	61.1±1.7	52.5±4.3	50.3±4.6	<0.005	NS	<0.001

Data represent mean±SD. The percentage contributed by Lc T_3 (T_4) to the total T_3 in a given tissue was calculated as outlined in the Methods section.

microsomal fraction, 10.6±3.6%; cytoplasmic fraction, 39.0±10.8%). Under slightly hypothyroid or euthyroid conditions, the relative contribution of Lc $T_3(T_4)$ was increased in the hepatic mitochondrial and microsomal fractions, not differing statistically from the values found for the cytoplasm in these rats (22–34%). In the kidney the small percentage contributed by Lc $T_3(T_4)$ was statistically the same for all subcellular fractions in all experiments (all values were between 1 and 11%).

Concentration of T_3 in the plasma and various tissues. Fig. 3 shows the total T_3 content in plasma (determined by the isotopic equilibrium technique) and various tissue homogenates. In addition, the quantity of Lc $T_3(T_4)$ is given.

In both experiments A and B, the plasma T_3 levels measured by RIA (Table I) were in close agreement with those derived from the isotopic equilibrium technique. For animals of experiment A, the mean T_3 content in the tissue was clearly reduced when compared with the values found in experiments B or C, although the differences for the pituitary gland between experiments A and B did not differ significantly (probably due to limited number of observations). Except for the cerebral cortex and cerebellum, tissue T_3 concentrations were lower in slightly hypothyroid rats than in the euthyroid rats. In all experiments, T_3 concentrations were higher in tissue (except in the thigh muscle) than in the plasma ($P < 0.001$).

Tissue/plasma T_3 gradients are listed in Table V. In contrast to the other organs the tissue/plasma T_3 concentration gradient for the liver increased gradually from hypothyroidism to euthyroidism. The gradient for the kidney decreased only in the hypothyroid rat. For both cerebral cortex and cerebellum the tissue/plasma T_3 gradient decreased significantly in euthyroid animals when compared with the values found for hypothyroid or slightly hypothyroid animals. Very low tissue/plasma T_3 gradients were encountered for the thigh muscle in all experiments. The amounts of T_3 (expressed as picograms per milligram of protein) in the subcellular fractions from the liver, kidney, and pituitary gland are shown in Fig. 4 *a*, *b*, and *c*, respectively.

In the hepatic subcellular fractions the quantity of T_3 per milligram of protein decreased from euthyroidism to hypothyroidism. This was also the case for

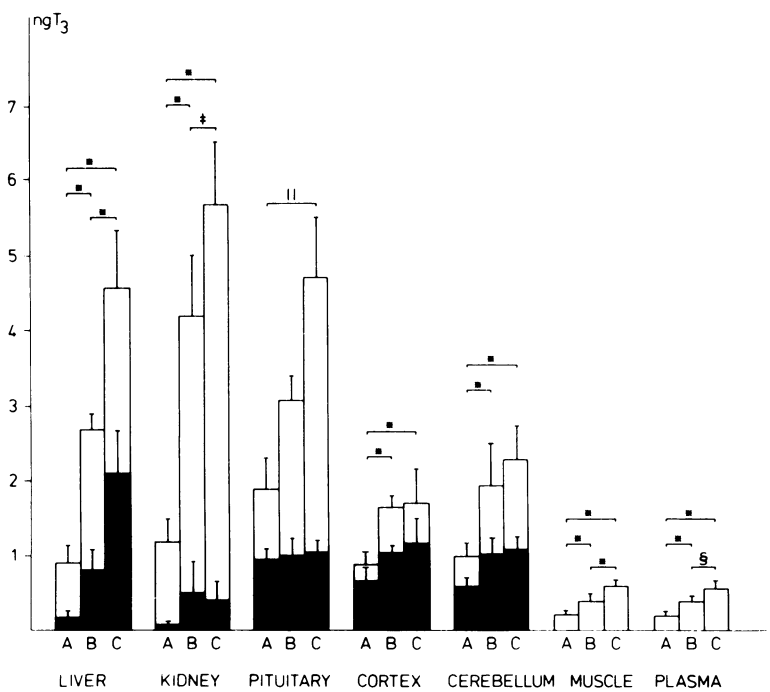

FIGURE 3 Concentrations of T_3 in plasma and tissues (per milliliter plasma and gram wet weight of tissue) from hypothyroid (experiment A), slightly hypothyroid (experiment B), and euthyroid (experiment C) rats, as determined by isotopic equilibrium technique; (■) contributed by Lc $T_3(T_4)$. Data represent the mean±SD. Unpaired *t* tests were used to compute the significance of differences between total T_3 concentrations. $^{*}P < 0.001$; $^{\ddagger}P < 0.01$; $^{\S}P < 0.025$; $^{\|}P < 0.05$.

TABLE V

Tissue/Plasma Gradients of T_3 Concentration in Hypothyroid (Experiment A), Slightly Hypothyroid (Experiment B), and Euthyroid (Experiment C) Rats

				P value (unpaired *t* test)		
	Exp. A	Exp. B	Exp. C	A vs. B	B vs. C	A vs. C
Liver	5.4±0.5	7.2±1.0	9.1±0.9	<0.01	<0.005	<0.001
Kidney	8.4±0.8	11.0±1.9	10.6±1.6	0.02	NS	<0.02
Thigh muscle	1.3±0.1	1.1±0.2	1.2±0.2	NS	NS	NS
Pituitary gland	12.7±2.5	8.0±1.3	8.9±1.8	NS	NS	NS
Cerebral cortex	4.9±0.9	4.3±0.5	3.3±1.0	NS	<0.05	<0.02
Cerebellum	7.1±1.4	7.0±0.9	4.1±0.5	NS	<0.001	<0.001

Data represent mean±SD. Concentrations of T_3 are expressed as nanograms per gram wet weight of tissue or milliliter plasma.

the nuclear fractions from the kidney and anterior pituitary gland. On the other hand, under slightly hypothyroid and euthyroid conditions, the concentration of T_3 in both the mitochondrial and the microsomal fraction of the kidney remained the same.

The nuclear T_3 content, expressed per milligram of DNA, for the liver, kidney, and pituitary gland (Table VI) was significantly less in the hypothyroid than in the euthyroid rat. In each experiment, the pituitary nuclear T_3 level was higher than either the liver or the

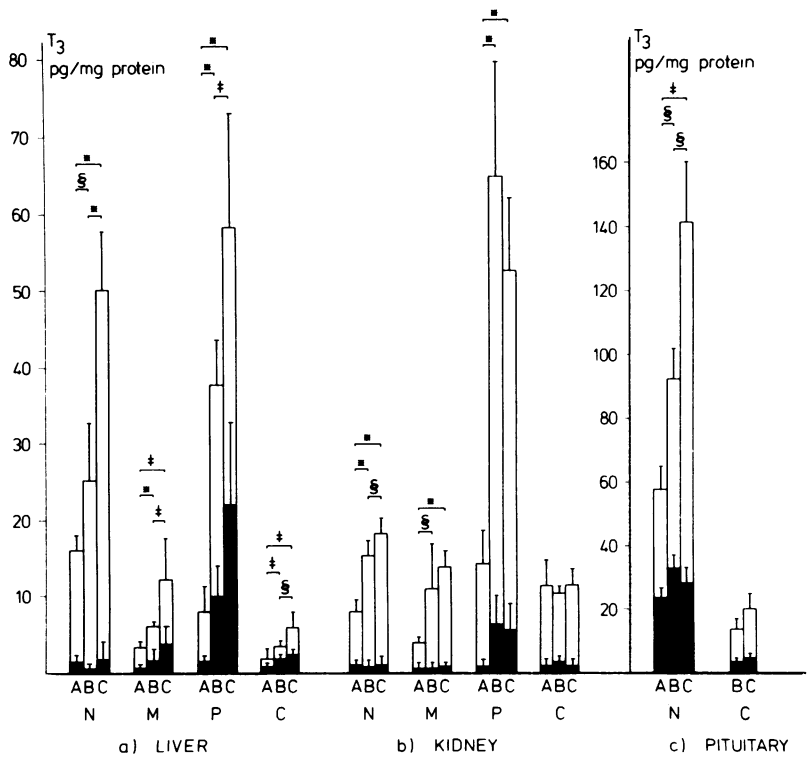

FIGURE 4 Concentration of T_3 (per milligram of protein) in subcellular fractions from liver (*a*), kidney (*b*), and anterior pituitary gland (*c*). Along the abscissa are the subcellular fractions in order of their isolation: N (nuclear), M (mitochondrial), P (microsomal), C (cytoplasmic). (■) contributed by Lc $T_3(T_4)$. Data represent the mean±SD. Unpaired *t* tests were used to compute the significance of differences between total T_3 content. $^{*}P < 0.001$; $\ddagger P < 0.01$; $\S P < 0.05$.

TABLE VI

Nuclear T_3 Levels in Liver, Kidney, and Anterior Pituitary Gland of Hypothyroid (Experiment A), Mildly Hypothyroid (Experiment B), and Euthyroid (Experiment C) Rats

	T_3			*P* value (unpaired *t* test)		
	Exp. A	Exp. B	Exp. C	A vs. B	B vs. C	A vs. C
	pg/mg DNA					
Liver	50±9	114±16	151±11	<0.001	<0.001	<0.001
Kidney	23±8	45±9	59±8	<0.005	<0.02	<0.001
Pituitary	304±47	506±17	580±10	<0.05	<0.05	<0.02

Data represent mean±SD.

kidney nuclear T_3 ($P < 0.001$), whereas hepatic nuclei contained more T_3 than nuclei from the kidney ($P < 0.001$). Negligibly small amounts of Lc T_3(T_4) were found in the nuclei of both liver and kidney cells. The quantity of Lc T_3 (T_4) in the pituitary nuclear fraction was higher in experiment B (224±51 pg/mg DNA) than in experiment A (119±2 pg/mg DNA) or experiment C (114±20 pg/mg DNA).

DISCUSSION

The prolonged administration of [^{125}I]T_4 and [^{131}I]T_3 by continuous intravenous infusion ensures a closer approximation of isotopic equilibrium than could be achieved by repeated injections or administration via the food. Comparison of radioactivity in excreta with daily input of ^{125}I and ^{131}I provides a convenient method for verifying the establishment of the steady state, although theoretically it is conceivable that small or slowly exchanging iodothyronine compartments will not yet have reached equilibrium. In each experiment, for practical reasons, the period of [^{125}I]T_4 infusion was extended from 10 to 11 (two animals) or 12 (two animals) d, while the [^{131}I]T_3 infusion lasted 7 instead of 6 d. In spite of these differences in the duration of the infusion of both labeled iodothyronines, the ratios of [^{125}I]T_4 to [^{131}I]T_3, as determined for the various tissue preparations and the plasma, were strikingly similar for different animals of the same experiment. This lack of variability with time provides additional evidence that the tissues (or subcellular preparations) investigated were in total isotopic equilibrium.

It was possible to determine the [^{125}I]T_3/[^{131}I]T_3 ratios for all tissue (subcellular) extracts directly, after thin-layer chromatography, since the [^{131}I]T_3 peak on the chromatogram coincided with the [^{125}I]T_3 peak. In contrast to our previous findings for severely hypothyroid animals (9), however, this was not the case for any of the plasma extracts from the rats of experiments A and C. The same difficulty with the quantitation of plasma [^{131}I]T_3 (after infusion of [^{131}I]T_4) was encountered by Obregon et al. (11) after paper chromatography using the Bellabarba system (28). Rapid semi-purification of the extracted plasma T_3 by column chromatography, and subsequent thin-layer chromatography of the ethanol extract of the eluted peak fractions of [^{131}I]T_3 solved this problem. This observation suggests the presence in the original extract of an ^{125}I-containing compound other than [^{125}I]T_3, moving ahead of the [^{131}I]T_3 peak and partially overlapping it. It might be argued that the plasma [^{125}I]T_3/[^{131}I]T_3 ratios were overestimated (and hence the contribution of locally generated T_3 to the total T_3 in a given tissue preparation underestimated) due to artifactual deiodination of [^{125}I]T_4. However, this is unlikely for the following reasons: (*a*) thin-layer chromatography (followed by column chromatography) of purified [^{125}I]T_4 added to plasma samples yielded no significant amounts of [^{125}I]T_3 (<0.01% [^{125}I]T_4 to [^{125}I]T_3 conversion; see Methods). The highest ratio of [^{125}I]T_4 to [^{125}I]T_3 in plasma at isotope equilibrium was found for Tx rats given 1.0 μg T_4/100 g body wt per d (240:1, Table I). Thus, for these animals an artifactual deiodination of 0.01% or less would lead to an overestimation of the plasma [^{125}I]T_3 of only 2.4% or less. In this study the [^{125}I]T_4/[^{125}I]T_3 ratios in the various tissues were not investigated systematically. However, inspection of the [^{125}I]T_4 region on the chromatograms of the various tissue extracts obtained from some of the euthyroid rats of experiment C, revealed that for all tissues the [^{125}I]T_4/[^{125}I]T_3 ratios were lower than those for the corresponding plasma samples. Therefore, it is reasonable to expect that in all experiments artifactual [^{125}I]T_4 to [^{125}I]T_3 conversion did not contribute significantly to tissue [^{125}I]T_3 either. (*b*) In

all experiments the plasma and tissue $[^{125}I]T_3/[^{131}I]T_3$ ratios were reproducible. (*c*) For Tx rats receiving T_4 replacement (experiments A and B) the concentrations of circulating T_3, measured by RIA, were in close agreement with those determined by the isotope equilibrium technique.

In all experiments, for both the liver and kidney substantial amounts of $[^{125}I]T_3$ and $[^{131}I]T_3$ were recovered in the microsomal and cytoplasmic fraction. Hence, these subcellular fractions (contrary to the nuclear and mitochondrial fractions) contributed mainly to the $[^{125}I]T_3/[^{131}I]T_3$ ratios in the whole liver or kidney homogenates (Tables II and III). However, in the liver of euthyroid rats (experiment C) none of the subcellular fractions had as high a $[^{125}I]T_3/[^{131}I]T_3$ ratio as that in the whole homogenate. Since artifactual deiodination during the handling of the samples is not likely, as discussed above, it is possible that the $[^{125}I]T_3/[^{131}I]T_3$ ratios in several discarded materials differed from those determined for the subcellular fractions prepared. Thus, if the $[^{125}I]T_3$ and/or $[^{131}I]T_3$ in discarded fractions had contributed significantly to the total radioactivity in the homogenates (by observation: presumably the so-called "fluffy layer," being discarded during purification of the mitochondrial fraction) this would explain the differences observed between whole homogenate and subcellular fractions.

Obregon et al. (29) used RIA to measure the concentrations of T_3 in plasma, liver, kidney, muscle, and brain of normal male rats. Our data from the isotopic equilibrium technique were in close agreement.

The present study provides additional evidence for the existence of so-called "hidden pools" of T_3 (11), i.e., for most tissues under both (slightly) hypothyroid and euthyroid conditions the intracellular T_3 is not totally exchangeable with circulating T_3. Considering the apparent difference in distribution between $[^{125}I]T_3$ from Lc $T_3(T_4)$ and $[^{131}I]T_3$ infused as such, this means that previous estimations of the total production rate of T_3 may be substantially underestimated, as discussed by others (3, 6). In contrast, muscular T_3 appears to be derived exclusively from plasma. Similar observations were reported by Obregon et al. (11) for cardiac and skeletal muscle. Furthermore, in all experiments, no significant thigh muscle/plasma T_3 gradient was found. The physiological implication of these findings might be that skeletal muscle depends directly on the plasma T_3 concentration, as far as the intracellular concentration of T_3 and hence the muscular response to thyroid hormone are concerned.

In the cerebral cortex and cerebellum, however, most of the intracellular T_3 derives from intracellular conversion of T_4 to T_3. Our conclusions regarding the importance of local monodeiodination of T_4 in these tissues essentially confirm recent observations by Crantz et al. (12) for euthyroid rats: they found that after a single injection of $[^{125}I]T_4$, the T_3 generated intracellularly from T_4 accounted for more than half of the specifically bound nuclear T_3 in the cortex and cerebellum. For both tissues the percent contributed by Lc $T_3(T_4)$ to the total T_3 was clearly increased in the hypothyroid state (experiment A), and in athyroid animals after infusion of tracer amounts of T_4 and T_3 (9), when compared with euthyroid animals. This could be explained by an increase in the rate of T_4 to T_3 conversion in the brain of the hypothyroid rat, as demonstrated in vitro by Kaplan and Yaskoski (30), although this did not prevent a decrease in total T_3 content in both cerebral cortex and cerebellum. On the other hand, in contrast to the other tissues investigated, the concentrations of T_3 in both the cerebral cortex and the cerebellum of slightly hypothyroid rats did not differ from those determined for euthyroid rats. It can be concluded that, despite lower plasma T_3 levels, local T_3 production reduced the loss of intracellular T_3 in the cerebral cortex and cerebellum when compared with the other organs. This suggests an important role for thyroid hormone in the brain, although no direct responsive event has as yet been identified in the adult rat brain (3).

For Tx rats receiving a low daily dose of T_4 (experiment A), about one-half of the total tissue T_3 content in the anterior pituitary gland was contributed by Lc $T_3(T_4)$, a proportion significantly higher than that found for slightly hypothyroid animals. In homogenates prepared from the pituitaries of intact euthyroid rats, locally produced T_3 accounted for only 24% of the total T_3. Although the total T_3 concentration in the pituitary gland was lower in the hypothyroid than in euthyroid state, the quantity of Lc $T_3(T_4)$ remained surprisingly constant, namely $\sim$1 ng/g wet wt of tissue in all experiments. Kaplan (31) and Cheron et al. (32) reported that the rate of T_4 5′-monodeiodination in vitro was much higher in anterior pituitaries from hypothyroid rats than in those from euthyroid controls. Therefore it seems likely that, despite the low plasma T_4 concentration in hypothyroid animals, the relatively large amount (gravimetric determination) of Lc $T_3(T_4)$ present in homogenates of the pituitary gland was caused by an enhancement of T_4 to T_3 conversion, which might be due to an increased number of thyrotropic cells (31).

For both hypothyroid and slightly hypothyroid rats the percentage contribued by Lc $T_3(T_4)$ to the total pituitary nuclear T_3 was about twice the value found for euthyroid animals. As can be seen from Fig. 4 *c* and deduced from Table VI the quantity of Lc $T_3(T_4)$ was identical in hypothyroid and euthyroid rats. Changes in the quantity of nuclear T_3 were merely

due to changes in plasma-derived T_3 in these animals. The gravimetric amount of pituitary nuclear Lc $T_3(T_4)$ was even somewhat higher in slightly hypothyroid rats than in euthyroid animals. However, despite the fact that plasma T_4 levels were the same in both groups of animals, the total nuclear T_3 content in these rats remained below the euthyroid value and hence the plasma TSH concentrations were somewhat elevated. The mean value found for pituitary nuclear T_3 (per milligram DNA) in the euthyroid rat agrees closely with that reported previously by others who used RIA (33). The present findings, regarding the source and quantity of pituitary nuclear T_3 strongly suggest that both plasma T_3 and T_4 (through its local conversion into T_3) play a role in the regulation of TSH secretion. The contribution of Lc $T_3(T_4)$ to the total pituitary nuclear T_3 was of minor importance in euthyroid rats, when compared with both groups of T_4-supplemented athyreotic rats. In contrast to the euthyroid state, under slightly hypothyroid conditions the pituitary nuclear and cytoplasmic T_3 pools were not completely exchangeable. It is possible that locally produced T_3 is preferentially transported to the nucleus in these rats. For both euthyroid and slightly hypothyroid rats the pituitary T_3 content (per milligram of protein) was significantly higher in the nucleus than in the cytoplasm, suggesting the presence of high capacity nuclear T_3 binding sites.

Previous estimations by Silva et al. (10) indicated that in the euthyroid rat plasma T_3 and intracellular T_4 5′-monodeiodination yield roughly equal quantities of pituitary nuclear T_3. Their study has relied on bolus injections of [^{125}I]T_4 and [^{131}I]T_3. Application of this technique requires several approximations and simplifying assumptions regarding, for example, the fractional disappearance rate of T_3 from both the nucleus and cytoplasm or plasma at the time of measurement. This may well have led to errors in the estimation of the proportion of the total nuclear T_3 contributed by Lc $T_3(T_4)$. The same might have occurred with respect to their estimation of Lc $T_3(T_4)$ in the nuclear fraction from both the liver and kidney. However, despite the differences in techniques, the total quantities of T_3 in the liver and kidney nuclei, as reported in the present study for euthyroid rats (Table VI), do not differ substantially from the data of Silva et al.: liver nuclei, 180 pg/mg DNA; kidney nuclei, 70 pg/mg DNA (10). Furthermore, our calculation for the hepatic nuclear fraction (euthyroid animals) agrees reasonably well with that of Lim et al. (34), 104 pg/mg DNA. These data, including our values (for liver nuclei) are different from RIA data reported by Surks and Oppenheimer (35), 330 pg/mg DNA. This apparent difference remains to be resolved.

The conversion of T_4 to T_3 appears to be an important function of the liver and kidney and perhaps other peripheral tissues. The T_3 produced within these tissues reenters the circulation and becomes available to all organs, accounting for about two-thirds of the plasma T_3 in the normal rat. Liver or kidney disease greatly modifies the metabolism of the thyroid hormones (36). Jennings et al. (37) showed that during perfusion of isolated liver with 10 μg T_4, the extraction of T_4 by the liver was associated with a significant increase in T_3 concentration in both the perfusate and the liver. The perfusate T_3 concentration increased in a linear fashion for 60–90 min before reaching a plateau; the liver T_3 concentration increased most dramatically in the first 30 min of perfusion, followed by a more gradual but linear increase thereafter. On the other hand, no net increase in the production of T_3 could be measured by van der Heide (38) during perfusion of isolated liver from euthyroid rats with plasma obtained from euthyroid rats after addition of 12 μg T_4. Furthermore, recent observations by van der Heide et al. (unpublished results) indicate that during prolonged perfusion of isolated rat liver with T_4 in the presence of high concentrations of T_3, the venous T_3 concentration decreases, reaching a plateau value after ~2 h. In our experiments the total T_3 concentration in the liver decreased from euthyroidism to hypothyroidism, in association with a decrease in the tissue/plasma T_3 concentration gradient. The contribution of Lc $T_3(T_4)$ to the total T_3 in the liver also decreased significantly from the euthyroid to the hypothyroid state. The aforementioned reports, together with the in vivo data of the present study, suggest that the liver has an important function in the regulation of the circulating T_3 concentration. During development of hypothyroidism it is possible that the liver may serve to moderate the effect of decreased thyroidal iodothyronine production. According to this concept in both groups of athyreotic T_4-replaced rats (with plasma T_3 levels below the euthyroid value) there would be a net increase in the release of locally produced T_3 by the liver into the plasma, resulting in a lower liver/plasma T_3 concentration gradient, and a relatively small contribution of Lc $T_3(T_4)$ to the total hepatic T_3 at dynamic equilibrium. However, at normal circulating T_3 levels, less locally produced T_3 would enter the plasma T_3 pool, hence resulting in higher hepatic intracellular T_3 concentrations and an increased percentage contributed by Lc $T_3(T_4)$ under steady-state conditions; disposal of T_3 might then become of greater importance. On the other hand, hypothyroidism has been shown to lead to a diminished T_4 5′-deiodinase activity of rat liver in vitro (3). This may provide an additional explanation for the observed decreased percent contribution of Lc $T_3(T_4)$ in the liver (both ho-

mogenate and microsomal fraction) of hypothyroid and slightly hypothyroid animals. Probably in vivo variations in hepatic T_4 5′-deiodinase activity, together with shifts of the balance between the disposal rate of hepatic intracellular T_3 and the release of T_3 by the liver into the vascular compartment, play an important part in the regulation of plasma T_3 levels. If the proposed regulatory function of the liver is correct, it would be likely that the underlying mechanisms respond directly to plasma T_3 concentrations. Indeed, the concentration of T_3 in the nucleus was a linear function of the plasma T_3 concentration and, in contrast to the other subcellular fractions investigated, in all experiments virtually all nuclear T_3 was derived from the plasma. A possible explanation for this lack of complete exchange of T_3 between nuclear and other subcellular compartments could be that T_3 from the plasma is transported directly to the nucleus via a binding protein located in the cell membrane (39, 40). In all experiments, for the liver the highest concentrations of T_3 were found in the nuclear and microsomal fractions. This reflects, probably, the presence of nuclear T_3-binding proteins (4, 5) and suggests the importance of the microsomal fraction as a site of T_3 and T_4 metabolism in vivo (41).

In the kidney, only a very small proportion of the total T_3 was contributed by Lc $T_3(T_4)$ in all experiments. Several investigations have demonstrated T_4 5′-monodeiodination activity in the kidney (3). Therefore, the observed small contribution of Lc $T_3(T_4)$ in the steady state could mean that the locally produced T_3 is rapidly exchanged with the plasma pool. As found for the liver, all nuclear T_3 appeared to be derived from the plasma. However, in contrast to the liver, the percentages contributed by Lc $T_3(T_4)$ in the subcellular fractions were not statistically different. Furthermore, the distribution pattern of the concentrations of T_3 in the renal subcellular fractions was also different from that observed for the liver. The physiological meaning of these tissue differences remains to be established.

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