Contributions of Plasma Triiodothyronine and Local Thyroxine Monodeiodination to Triiodothyronine to Nuclear Triiodothyronine Receptor Saturation in Pituitary, Liver, and Kidney of Hypothyroid Rats: FURTHER EVIDENCE RELATING SATURATION OF PITUITARY NUCLEAR TRIIODOTHYRONINE RECEPTORS AND THE ACUTE INHIBITION OF THYROID-STIMULATING HORMONE RELEASE

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FURTHER EVIDENCE RELATING SATURATION OF PITUITARY NUCLEAR TRIIODOTHYRONINE RECEPTORS AND THE ACUTE INHIBITION OF THYROID-STIMULATING HORMONE RELEASE

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**Abstract**

Injections of triiodothyronine (T₃) and thyroxine (T₄) into chronically hypothyroid rats were used to evaluate the contribution of intracellular T₄ to T₃ conversion to nuclear T₃ in pituitary, liver, and kidney, and to correlate the occupancy of pituitary nuclear T₃ receptors with inhibition of thyroid-stimulating hormone (TSH) release. Injection of a combination of 70 ng T₃ and 400 ng T₄/100 g body wt resulted in plasma T₃ concentrations of 45±7 ng/dl (mean±SD) and 3.0±0.4 μg/dl T₄, 3 h later. At that plasma T₃ level, the contribution of plasma T₃ to the nuclear receptor sites resulted in saturation of 34±7% for pituitary, 27±5% for liver, and 33±2% for kidney. In addition to the T₃ derived from plasma T₃, there was additional T₃ derived from intracellular monodeiodination of T₄ in all three tissues that resulted in total nuclear occupancy (as percent saturation) of 58±11% (pituitary), 36±8% (liver), and 41±11% (kidney), respectively. The percent contribution of T₃ derived from cellular T₄ added 41% of the total nuclear T₃ in the pituitary which was significantly higher than the contribution of this source in the liver (24%) or the kidney (19%). 3 h after intravenous injection of increasing doses of T₃, the plasma T₃ concentration correlated well with both the change in TSH and the nuclear occupancy, suggesting a linear relationship between the integrated nuclear occupancy by T₃ and TSH release rate. The contribution of intrapituitary T₄ to T₃ conversion to nuclear T₃ was accompanied by an appropriate decrease in TSH, supporting the biological relevance of nuclear T₃. Pretreatment of the animals with 6-n-propylthiouracil before T₄ injection decreased neither the nuclear T₃ derived from intrapituitary T₄ nor the subsequent decrease in TSH.

These results indicate that intracellular monodeiodination of T₄ contributes substantially to the nuclear T₃ in the pituitary of the hypothyroid rat, and suggest a linear inverse relationship between nuclear receptor occupancy by T₃ in the pituitary and TSH release rate. The data further indicate that T₄ to T₃ monodeiodination is considerably more important as a source of nuclear T₃ in the pituitary than in the liver and kidney. This provides a mechanism whereby the TSH secretion could respond promptly to a decrease in thyroid secretion (predominantly T₄) before a decrease in plasma T₃ would be expected to lead to significant metabolic hypothyroidism.

**Introduction**

There is considerable indirect evidence suggesting that interaction of triiodothyronine (T₃) with a specific

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1Abbreviations used in this paper: aGPD, α-glycerophosphate dehydrogenase; MBC, maximal binding capacity; N/P, nuclear/plasma ratio; PTU, 6-n-propylthiouracil; T₃, triiodothyronine; T₄, thyroxine; TSH, thyroid-stimulating hormone.
nuclear thyroid hormone receptor is a critical event in the mechanism by which thyroid hormones produce many of their effects (1-9). This event has specifically been demonstrated for induction of growth hormone synthesis and inhibition of prolactin production in growth hormone (GH-1) cells and for stimulation of hepatic mitochondrial α-glycerophosphate dehydrogenase (αGPD) and malic enzyme by T₃ in rats (10-13). We have recently observed (14) that there is a similar excellent chronological and quantitative correlation between nuclear occupancy by T₃ and subsequent suppression of thyroid-stimulating hormone (TSH) after intravenous injection of 70 ng/100 g body wt T₃ to hypothyroid rats. When 800 ng thyroxine (T₄)/100 g body wt was injected, the identical TSH response was correlated with the same nuclear T₃ content as after 70 ng T₃/100 g body wt. Negligible quantities of T₄ were found bound to the pituitary nuclei. These data suggested that nuclear occupancy by T₃ may be the initial event in the process leading to the acute suppression of TSH release in hypothyroid animals after thyroid hormone administration (14-16).

Furthermore, because negligible amounts of T₃ were found in the plasma during the first few hours after T₃ injection, the data suggested that there was a very active conversion of T₃ to T₂ in the pituitary with subsequent binding of the T₂ produced to the pituitary nuclei.

Because our laboratory has recently demonstrated (17) that some thyromimetic effects of T₄ do not appear to be associated with appropriate increases in serum T₃, the question of whether or not there is a significant quantity of intracellular T₄ monodeiodination to T₃ has arisen. The T₃ produced therefrom could then be bound to critical sites in the cell and produce hormone effects not manifested by an increase in the concentration of plasma T₃. The present studies were performed to determine the relative quantitative importance of this source of nuclear T₃ in three thyroid hormone-responsive tissues of hypothyroid rats.

METHODS

Male Sprague-Dawley rats were obtained from Zivic-Miller (Allison Park, Pa.) where they had thyroidectomy with parathyroid gland reimplantation 2-3 months before performance of all experiments. Hypothyroidism was established by plateau of weight gain, serum T₃ concentrations <10 ng/dl (normal rats, mean ± SD, 38 ± 19 ng/dl), and plasma or serum TSH concentrations >1,500 μU/ml (normal range, 50-150 μU/ml). ¹²⁵I-T₃ was obtained from Amersham Corp. (Arlington Heights, Ill.; 30 μCi/μg). ¹²⁵I-T₄ from Cambridge Nuclear Corp. (Billerica, Mass.; ≈5,500 μCi/μg). T₂ contamination of T₃ was <1% as estimated by affinity chromatography using specific T₂ antibody coupled to Sepharose (Pharmacia Fine Chemicals, Piscataway, N. J.) by a method we recently described (18). All injections were given to animals in a small aliquot of hypothyroid rat serum. In general, the volume of injection was <0.1 ml/10 g body wt. T₁/T₂ (both in free acid form) were obtained from Sigma Chemical Co. (St. Louis, Mo.). T₁ was <0.5% T₂ by weight as determined by radioimmunoassay (19, 20).

Estimation of plasma or serum T₃, T₂, and TSH. Plasma basal T₃ concentrations were measured by radioimmunoassay (19). The method previously used in our laboratory has been modified to use 25-μl aliquots of rat serum or plasma. Because of differences in nonspecific binding between rat and human serum, the standard curve included 25 μl of T3-free rat serum prepared using a CG-400 anion-exchange resin (Mallinckrodt Inc., St. Louis, Mo.). Plasma or serum T₄ was measured as described previously using human serum in the standard curve (20). Serum TSH was assayed using reagents provided by the Pituitary Hormone Distribution Program of the National Institute of Arthritis, Metabolic, and Digestive Diseases using the modifications for tracer preparation and purification which we have recently described (15). All other estimates of plasma or serum T₃ and T₄ concentrations were based on the specific activity of injected hormones (see below).

Preparation of cell nuclei. Nuclei from pituitary, hepatic, and renal tissues were prepared by centrifugation through 2.4 M sucrose, 3 mM MgCl₂ (21, 22). Before the 2.4 M sucrose centrifugation, the 1,000-g pellet was washed once with 0.32 M sucrose, 3 mM MgCl₂ containing 0.5% Triton X-100. Also, the 2.4 M sucrose nuclear pellet was washed once with 0.14 M unbuffered NaCl to remove loosely bound protein. This procedure minimized the nonspecific binding and decreased the specifically bound T₃ by <10% (9.5±1.0%, SD). The purity of nuclear preparations was verified by phase-contrast microscopy. In general, 2-g aliquots of liver and both kidneys were used for analyses. DNA was measured by Burton's method (23) modified by Giles and Myers (24). The yield of pituitary nuclear DNA averaged 6±1.4 (mean ± SD, n = 15) mg/g wet wt with recoveries ranging from 75 to 83%. Pituitary glands weighed from 8 to 12 mg. Hepatic nuclear DNA was 2.0±0.3 mg/g wet wt (recovery of 50±10%), and kidney nuclear DNA was 3.9±0.7 mg/g wet wt (recovery 58±10%).

Determination of the quantity of trapped plasma in pituitary tissues. The contribution of trapped plasma to tissue T₃ radioactivity was negligible in agreement with previous reports (25). In T₄-injected animals, however, the contamination by plasma ¹²⁵I-T₄ was significant. In studies of pituitary T₃ and T₄ content, the trapped plasma volume was evaluated by simultaneous injection of ¹²⁵I-bovine serum albumin labeled in our laboratory (14, 25). The contribution of plasma T₃ to pituitary nuclear radioactivity was shown to be eliminated by perfusion of the intact animals with 30 ml of cold saline (retrograde) through the abdominal aorta immediately after exsanguination. The plasma ¹²⁵I-T₄ still provided a considerable contribution to the extranuclear radioactivity. However, because the goal of the present studies was to evaluate the intracellular T₂ (not T₃), and this was not significantly influenced by trapped plasma, this perfusion technique was used throughout. This allowed us to inject ¹²⁵I-T₁ and ¹²⁵I-T₄ simultaneously. Our previous studies have shown that from 0 to 10% of nuclear radioactivity in the pituitary gland was T₂ 2-4 h after ¹²⁵I-T₁ administration (14).

Specificity of nuclear thyroid hormone binding. The specificity of nuclear binding was assessed in pilot experiments. These showed that in the nuclei from pituitary, liver, and kidney, the binding of tracer T₃ was decreased to <2.5% of its value by simultaneous injection of 20 μg T₂/100 g body wt. In similar experiments performed with ¹²⁵I-T₁-injected animals, the pituitary nuclear radioactivity was reduced to <1.5% of the value found with tracer T₃ alone by injection of 20 μg of T₃. In liver and kidney, injection of 20 μg T₃ with
$^{125}$I-$T_3$, led to a reduction of total radioactivity to 37 and 30%, respectively, of the control values, but chromatographic analysis of the nuclear radioactivity showed that this was virtually all $T_4$, i.e., all the $^{125}$I-$T_3$ was specifically bound to limited capacity binding sites. The $^{125}$I-$T_4$ found in the nuclear preparation under these circumstances presumably represents contamination of the nuclei with plasma and/or cytosol. Assuming that this did represent plasma contamination, it corresponded to 0.25 μl of plasma/mg DNA in the liver (0.52 μl/g wet wt) and 0.12 μl/mg DNA in the kidney (0.39 μl/g wet wt).

Identification of labeled compounds in nuclei, plasma, and injected $^{125}$I-$T_4$. Radioactivity was extracted from nuclei using acid-butanol as previously described (14). $T_3$ was separated from $T_4$ and identified by paper chromatography in tertiary amyl alcohol/hexane/2-N NH$_4$OH (6:1:5), as we have previously described (26, 27). Nuclear radioactivity was also chromatographed in n-butanol saturated with 2 N NH$_4$OH in some studies. The locations of $^{125}$I-$T_3$ and $^{125}$I-$T_4$ were determined by staining unlabeled $T_3$ and $T_4$ (which had been added to the origin) with diazotized sulfanilic acid. $^{125}$I-$T_4$, either injected simultaneously or added at the beginning of the extraction procedure, was used as an additional marker and to monitor recovery.

2–3 h after $^{125}$I-$T_3$ injection, the plasma $^{125}$I-$T_3$/$^{125}$I-$T_4$ ratio was very high (150–200:1). Accurate determination of the $^{125}$I-$T_3$ present required prior elimination of most of the $^{125}$I-$T_3$ because chromatographic separation of $T_3$ and $T_4$ on paper generates 0.6–0.8% of the $T_4$ as $T_2$ (27). Elimination of $T_4$ was achieved by extracting 100 μl of plasma with specific anti-$T_3$ antibody conjugated to Sepharose as has been described previously (18). In the washed Sepharose pellet, $^{125}$I-$T_3$ was reduced to <1% of the original quantity while >90% of the $T_4$ was retained as judged by the $^{125}$I-$T_4$ present (Appendix). The anti-$T_3$-antibody-Sepharose conjugate was then extracted with methanol:2 N NH$_4$OH (90:10) and chromatographed on paper in tertiary amyl alcohol:hexane:2-N NH$_4$OH as above. The contribution of artificial $T_3$ to $T_4$ conversion during chromatography to the measured $T_3$ was therefore negligible. The center of the $^{125}$I-$T_3$ peak on paper chromatography was used for quantitation of $^{125}$I-$T_3$. The fraction of the original $^{125}$I-$T_3$ present in this area was assessed by comparison with standards appropriately corrected for geometry and counting efficiency. The $^{125}$I-$T_4$ originally present could then be estimated from this $^{125}$I-$T_3$ recovery figure.

Inasmuch as the goal of the study was to estimate what fraction of nuclear $^{125}$I-$T_3$ was derived from intracellular $T_3$ to $T_4$ conversion, that portion of nuclear $^{125}$I-$T_3$ coming from plasma had to be quantitated and subtracted. The sources of plasma $^{125}$I-$T_3$ were two: one was a fraction of the $^{125}$I-$T_4$ contaminating the injected $^{125}$I-$T_3$ still present in plasma, and the second was $^{125}$I-$T_4$ derived from intracellular monodeiodination of $^{125}$I-$T_4$ which had entered the plasma compartment. Because $^{125}$I-$T_3$ was injected simultaneously with $^{125}$I-$T_4$, in most experiments, and because the percent contamination of each $^{125}$I-$T_3$ tracer with $T_3$ was known, it was possible to determine the relative contribution of each of these two sources to the plasma $^{125}$I-$T_3$ present. At 2 h after injection of $^{125}$I-$T_3$, >95% of the $^{125}$I-$T_3$ was derived from contamination (14); at 3 h, contaminating $^{125}$I-$T_3$ accounted for >40% of the plasma $^{125}$I-$T_3$. To determine what fraction of the nuclear $^{125}$I-$T_3$ was derived from the total plasma $^{125}$I-$T_3$, the simultaneously injected $^{125}$I-$T_3$ was used to determine the nuclear $^{125}$I-$T_3$ (counts per milligram DNA) to plasma $^{125}$I-$T_3$ (counts per milliliter) ratio. The total plasma $^{125}$I-$T_3$ (counts/milliliter) × $^{125}$I-$T_3$ nuclear/plasma ratio gave the number of $^{125}$I-$T_3$ (counts per milligram DNA) present in the nucleus that was derived from plasma $^{125}$I-$T_3$. These counts were subtracted from the total nuclear $^{125}$I-$T_3$ and the locally generated $T_3$ was estimated as follows: nuclear $^{125}$I-$T_3$ total $^{125}$I-$T_3$ × 2 x dose of $T_4$ (nanograms) × 651/777 based on previous considerations (14). A complete example of these calculations is presented in the Appendix.

### Specific experimental protocols

Assessment of the maximal nuclear $T_3$-binding capacity (MBC). MBC was measured in vivo in hypothyroid rats using an isotopic approach (3). In previous experiments it was found that from 2 h on after the injection of 70 ng $T_3$/100 g body wt the nuclear/plasma (N/P) ratio of $T_3$ was maximal and constant representing equilibrium of $T_3$ between receptor sites and plasma (14). Thus, plasma $T_3$ concentration is proportional to the concentration of $T_3$ to which the nuclear binding sites are exposed in animals with negligible plasma $T_4$ concentrations. Therefore, the bound $T_3$ is in rapid exchange with the free $T_3$ around the receptor, and between this $T_3$ pool and plasma. This type of equilibrium is a requisite for analysis of the nuclear $T_3$ content by isotopic techniques (28). 3 h was thus chosen as a reliable time-point mainly because a constant N/P ratio was clearly established and because it allowed enough time for plasma TSH to reflect the final result of integrated changes in the rate of TSH release. After suitable pilot experiments, groups of three hypothyroid rats were injected intravenously with $^{125}$I-$T_3$ containing increasing doses of unlabeled $T_3$. The doses used were 19, 70, 210, and 630 ng/100 g body wt. An additional dose of 20 μg/100 g body wt was injected into another group to allow calculation of nondisplaceable (nonspecific) binding. At 3 h after injection, plasma, pituitary, liver, and kidney tissue was obtained, and nuclei were prepared as described. Plasma samples were obtained at time 0, and TSH measured in the same assay (using $^{125}$I-TSH) as those samples obtained at the time the animals were sacrificed.

Based on the principles of reversible binding, and the law of mass action, the reaction of $T_3$ with nuclear receptor (N) can be viewed as:

$$[T_3] + [N] \xrightarrow{k_1} [NT_3], \quad (1)$$

and at equilibrium

$$K_d = \frac{[T_3][N]}{[NT_3]}, \quad (2)$$

where $N$ represents the number of unoccupied sites and $NT_3$ the number of occupied sites or bound $T_3$, and $K_d$ the dissociation constant or half-maximal saturation concentration of free $T_3$ at the receptor. Because the in vivo system is in a rapid exchange at equilibrium, and assuming no rapid variations of dialyzable fraction of the hormone, the total plasma $T_3$ concentration that saturates half of the sites is proportional to the concentration of free hormone around the receptors so that $K_d$ can be replaced by $[T_3]_{50}$. On the other hand, since $N$ is unknown and the goal is to know the total number of sites, i.e. the MBC, $N$ can be replaced by $[MBC - T_3]$. Substitution in Eq. 2 yields the following expression:

$$[T_3]_{50} = \frac{[T_3][MBC - NT_3]}{[NT_3]} \cdot \frac{[T_3]}{[T_3]_{50}}, \quad (3)$$

which on rearrangement gives a linear equation:

$$NT_3 = MBC - \frac{[T_3]_{50} \cdot NT_3}{[T_3]}, \quad (4)$$

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Thus the experimental data can be fitted to a straight line with the y intercept being the MBC and the slope the halfmaximal saturation plasma concentration (29). Further rearrangements of the above give

\[
\frac{N_{T_3}}{MBC} = \frac{[T_3]}{[T_3] + [T_3]_{50}},
\]

which allows the estimation of the nuclear saturation at any plasma concentration.

Analysis of the correlations of nuclear T3 receptor occupancy and suppression of TSH release in hypothyroid rats. A plot of the plasma T3 and TSH concentrations 3 h after T3 injection and the fact that there is a short latency between thyroid hormone injection and TSH suppression (14, 15) suggested that a saturable system might be involved relating plasma T3 and the TSH release rate. If so, the data could then be fitted in the following equation as has been done for aGPD and malic enzyme (13):

\[
\frac{\Delta TSH}{\Delta MAX} = \frac{[T_3]}{[T_3] + [T_3]_{50}},
\]

where \(\Delta TSH\) is the decrease in TSH at 3 h after T3 and \([T_3]\) is plasma T3. \([T_3]_{50}\) is the plasma \([T_3]\) at 3 h after injection of a T3 dose which causes 50% decrease in TSH release. To determine the maximal attainable \(\Delta TSH(\Delta MAX)\), Eq. 6 can be written

\[
\frac{\Delta TSH}{\Delta MAX} = \frac{[T_3]}{[T_3] + [T_3]_{50}},
\]

and this can be rearranged to yield

\[
\Delta TSH = \Delta MAX - \frac{\Delta TSH}{[T_3]} \cdot [T_3]_{50}.
\]

Again the data could be fitted to this linearized relationship and \(\Delta MAX\) and \([T_3]_{50}\) can be obtained.

To compare the plasma T3 concentration at 3 h with both the fraction of maximal suppressible TSH and of nuclear occupancy, both \(N_{T_3}\) and \(\Delta TSH/\Delta MAX\) were plotted against the log of \([T_3]\). The \([T_3]_{50}\)s determining the shapes of the two curves were obtained by the least squares method for Eq. 4 and 7 for \(N_{T_3}\) and \(\Delta TSH\), respectively.

Effect of 6-\(n\)-propylthiouracil (PTU) on nuclear T3 derived from intracellular deiodination of T4. PTU was given to hypothyroid rats in a dose of 1 mg/100 g body wt 16 and 2 h before the experiments. Two groups of animals, control and PTU treated, were injected with 800 ng/100 g body wt \(^{131}\)I-T3 and killed 2 h later. Pituitary glands and livers were removed and analyzed for nuclear T3 as described. This dose of PTU has been shown previously to significantly decrease plasma T3 concentrations in euthyroid rats 14 and 24 h after injection, and to abolish most of the mitochondrial aGPD response of hypothyroid rats to 800 ng/100 g body wt T4/day for 12-13 days (17). Statistical analyses were performed by Student's t test on paired or unpaired samples as stated (30).

RESULTS

MBC for T3 in the nuclei of pituitary, liver, and kidney tissues from hypothyroid rats. The nuclear content of specifically bound T3 in pituitary, liver, and kidney tissues was significantly related to the plasma T3 concentration. When nuclear T3 was plotted against the N/P T3 ratio, a linear relationship was apparent with \(r = -0.90\) for pituitary (\(P < 0.001\)), -0.93 for liver (\(P < 0.001\)), and -0.87 for kidney (\(P < 0.001\); Fig. 1). The MBC were 0.96, 0.43, and 0.13 ng T3/mg DNA for pituitary, liver, and kidney, respectively (Fig. 1). In the same figure, the [T3]50 was 0.92, 0.98, and 0.66 ng T3/ml for pituitary, liver, and kidney, respectively. Both pituitary and liver nuclei have a lower apparent affinity for T3 than do kidney nuclei.

To emphasize the relationships between plasma T3 and nuclear T3 content, in Fig. 2 both variables were plotted for the three tissues examined. The dotted lines represent the 95% confidence limits of the regression coefficient ([T3]50). The curves are quite similar considering the dispersion obtained. The results indicate that over 90% of nuclear T3 saturation is obtained with plasma levels of 10 ng/ml, and one-half maximal occupancy is obtained with plasma T3 concen-

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**Figure 1** Nuclear T3 vs. the nuclear T3/plasma T3 ratio 3 h after injection of various doses of T3 to chronically hypothyroid rats. Lines were constructed by least squares regression analysis. Each point represents results of a single animal.

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nulations of 0.92 ng/ml in the pituitary, 0.98 in the liver, and 0.66 in the kidney. The bar graphs superimposed on this figure are discussed below.

Relative contribution of injected T4 to the nuclear T3 in hypothyroid animals given 70 ng/100 g body wt T3. When 400 ng/100 g body wt 123I-T4 was given together with 70 or 210 ng 123I-T3, the degree of nuclear saturation obtained for T3 alone was similar for all of these tissues (bar graph in Fig. 2). When the contribution of 123I-T3 derived from local 123I-T4 tissue conversion is considered, it can be appreciated that in the pituitary it raised the nuclear saturation beyond the expected 95% confidence limits, whereas in the liver and kidney the increments were within the expected experimental variation (although by paired t test at the 70 ng T3-400 ng T4 dose this contribution was significant for both tissues (Table I)). The plasma concentrations of T3 and T4, 3 h after intravenous injection of 70 ng T3 + 400 ng T4/100 g body wt were in the physiological range (0.45±0.07 ng T3/ml and 3.0±0.4 μg T4/dl, mean±SD) as estimated by the specific activity of injected isotopes.

The results of this experiment are analyzed in greater detail in Tables I–III. In Table I it is shown that at both T3 dose levels, 123I-T4 contributed more T3 to the pituitary nuclear T3 receptors than it did in the liver and kidney (P < 0.005). Thus the degree of saturation of the pituitary nuclei was increased from 34 to 58% by injection of 400 ng/100 g body wt of T4, whereas this source of T3 only results in an increase in saturation from 27 to 36% in the liver and from 33 to 41% in the kidney. All contributions of T4 to nuclear T3 are reduced proportionately when the dose of T3 given is trebled, suggesting that the T3 derived from these two sources can mix in an intracellular compartment before binding to the nuclear receptor.

An evaluation of the N/P T3 ratios in the three tissues is presented in Table II. The pituitary N/P ratio for T3 derived from plasma alone was 0.73±0.22 but when the contribution of T3 from T4 was added, this was significantly higher, 1.03±0.26 (P < 0.005). The increase in the N/P for liver nuclei due to T3 derived from T4 was also significant (P < 0.025) but modest (0.25–0.27), and there was no significant effect in the kidney.

Because the contribution of T4 to nuclear T3 from intracellular monodeiodination is likely to be dependent on the T4 concentration within the cell, and this in turn is related to plasma T4 concentration, it was of interest to evaluate the nuclear 123I-T3 to plasma 123I-T4 ratio under the same circumstances (Table III). This ratio, although smaller than the N/P T3 ratio by a factor of 100, is significantly higher for pituitary nuclei than it is for those of either liver or kidney at either dose of T3 given (P < 0.005). As expected, the nuclear T3/plasma T4 ratio decreased in all three tissues when the dose of T3 was increased, reflecting the limited binding capacity of the nuclear T3 receptors.

Correlation of TSH suppression with nuclear and plasma T3. Severely hypothyroid rats seemed suitable models to evaluate the quantitative relationships between nuclear T3 and TSH suppression because the component of nuclear T3 derived from local T4 to T3 conversion is negligible. Fig. 3 shows the correlation between ΔTSH and ΔTSH/[T3] as discussed in Methods. The maximal theoretical suppression obtained 3 h after the T3 injection was 95%, and the [T3]bio was 0.62 ng/ml (r = −0.91, P < 0.001).

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TABLE I
Source of Nuclear T₃ in Pituitary, Liver, and Kidney of Hypothyroid Rats 3 h after Intravenous ¹³¹I-T₃ (70 or 210 ng/100 g Body wt) and 400 ng/100 g Body wt ¹²⁵I-T₄

<table>
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<tr>
<th>T₃ from plasma</th>
<th>P values</th>
<th>T₄ from intracellular T₄ to T₃ monodeiodination</th>
<th>P values</th>
<th>Nuclear T₃ from both sources % of MBC</th>
<th>P values</th>
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<tr>
<td>T₃ in pituitary (P), liver (L), and kidney (K) % of MBC</td>
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<tr>
<td>Dose</td>
<td>Plasma hormone concentrations 3 h after dose</td>
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<td>T₃* T₄* L K</td>
<td>T₃* T₄* L K</td>
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<td>15</td>
<td>14</td>
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</tr>
</tbody>
</table>

* Estimated from the specific activity of the respective injected iodothyronine.
1 Pair t test.

Fig. 4 was generated by plotting the 3-h ΔTSH/ΔMAX (i.e. the fraction of maximal attainable suppression) and nuclear T₃ as a function of plasma T₃. The regression lines were obtained from Eqs. 4 and 7 (Figs. 1 and 3). The 95% confidence limit (dotted lines) of nuclear T₃ as a function of plasma T₃ is plotted as an index of the expected experimental variation. It can be appreciated that the plasma T₃ concentration at 3 h bears an almost superimposable relationship with both the nuclear occupancy and the maximal suppression of TSH obtained 3 h after each dose. 95% nuclear saturation was obtained with a plasma T₃ concentration of approximately 20 ng/ml, similar to that which produces 95% inhibition of TSH release.

Effect of the increment in nuclear T₃ provided by T₄ to the inhibition of pituitary TSH release. In Table IV are shown the biological effects of administration of 70 ng T₄/100 g body wt with or without 400 ng/100 g body wt T₄. The percent saturation of the nuclear T₃ receptors by T₄ from both plasma and intracellular T₄ to T₃ conversion is also presented. 70 ng T₄ alone resulted in 32% saturation of the nuclear T₃ receptors at 3 h after administration, and a 39% decrease in plasma TSH. Injection of T₄ 70 + T₄ 400 ng/100 g body wt resulted in a higher nuclear receptor occupancy of 58% (P < 0.025), with a concomitant reduction in plasma TSH equivalent to 61% of the basal TSH concentration (P < 0.025 compared with T₃ alone). At a dosage level of 210 ng T₄/100 g body wt, the nuclear T₃ saturation was 60% and the decrease in plasma TSH was equivalent to 77% of the basal TSH concentration, probably reflecting a higher previous nuclear occupancy as has been discussed (14). Addition of 400 ng T₄/100 g body wt did not result in a significantly higher nuclear saturation, nor in a further decrease in plasma TSH. However, changes at this level of nuclear T₃ and ΔTSH are more difficult to detect.

Effect of PTU pretreatment on the acute contribution of injected T₄ to pituitary and hepatic nuclear T₃. The results of T₄ injections given to control and PTU-treated hypothyroid rats are presented in Table V. In the pituitary, 800 ng T₄/100 g body wt increased nuclear T₃ 0.42 ng/mg DNA. This was identical to the quantity of nuclear T₃ derived from 800 ng T₄/100 g body wt in PTU-pretreated rats. Thus, the lack of effect of PTU pretreatment on T₄-induced TSH suppression was reflected in the absence of effect on nuclear T₃ as well. Under the acute conditions of the

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the maximal obtainable contribution of T3 to hepatic nuclear T3.

**DISCUSSION**

To analyze the relationship between nuclear content of T3 in the pituitary and the resulting suppression of TSH, the maximal T3-binding capacity of the pituitary nuclei must be known. Using an approach similar to that of Oppenheimer et al. (3, 4), we have observed that the MBC of the pituitary nuclei of hypothyroid rats is 0.96 ng T3/mg DNA, whereas it is 0.43 in hepatic nuclei and 0.13 in nuclei from kidney tissue (Fig. 1). The results for MBC and apparent Kd in hypothyroid animals differ slightly in some tissues (liver and kidney) from those previously reported in euthyroid animals (21), but these differences do not affect the conclusions of the present study. Because the T3 specifically bound to the nucleus either coming from plasma or from local T4 to T3 conversion can be fully displaced by an excess of cold T3, we may conclude that all the nuclear binding sites are available to both sources of T3. It follows that the approach of saturation analysis gives a good estimation of the MBC. How-

![Figure 3](image1)

**FIGURE 3** Δ Plasma TSH as a function of ΔTSH/[T3] ratio in hypothyroid animals given the quantities of T3 depicted in Fig. 13 h before analysis. Each point is the result of the response of a single animal. The maximum ΔTSH in the equation corresponds to the maximal obtainable suppression of TSH, and the [T3]50 is the plasma T3 concentration associated with the 50% decrease in plasma TSH concentration.

![Figure 4](image2)

**FIGURE 4** Pituitary nuclear saturation and maximal TSH suppression plotted as a function of plasma T3 concentration in chronically hypothyroid rats injected with various doses of T3 3 h before analysis of pituitary T3 and plasma TSH. The heavy line (C) is the curve describing nuclear saturation vs. plasma T3, and the dotted lines indicate the 95% confidence limits of the regression coefficient. The thin line (O) relates TSH suppression to plasma T3 in the same animals.

**TABLE III**

<table>
<thead>
<tr>
<th>Iodothyronine dose</th>
<th>Mean±SD</th>
<th>P values*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pituitary × 10⁻²</td>
<td>Liver × 10⁻²</td>
</tr>
<tr>
<td>T1 ng/100 g body wt</td>
<td></td>
<td></td>
</tr>
<tr>
<td>70</td>
<td>7.9±1.3</td>
<td>1.2±0.2</td>
</tr>
<tr>
<td>210</td>
<td>5.3±1.0</td>
<td>0.6±0.2</td>
</tr>
</tbody>
</table>

* P (unpaired t test).
ever, as discussed below, this approach may not give an accurate estimate of the degree of saturation at endogenous levels of plasma T₃ in euthyroid animals.

For reasons stated in the Introduction, we have speculated that a local mechanism for generation of T₃ from T₄ should be quantitatively more important as a source of nuclear T₃ in the pituitary than in the liver of the rat. The results in Fig. 2 and Tables I–III indicate that when T₄ is given together with T₃, the increment in the pituitary nuclear T₃ content is three- to fourfold that found in the liver and kidney. Tables I–III show in detail the relative importance of this mechanism for T₄ deiodination in these three tissues. Several "artificial" explanations can be readily excluded for the presence of T₃ in the nuclei of these tissues soon after T₄ injection. The quantity of locally derived T₃ in the nuclei is determined from the net ¹²⁵I-T₃ present over that which can be accounted for by

---

### TABLE IV

<table>
<thead>
<tr>
<th>Dose</th>
<th>T₃</th>
<th>T₄</th>
<th>n</th>
<th>Nuclear T₃</th>
<th>Decrease in plasma TSH</th>
</tr>
</thead>
<tbody>
<tr>
<td>ng/100 g body wt</td>
<td></td>
<td></td>
<td></td>
<td>(mg saturation)</td>
<td>(% of basal TSH concentration)</td>
</tr>
<tr>
<td>70</td>
<td>0</td>
<td>3</td>
<td>32±3</td>
<td>39±1</td>
<td></td>
</tr>
<tr>
<td>70</td>
<td>400</td>
<td>4</td>
<td>58±11</td>
<td>61±10</td>
<td></td>
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<tr>
<td>P</td>
<td></td>
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<td>&lt;0.025</td>
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<tr>
<td>210</td>
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<td>3</td>
<td>60±4</td>
<td>77±4</td>
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<td>4</td>
<td>81±15</td>
<td>78±9</td>
<td></td>
</tr>
<tr>
<td>P</td>
<td>NS</td>
<td>NS</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Mean±SD.

---

### TABLE V

<table>
<thead>
<tr>
<th>Pituitary</th>
<th>Liver</th>
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<tr>
<td>Control</td>
<td>PTU</td>
</tr>
<tr>
<td>Nuclear T₃, ng/mg DNA</td>
<td></td>
</tr>
<tr>
<td>0.42±0.04</td>
<td>0.42±0.08</td>
</tr>
<tr>
<td>Plasma TSH response, % of basal concentration</td>
<td></td>
</tr>
<tr>
<td>67.5±6.1</td>
<td>59.6±15.3</td>
</tr>
</tbody>
</table>

Mean±SD.

* Hypothyroid rats received 800 ng/100 g body wt T₄, with or without intraperitoneal injections of 1 mg/100 g PTU 16 and 2 h previously. Animals were killed 2 h after T₄ injection.

the small quantities of ¹³¹I-T₃ present in plasma. Therefore, the accurate determination of plasma ¹³¹I-T₃ is quite critical. Inasmuch as the ¹³¹I-T₃ can be followed throughout the identification and quantitation procedure using the injected ¹³¹I-T₃ (see Appendix for calculations), we are unable to postulate a mechanism by which the recoveries of the two T₃ molecules, labeled with different isotopes, could be different. Because the ¹³¹I-T₃ N/P ratios determined for at least pituitary and liver are quite similar to those reported by other laboratories (21), it seems unlikely that both could be underestimated. If, in some way, plasma ¹³¹I-T₃ were underestimated, it would result in an overestimation of locally produced nuclear T₃ from T₄. This overestimation would be greatest in pituitary nuclei due to the higher N/P ratio present in this tissue in comparison with liver and kidney. It would be impossible to interpret the present experiments accurately without a precise method for specific isolation of small amounts of ¹³¹I-T₃ from the large quantities of plasma ¹³¹I-T₃, such as the T₃-antibody-Sepharose conjugate technique used here (18). The possibility of a rapid and disproportionately greater ¹²⁵I-T₃ monodeiodination (with a subsequent peak of plasma ¹³¹I-T₃) on the first passage of T₄ through the tissues can also be eliminated. Because the plasma ¹³¹I-T₃ derived from this reaction would disappear at the same rate as the initially injected ¹³¹I-T₃, we should have seen fivefold higher quantities of ¹³¹I-T₃ than were actually observed 2–3 h after ¹³¹I-T₄ injection. As the unlabeled T₄ contained even less contaminating T₃ than did the tracer (<1% as opposed to <0.5%), no significant physiological effect can be attributed to this source.

The data demonstrating an apparently rapid rate of T₄ to T₃ conversion in pituitary tissue is at variance with recent in vitro studies. These have indicated that the rat kidney and liver are the most active on a weight basis (31), and, in fact, in vitro T₄ to T₃ conversion has not been previously demonstrated in pituitary tissue (32) despite suggestive in vivo data in other species (33–36). However, using improved technology (18), we have recently observed T₄ to T₃ conversion in pituitary homogenates at rates at least similar to the rat liver on a weight basis under certain in vitro circumstances.

Of the total nuclear T₃ in animals receiving 70 ng T₃ and 400 ng T₄, 41% is derived from local T₄ monodeiodination in pituitary, whereas only 23 and 19% of nuclear T₃, respectively, is derived from this source in liver and kidney. In rats given 210 ng T₃ plus 400 ng T₄/100 g body wt, the relative contribution of T₄ is less, but again, much higher in the pituitary than in the liver. Correspondingly, the N/P

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² Silva, J. E., T. E. Dick, and P. R. Larsen. Manuscript submitted for publication.
Although the biological ratio for T₃ after T₄ is markedly higher for pituitary nuclei than it is for those of the liver and kidney. Although the biological significance of this T₃ is apparent in terms of pituitary nuclei, because it is associated with TSH suppression (14 and Table IV), there are no known rapid effects of T₃ on the hepatic or renal tissues to allow early estimates of the possible physiological significance of the T₃ derived from T₄ in these tissues. Thus, despite the in vitro capacity of hepatic and renal tissues to produce T₃ from T₄, the T₃ generated does not appear to contribute substantially to the nuclear T₃ in these tissues.

Because the apparent affinity of nuclear receptors for T₃ has been shown in vitro to be approximately the same in all tissues (21, 37), a fact we have also confirmed in vivo for the pituitary and liver in the present studies, the higher nuclear T₃ content after T₄ in the pituitary has to be explained on a different basis. The higher MBC of the pituitary (0.96 ng T₃/mg DNA) compared to the liver (0.43 ng T₃/mg DNA) cannot account for the difference either because expressing nuclear T₃ derived from T₄ as a percent of the MBC, i.e., in terms of saturation, the contribution of T₃ to nuclear T₃ in the pituitary is still significantly higher (threefold) than in the liver.

It can also be proposed that local T₄ to T₃ conversion and subsequent binding to the nucleus reaches equilibrium at very different intervals after T₄ injection in the tissues studied. This possibility also seems unlikely because it has been estimated that 10 min are required for establishment of an equilibrium between tissue T₄ and plasma T₄ in rat pituitary, liver, and kidney (25, 38). We have found that the nuclear ²³¹I-T₃/plasma ²³¹I-T₄ ratio is about 20% higher at 3 h than at 2 h after injection of ²³¹I-T₄ in both pituitary and liver suggesting that neither tissue has reached equilibrium at 3 h. Therefore, calculation of nuclear T₃ from T₄ at 3 h does not overestimate (but could underestimate) the contribution of this process to the nuclear T₃. That this is, in fact, an underestimate is suggested by our recent results in euthyroid rats indicating that the peak of the ratio of intracellularly derived nuclear ²³¹I-T₃ to plasma ²³¹I-T₄ is not attained until 16–18 h after ²³¹I-T₄ injection.³

We have demonstrated that nuclear binding of the T₃ derived from T₄ to T₃ conversion can be completely blocked by 20 μg T₃/100 g body wt (14). These results and those of the present study showing a decrease in the fraction of nuclear T₃ derived from T₄ as plasma T₃ concentration increases (Table II) indicate that the nuclear pools of T₃ derived from both sources are miscible. We would speculate that the relative proportions of each source of T₃ in the nuclei of the various tissues would be determined by the plasma concentrations of T₄ and T₃, the transfer rates of these hormones into the cell, the rate of intracellular T₄ to T₃ conversion in each tissue, and the characteristic subcellular distribution of the T₃ derived from both sources in that particular tissue. The weight of each of these factors in determining the sources of nuclear T₃ is not known and is currently under investigation.⁴ Hepatic and renal tissues have been demonstrated to be quite active in T₄ monodeiodination, and yet our studies indicate that relatively small amounts of this locally produced T₃ are bound to the nuclear T₃ receptor. This is certainly in part influenced by the fact that <10% of total tissue T₃ is found in the nucleus in these tissues, whereas more than 40% of pituitary T₃ is nuclear (21).

There is a striking correlation between plasma T₃ 3 h after injection with the nuclear occupancy and with TSH suppression (Fig. 4). Three facts allow us to extend this observation to say that there is an inverse linear relationship between the nuclear occupancy by T₃ and the rate of TSH release. First, there is a short delay (<1 h) between nuclear occupancy by T₃ and changes of TSH release rate (14, 15). Second, because of the short half-life of TSH (30 min), decreases in TSH release rate are rapidly reflected in the plasma TSH concentration. Third, the nuclear receptor occupancy by T₃ at 3 h follows closely the time of the peak nuclear occupancy, and this time is not affected by increasing T₃ doses over the range used here (14, 28). Therefore, the integrated nuclear occupancy from 0–3 h is linearly related to the nuclear occupancy at 3 h after T₃ injection. These considerations provide strong support for a linear relationship between nuclear T₃ receptor occupancy and the rate of TSH release and suggest that the correlation apparent in Fig. 4 is more than fortuitous.

The slight deviation to the left observed in the TSH suppression curve (Fig. 4) ([T₃]₀₀₀ 0.62 ng/ml for TSH suppression as opposed to the [T₃]₀₀₀ of 0.92 ng/ml for nuclear occupancy) can be accounted for by the latency between changes in nuclear occupancy and in the rate of release of TSH (14). Thus, the plasma TSH concentration at 3 h reflects the nuclear occupancy ≈60 min previously. For the range of T₃ doses examined, this is ≈20% higher at 2 than at 3 h. These data add further support to the concept that T₃ effects on TSH release, like those on growth hormone stimulation and prolactin suppression in pituitary cell cul-

³ Silva, J. E., T. E. Dick, and P. R. Larsen. Manuscript submitted for publication. These data showed that in euthyroid rats the ratio of nuclear T₃ from local T₄ monodeiodination to plasma T₄ had equilibrated in pituitary, liver, and kidney by 16–18 h. At that time, the relative contributions of local T₄ monodeiodination to nuclear T₃ showed the pattern observed in the present experiments.

turies (5, 11), and α-GPD and malic enzyme induction in liver tissue (12, 13) are initiated by events occurring in the nucleus. As was demonstrated in our earlier report (14) and confirmed above, the acute suppression of TSH after T₄ can also be correlated with the quantity of pituitary nuclear T₃, further supporting this hypothesis. However, as with the data for α-GPD and malic enzyme and growth hormone stimulation and prolactin suppression, the fact that two events are correlated in time and magnitude cannot be used as evidence of causality because the precise nature of the interrelationship between the two events is not known.

As mentioned, hepatic and renal tissues are thought to be the major sources of extrathyroidal T₃ production from T₄. In euthyroid rats or T₄-maintained hypothyroid rats, this deiodination process has been shown to be partly sensitive (~70%) to inhibition by PTU in doses similar to those used in these experiments (17, 39). However, we have repeatedly failed to demonstrate inhibition of acute local pituitary T₄ to T₃ conversion in PTU-treated animals. The explanation for this is not clear. It is possible that the mechanism for converting T₄ to T₃ in the hypothyroid pituitary is less sensitive to acute inhibition by PTU. Alternatively, PTU may not penetrate pituitary tissue, or may be inactivated therein. Whatever the reason, if our hypothesis that nuclear T₃ receptor binding is involved in suppression of TSH release is correct, then the contribution of T₄ to pituitary nuclear T₃ could not be depressed by PTU pretreatment, because this does not affect the acute TSH response to T₄ as we have shown previously (17) and confirmed in the present study. The lack of a significant decrease in hepatic nuclear T₃ due to local T₄ monodeiodination in acutely PTU-treated hypothyroid rats could be explained in a similar way and by the fact that the fraction of the T₃ derived from T₄ that binds to liver nuclei is so small. The often demonstrated inhibition of induction of hepatic αGPD associated with chronic T₄ + PTU administration to hypothyroid rats is better explained by a decrease in the nuclear T₃ fraction derived from plasma T₃ (substantially depressed under these circumstances) than by a decrease in the nuclear T₃ derived directly from intracellular T₄ monodeiodination. Likewise, the small increment in TSH or in thyroid releasing hormone responsiveness observed in PTU-treated euthyroid or hypothyroid rats and man seems better explained by the demonstrated decrease in serum T₃ than by inhibition of T₄ to T₃ conversion in the pituitary (17, 40–42). More data are needed in euthyroid rats to exclude completely a PTU effect on intrapituitary T₄ to T₃ conversion under these circumstances.

If local pituitary T₄ to T₃ conversion is an important source of pituitary nuclear T₃, if there is a linear relationship between nuclear T₃ receptor occupancy and the rate of TSH release, and if in the euthyroid rat the TSH secretion rate is only ~10% of the maximal attainable in hypothyroidism (15), one must consider the possibility that in euthyroid rats, the pituitary nuclear receptor sites are saturated to a significantly greater extent than has been estimated previously (21). The latter estimate of 48% saturation of pituitary nuclear receptors was determined by saturation analysis using increasing doses of 125I-T₃. This methodological approach is adequate to calculate the MBC because all the receptor sites are available to plasma T₃ as demonstrated by the full blockade of tracer T₃ binding (from plasma T₃ or tissue T₄) by an excess of cold T₃ (14). However, because the intrinsic in vivo affinity of these sites is unknown and the degree of the dilution of the T₃ entering the cell from plasma by the T₃ derived from tissue T₄ is also unknown, this method might considerably underestimate nuclear T₃ saturation in the pituitary, though probably only modestly underestimate that in the liver and kidney. An excellent example of this is seen in Fig. 2 where, using plasma T₃ concentrations and the N/P T₃ ratio alone, one would have estimated that pituitary nuclear receptors were only 34% saturated. The true pituitary nuclear saturation at this time was 58% due to the additional contribution of the T₃ derived locally from tissue T₄. This significant contribution was found in association with a plasma T₄ concentration in the physiological range for the rat (3 μg/dl). Using techniques similar to those of the present study, we have recently estimated that pituitary nuclear receptor saturation is ~78% in euthyroid rats in agreement with this hypothesis. One-half of this nuclear T₃ was derived from intrapituitary T₄ monodeiodination.

The above considerations have important physiological consequences. We have shown that a decrease in saturation of nuclear binding sites is associated with an increase in the acutely suppressed plasma TSH in hypothyroid animals (14). Presumably, a decrease in pituitary nuclear saturation due to a decrease in T₃ derived locally from T₄ would be similarly followed by an increased TSH release from the euthyroid pituitary. Thus, a decrease in plasma T₄ alone could lead to an increase in TSH release. Our present studies suggest that the contribution of T₄ to nuclear T₃ in the liver and kidney is so small that significant nuclear desaturation would not occur in these tissues as a result of a modest decrease in plasma T₄ alone. This could explain the circumstances in the iodine-deficient rat where an apparently euthyroid state is associated with a low plasma T₄, normal plasma T₃, and an elevated TSH (17, 43). Similar arguments can be applied to explain why patients with endemic goiter, a high plasma TSH, and normal plasma T₃ can appear euthyroid as well as why patients with early thyroid dysfunction with reduced plasma T₄, normal plasma T₃, and elevated TSH.
are often asymptomatic in metabolic terms (44–47). It would appear that the presence of a system in the thyrotroph responsive to decreases in either plasma T₃ or T₄ would provide maximum protection against the onset of metabolic hypothyroidism in tissues such as the liver and kidney whether the threat to the euthyroid state is a result of primary thyroid disease or iodine deficiency.

APPENDIX

A sample calculation of nuclear T₃ in animals given ¹³¹I-T₃ and ¹²⁵I-T₄ is given below. Suitable corrections for ¹³¹I appearing in the ¹²⁵I spectrometer window and changes in geometry have been performed where indicated. The nuclear T₃ content was calculated based on the specific activity of injected iodothyronines as follows:

Because the nuclear ¹³¹I was >95% ¹³¹I-T₃, the nuclear T₃ from injected T₃ was:

\[
\frac{\text{Nuclear } ¹³¹I \text{ counts}}{\text{Total injected } ¹³¹I} \times \frac{1}{\text{mg DNA}} \times \text{dose } T₃ (\text{ng}) = \text{ng T₃/mg DNA}. \tag{1}
\]

The ¹³¹I-T₃ in the nucleus was determined by paper chromatography using ¹³¹I-T₃ in the nucleus as a recovery and localization standard (Methods).

Nuclear ¹³¹I-T₃ (percent of ¹²⁵I-T₄ dose/mg DNA)

\[
= \frac{¹³¹I-T₃ \text{ counts selected area}}{¹²⁵I-T₄ \text{ counts selected area}} \times \frac{1}{\text{mg DNA}} \times \frac{100}{\text{total injected } ¹³¹I}.
\]

To deduct from the above ¹³¹I-T₃ that coming from the plasma, quantitation of the plasma ¹³¹I-T₃ content was required. The T₄-Ab Sepharose conjugate (T₄-Ab-S) was used to reduce the ¹³¹I-T₄ in plasma as described (14, 18). As an example in one rat:

<table>
<thead>
<tr>
<th>counts/100 μl plasma</th>
</tr>
</thead>
<tbody>
<tr>
<td>acid precipitable</td>
</tr>
<tr>
<td>689,598 counts/ min (100%)</td>
</tr>
<tr>
<td>T₄-AB-S</td>
</tr>
<tr>
<td>7,240 counts/ min (0.21%)</td>
</tr>
<tr>
<td>T₃ area on paper</td>
</tr>
<tr>
<td>2,870 counts/ 10 min</td>
</tr>
</tbody>
</table>

Plasma ¹³¹I-T₃ (percent ¹³¹I dose/ml)

\[
\times \frac{2,870 \times 10}{0.28} \times \frac{1}{\text{Total } ¹³¹I \text{ counts injected}} \times 100 = 0.0039\% ¹³¹I/ml
\]

Since the N/P ¹³¹I was known, the contribution of plasma ¹³¹I-T₃ to the nucleus could be determined. In the above rat,

\[
\text{N/P } ¹³¹I-T₃ = \frac{3,379 \text{ (counts/10 min per nucleus)}}{0.054 \text{ (mg DNA)}} + 102,420 \text{ (counts/10 min per ml)} = 0.61.
\]

The N/P for the 20 μg/100 g-injected rat was 0.016±0.003 (nonspecific binding). Therefore, the nuclear ¹³¹I-T₃ from plasma ¹³¹I-T₃ was:

\[
(0.61 - 0.016) \times 0.0039\%/\text{ml} = 0.0023\% \text{ dose/mg DNA}.
\]

In this example, the total nuclear ¹³¹I-T₃ was 0.018% dose/mg DNA. Therefore the ¹³¹I-T₄ derived from local T₄ monodeiodination was:

\[
0.018 - 0.0023 = 0.0157\% \text{ dose/mg DNA}.
\]

To convert to nanograms T₃:

\[
0.0157 \times \frac{1}{100} \times 2 \times \frac{651}{777} \times 400 (\text{ng}) T₄ \times \frac{315 (g)}{100} = 0.33 (\text{ng T₃/mg DNA}).
\]

To allow estimates of the dispersion of the data, the detailed calculation of pituitary nuclear T₃ in the experiment in which 70 ng ¹³¹I-T₃ plus 400 ng ¹²⁵I-T₄ was given is presented as follows: (results are the mean ± (SD) of four rats): Injected ¹³¹I counts, 61,688,400 cpm/100 g body wt; Injected ¹³¹I counts, 657,800 cpm/100 g body wt; Animal weight, 340±17 g; Pituitary nuclear DNA, 0.047±0.009 mg; Total N ¹³¹I-T₃, 11,063±2,210 (counts/10 min); Total N ¹³¹I-T₃, 1,470±372 (counts/10 min); Total plasma trichloroacetic acid-precipitable counts at 3 h: ¹³¹I, 45,199,090±1,852,365 counts/10 min per ml; ¹¹¹I, 61,326±9,015 counts/10 min per ml; Plasma ¹³¹I-T₃: (a) from contamination, 63,446±10,881 counts/10 min per ml (1% of ¹³¹I-T₃ was ¹³¹I-T₄), (b) from T₄ to T₃ in extrapituitary tissues, 98,578±31,992 counts/10 min per ml (0.15 ng/ml), Total, 161,500±39,905 counts/10 min per ml. ¹³¹I-T₃, N/P ratio, 0.51±0.12; Total nuclear ¹³¹I-T₃, 283,150±50,337 counts/10 min per mg DNA; From plasma, 82,847±26,996 counts/10 min per mg DNA; Local T₄ to T₃ conversion, 201,350±41,948 counts/10 min per mg DNA, (9.6±2)×10⁻³ ¹²⁵I dose/mg DNA, 0.23±0.05 ng T₃/mg DNA; From injected T₃, 0.33±0.08 ng T₃/mg DNA.

ACKNOWLEDGMENTS

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