

**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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# 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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**ABSTRACT** Injections of triiodothyronine ( $T_3$ ) and thyroxine ( $T_4$ ) into chronically hypothyroid rats were used to evaluate the contribution of intracellular  $T_4$  to  $T_3$  conversion to nuclear  $T_3$  in pituitary, liver, and kidney, and to correlate the occupancy of pituitary nuclear  $T_3$  receptors with inhibition of thyroid-stimulating hormone (TSH) release. Injection of a combination of 70 ng  $T_3$  and 400 ng  $T_4$ /100 g body wt resulted in plasma  $T_3$  concentrations of  $45 \pm 7$  ng/dl (mean  $\pm$  SD) and  $3.0 \pm 0.4$   $\mu$ g/dl  $T_4$  3 h later. At that plasma  $T_3$  level, the contribution of plasma  $T_3$  to the nuclear receptor sites resulted in saturation of  $34 \pm 7\%$  for pituitary,  $27 \pm 5\%$  for liver, and  $33 \pm 2\%$  for kidney. In addition to the  $T_3$  derived from plasma  $T_3$ , there was additional  $T_3$  derived from intracellular monodeiodination of  $T_4$  in all three tissues that resulted in total nuclear occupancy (as percent saturation) of  $58 \pm 11\%$  (pituitary),  $36 \pm 8\%$  (liver), and  $41 \pm 11\%$  (kidney), respectively. The percent contribution of  $T_3$  derived from cellular  $T_4$  added 41% of the total nuclear  $T_3$  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  $T_3$ , the plasma  $T_3$  concentration correlated

well with both the change in TSH and the nuclear occupancy, suggesting a linear relationship between the integrated nuclear occupancy by  $T_3$  and TSH release rate. The contribution of intrapituitary  $T_4$  to  $T_3$  conversion to nuclear  $T_3$  was accompanied by an appropriate decrease in TSH, supporting the biological relevance of nuclear  $T_3$ . Pretreatment of the animals with 6-*n*-propylthiouracil before  $T_4$  injection decreased neither the nuclear  $T_3$  derived from intrapituitary  $T_4$  nor the subsequent decrease in TSH.

These results indicate that intracellular monodeiodination of  $T_4$  contributes substantially to the nuclear  $T_3$  in the pituitary of the hypothyroid rat, and suggest a linear inverse relationship between nuclear receptor occupancy by  $T_3$  in the pituitary and TSH release rate. The data further indicate that  $T_4$  to  $T_3$  monodeiodination is considerably more important as a source of nuclear  $T_3$  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  $T_4$ ) before a decrease in plasma  $T_3$  would be expected to lead to significant metabolic hypothyroidism.

## INTRODUCTION

There is considerable indirect evidence suggesting that interaction of triiodothyronine ( $T_3$ )<sup>1</sup> with a specific

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<sup>1</sup> Abbreviations used in this paper:  $\alpha$ GPD,  $\alpha$ -glycerophosphate dehydrogenase; MBC, maximal binding capacity; N/P, nuclear/plasma ratio; PTU, 6-*n*-propylthiouracil;  $T_3$ , triiodothyronine;  $T_4$ , 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  $\alpha$ -glycerophosphate dehydrogenase ( $\alpha$ GPD) and malic enzyme by  $T_3$  in rats (10-13). We have recently observed (14) that there is a similar excellent chronological and quantitative correlation between nuclear occupancy by  $T_3$  and subsequent suppression of thyroid-stimulating hormone (TSH) after intravenous injection of 70 ng/100 g body wt  $T_3$  to hypothyroid rats. When 800 ng thyroxine ( $T_4$ )/100 g body wt was injected, the identical TSH response was correlated with the same nuclear  $T_3$  content as after 70 ng  $T_3$ /100 g body wt. Negligible quantities of  $T_4$  were found bound to the pituitary nuclei. These data suggested that nuclear occupancy by  $T_3$  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  $T_3$  were found in the plasma during the first few hours after  $T_4$  injection, the data suggested that there was a very active conversion of  $T_4$  to  $T_3$  in the pituitary with subsequent binding of the  $T_3$  produced to the pituitary nuclei.

Because our laboratory has recently demonstrated (17) that some thyromimetic effects of  $T_4$  do not appear to be associated with appropriate increases in serum  $T_3$ , the question of whether or not there is a significant quantity of intracellular  $T_4$  monodeiodination to  $T_3$  has arisen. The  $T_3$  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  $T_3$ . The present studies were performed to determine the relative quantitative importance of this source of nuclear  $T_3$  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  $T_3$  concentrations <10 ng/dl (normal rats, mean  $38 \pm 19$  ng/dl), and plasma or serum TSH concentrations >1,500  $\mu$ U/ml (normal range, 50-150  $\mu$ U/ml).  $^{131}$ I- $T_3$  was obtained from Amersham Corp. (Arlington Heights, Ill.; 30  $\mu$ Ci/ $\mu$ g),  $^{125}$ I- $T_4$  from Cambridge Nuclear Corp. (Billerica, Mass.;  $\approx 5,600$   $\mu$ Ci/ $\mu$ g).  $T_3$  contamination of  $T_4$  was <1% as estimated by affinity chromatography using specific  $T_3$  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/100 g body wt.  $T_4$   $T_3$  (both in free acid form)

were obtained from Sigma Chemical Co. (St. Louis, Mo.).  $T_4$  was <0.5%  $T_3$  by weight as determined by radioimmunoassay (19, 20).

*Estimation of plasma or serum  $T_3$ ,  $T_4$ , and TSH.* Plasma basal  $T_3$  concentrations were measured by radioimmunoassay (19). The method previously used in our laboratory has been modified to use 25- $\mu$ l aliquots of rat serum or plasma. Because of differences in nonspecific binding between rat and human serum, the standard curve included 25  $\mu$ l of  $T_3$ -free rat serum prepared using a CG-400 anion-exchange resin (Mallinckrodt Inc., St. Louis, Mo.). Plasma or serum  $T_4$  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  $T_3$  and  $T_4$  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  $MgCl_2$  (21, 22). Before the 2.4 M sucrose centrifugation, the 1,000-g pellet was washed once with 0.32 M sucrose, 3 mM  $MgCl_2$  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  $T_3$  by <10% ( $9.5 \pm 1.0\%$ , 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  $6 \pm 1.4$  (mean  $\pm$ 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  $2.0 \pm 0.3$  mg/g wet wt (recovery of  $50 \pm 10\%$ ), and kidney nuclear DNA was  $3.9 \pm 0.7$  mg/g wet wt (recovery  $58 \pm 10\%$ ).

*Determination of the quantity of trapped plasma in pituitary tissues.* The contribution of trapped plasma to tissue  $T_3$  radioactivity was negligible in agreement with previous reports (25). In  $T_4$ -injected animals, however, the contamination by plasma  $^{125}$ I- $T_4$  was significant. In studies of pituitary  $T_3$  and  $T_4$  content, the trapped plasma volume was evaluated by simultaneous injection of  $^{131}$ I-bovine serum albumin labeled in our laboratory (14, 25). The contribution of plasma  $T_4$  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  $^{125}$ I- $T_4$  still provided a considerable contribution to the extranuclear radioactivity. However, because the goal of the present studies was to evaluate the intracellular  $T_3$  (not  $T_4$ ), and this was not significantly influenced by trapped plasma, this perfusion technique was used throughout. This allowed us to inject  $^{131}$ I- $T_3$  and  $^{125}$ I- $T_4$  simultaneously. Our previous studies have shown that from 0 to 10% of nuclear radioactivity in the pituitary gland was  $T_4$  2-4 h after  $^{125}$ I- $T_4$  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  $T_3$  was decreased to <2.5% of its value by simultaneous injection of 20  $\mu$ g  $T_3$ /100 g body wt. In similar experiments performed with  $^{125}$ I- $T_4$ -injected animals, the pituitary nuclear radioactivity was reduced to <1.5% of the value found with tracer  $T_4$  alone by injection of 20  $\mu$ g of  $T_3$ . In liver and kidney, injection of 20  $\mu$ g  $T_3$  with

$^{125}\text{I-T}_4$  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  $\text{T}_4$ , i.e., all the  $^{125}\text{I-T}_3$  was specifically bound to limited capacity binding sites. The  $^{125}\text{I-T}_4$  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  $\mu\text{l}$  of plasma/mg DNA in the liver (0.52  $\mu\text{l/g}$  wet wt) and 0.12  $\mu\text{l/mg}$  DNA in the kidney (0.39  $\mu\text{l/g}$  wet wt).

**Identification of labeled compounds in nuclei, plasma, and injected  $^{125}\text{I-T}_4$ .** Radioactivity was extracted from nuclei using acid-butanol as previously described (14).  $\text{T}_3$  was separated from  $\text{T}_4$  and identified by paper chromatography in tertiary amyl alcohol/hexane/2-N  $\text{NH}_4\text{OH}$  (6:1:5), as we have previously described (26, 27). Nuclear radioactivity was also chromatographed in *n*-butanol saturated with 2 N  $\text{NH}_4\text{OH}$  in some studies. The locations of  $^{125}\text{I-T}_3$  and  $^{125}\text{I-T}_4$  were determined by staining unlabeled  $\text{T}_3$  and  $\text{T}_4$  (which had been added to the origin) with diazotized sulfanilic acid.  $^{131}\text{I-T}_3$ , 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  $^{125}\text{I-T}_4$  injection, the plasma  $^{125}\text{I-T}_4/^{125}\text{I-T}_3$  ratio was very high (150–200:1). Accurate determination of the  $^{125}\text{I-T}_3$  present required prior elimination of most of the  $^{125}\text{I-T}_4$  because chromatographic separation of  $\text{T}_3$  and  $\text{T}_4$  on paper generates 0.6–0.8% of the  $\text{T}_4$  as  $\text{T}_3$  (27). Elimination of  $\text{T}_4$  was achieved by extracting 100  $\mu\text{l}$  of plasma with specific anti- $\text{T}_3$  antibody conjugated to Sepharose as has been described previously (18). In the washed Sepharose pellet,  $^{125}\text{I-T}_4$  was reduced to <1% of the original quantity while >30% of the  $\text{T}_3$  was retained as judged by the  $^{131}\text{I-T}_3$  present (Appendix). The anti- $\text{T}_3$ -antibody-Sepharose conjugate was then extracted with methanol:2 N  $\text{NH}_4\text{OH}$  (90:10) and chromatographed on paper in tertiary amyl alcohol: hexane:2-N  $\text{NH}_4\text{OH}$  as above. The contribution of artifactual  $\text{T}_4$  to  $\text{T}_3$  conversion during chromatography to the measured  $\text{T}_3$  was therefore negligible. The center of the  $^{131}\text{I-T}_3$  peak on paper chromatography was used for quantitation of  $^{125}\text{I-T}_3$ . The fraction of the original  $^{131}\text{I-T}_3$  present in this area was assessed by comparison with standards appropriately corrected for geometry and counting efficiency. The  $^{125}\text{I-T}_3$  originally present could then be estimated from this  $^{131}\text{I-T}_3$  recovery figure.

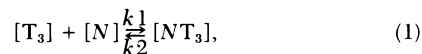
Inasmuch as the goal of the study was to estimate what fraction of nuclear  $^{125}\text{I-T}_3$  was derived from intracellular  $\text{T}_4$  to  $\text{T}_3$  conversion, that portion of nuclear  $^{125}\text{I-T}_3$  coming from plasma had to be quantitated and subtracted. The sources of plasma  $^{125}\text{I-T}_3$  were two: one was a fraction of the  $^{125}\text{I-T}_3$  contaminating the injected  $^{125}\text{I-T}_4$  still present in plasma, and the second was  $^{125}\text{I-T}_3$  derived from intracellular monodeiodination of  $^{125}\text{I-T}_4$  which had entered the plasma compartment. Because  $^{131}\text{I-T}_3$  was injected simultaneously with  $^{125}\text{I-T}_4$ , in most experiments, and because the percent contamination of each  $^{125}\text{I-T}_4$  tracer with  $\text{T}_3$  was known, it was possible to determine the relative contribution of each of these two sources to the plasma  $^{125}\text{I-T}_3$  present. At 2 h after injection of  $^{125}\text{I-T}_4$ , >95% of the  $^{125}\text{I-T}_3$  was derived from contamination (14); at 3 h, contaminating  $^{125}\text{I-T}_3$  accounted for  $\approx 40\%$  of the plasma  $^{125}\text{I-T}_3$ . To determine what fraction of the nuclear  $^{125}\text{I-T}_3$  was derived from the total plasma  $^{125}\text{I-T}_3$ , the simultaneously injected  $^{131}\text{I-T}_3$  was used to determine the nuclear  $^{131}\text{I-T}_3$  (counts per milligram DNA) to plasma  $^{131}\text{I-T}_3$  (counts per milliliter) ratio. The total plasma  $^{125}\text{I-T}_3$  (counts/milliliter)  $\times$   $^{131}\text{I-T}_3$  nuclear/plasma ratio gave the number of  $^{125}\text{I-T}_3$  (counts per milligram DNA) present in the nucleus that was

derived from plasma  $^{125}\text{I-T}_3$ . These counts were subtracted from the total nuclear  $^{125}\text{I-T}_3$  and the locally generated  $\text{T}_3$  was estimated as follows: nuclear  $^{125}\text{I-T}_3/\text{total } ^{125}\text{I-T}_4 \times 2 \times \text{dose of } \text{T}_4$  (nanograms)  $\times 651/777$  based on previous considerations (14). A complete example of these calculations is presented in the Appendix.

### Specific experimental protocols

**Assessment of the maximal nuclear  $\text{T}_3$ -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  $\text{T}_3/100$  g body wt the nuclear/plasma (N/P) ratio of  $\text{T}_3$  was maximal and constant representing equilibrium of  $\text{T}_3$  between receptor sites and plasma (14). Thus, plasma  $\text{T}_3$  concentration is proportional to the concentration of  $\text{T}_3$  to which the nuclear binding sites are exposed in animals with negligible plasma  $\text{T}_4$  concentrations. Therefore, the bound  $\text{T}_3$  is in rapid exchange with the free  $\text{T}_3$  around the receptor, and between this  $\text{T}_3$  pool and plasma. This type of equilibrium is a requisite for analysis of the nuclear  $\text{T}_3$  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  $^{125}\text{I-T}_3$  containing increasing doses of unlabeled  $\text{T}_3$ . The doses used were 19, 70, 210, and 630 ng/100 g body wt. An additional dose of 20  $\mu\text{g}/100$  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  $^{131}\text{I-TSH}$ ) 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  $\text{T}_3$  with nuclear receptor (N) can be viewed as:



and at equilibrium

$$Kd = \frac{[\text{T}_3] \cdot [\text{N}]}{[\text{NT}_3]}, \quad (2)$$

where  $N$  represents the number of unoccupied sites and  $\text{NT}_3$  the number of occupied sites or bound  $\text{T}_3$ , and  $Kd$  the dissociation constant or half-maximal saturation concentration of free  $\text{T}_3$  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  $\text{T}_3$  concentration that saturates half of the sites is proportional to the concentration of free hormone around the receptors so that  $Kd$  can be replaced by  $[\text{T}_3]_{50}$ . 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  $[\text{MBC} - \text{T}_3\text{N}]$ . Substitution in Eq. 2 yields the following expression:

$$[\text{T}_3]_{50} = \frac{[\text{T}_3] \cdot [\text{MBC} - \text{NT}_3]}{[\text{NT}_3]}, \quad (3)$$

which on rearrangement gives a linear equation:

$$\text{NT}_3 = \text{MBC} - [\text{T}_3]_{50} \cdot \frac{\text{NT}_3}{[\text{T}_3]}. \quad (4)$$

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

$$\frac{NT_3}{MBC} = \frac{[T_3]}{[T_3] + [T_3]_{50}}, \quad (5)$$

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

*Analysis of the correlations of nuclear  $T_3$  receptor occupancy and suppression of TSH release in hypothyroid rats.* A plot of the plasma  $T_3$  and TSH concentrations 3 h after  $T_3$  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  $T_3$  and the TSH release rate. If so, the data could then be fitted in the following equation as has been done for  $\alpha$ GPD and malic enzyme (13):

$$\Delta TSH = \frac{[T_3]}{[T_3] + [T_3]_{50}}, \quad (6)$$

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

$$\frac{\Delta TSH}{\Delta MAX} = \frac{[T_3]}{[T_3] + [T_3]_{50}}, \quad (7)$$

and this can be rearranged to yield

$$\Delta TSH = \Delta MAX - \frac{\Delta TSH}{[T_3]} \cdot [T_3]_{50}. \quad (8)$$

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

To compare the plasma  $T_3$  concentration at 3 h with both the fraction of maximal suppressible TSH and of nuclear occupancy, both  $NT_3$  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  $NT_3$  and  $\Delta TSH$ , respectively.

*Effect of 6-n-propylthiouracil (PTU) on nuclear  $T_3$  derived*

*from intracellular deiodination of  $T_4$ .* 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  $^{125}I$ - $T_4$  and killed 2 h later. Pituitary glands and livers were removed and analyzed for nuclear  $T_3$  as described. This dose of PTU has been shown previously to significantly decrease plasma  $T_3$  concentrations in euthyroid rats 14 and 24 h after injection, and to abolish most of the mitochondrial  $\alpha$ GPD response of hypothyroid rats to 800 ng/100 g body wt  $T_4$ /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  $T_3$  in the nuclei of pituitary, liver, and kidney tissues from hypothyroid rats.* The nuclear content of specifically bound  $T_3$  in pituitary, liver, and kidney tissues was significantly related to the plasma  $T_3$  concentration. When nuclear  $T_3$  was plotted against the N/P  $T_3$  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  $T_3$ /mg DNA for pituitary, liver, and kidney, respectively (Fig. 1). In the same figure, the  $[T_3]_{50}$  was 0.92, 0.98, and 0.66 ng  $T_3$ /ml for pituitary, liver, and kidney, respectively. Both pituitary and liver nuclei have a lower apparent affinity for  $T_3$  than do kidney nuclei.

To emphasize the relationships between plasma  $T_3$  and nuclear  $T_3$  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 ( $[T_3]_{50}$ ). The curves are quite similar considering the dispersion obtained. The results indicate that over 90% of nuclear  $T_3$  saturation is obtained with plasma levels of 10 ng/ml, and one-half maximal occupancy is obtained with plasma  $T_3$  concen-

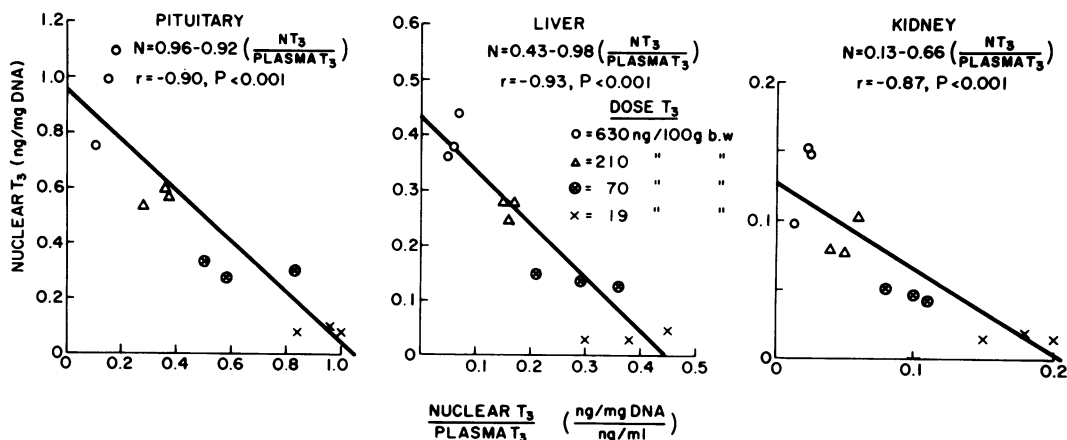


FIGURE 1 Nuclear  $T_3$  vs. the nuclear  $T_3$ /plasma  $T_3$  ratio 3 h after injection of various doses of  $T_3$  to chronically hypothyroid rats. Lines were constructed by least squares regression analysis. Each point represents results of a single animal.

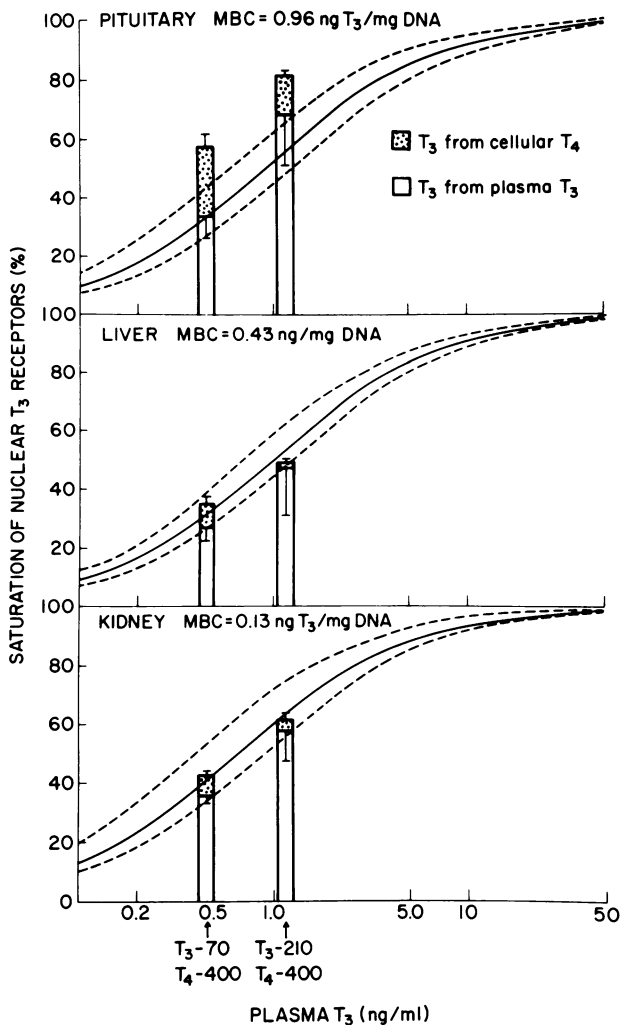


FIGURE 2 Comparison of the saturation of nuclear  $T_3$  receptors in pituitary, liver, and kidney 3 h after injection of various doses of  $T_3$  with that found after injection of 400 ng  $T_4$  + 70 or 210 ng  $T_3$ /100 g body wt in chronically hypothyroid rats. The bar graphs depicting total nuclear  $T_3$  (from plasma  $T_3$  and  $T_3$  derived from local  $T_4$  to  $T_3$  conversion) are superimposed on the curves obtained from the data presented in Fig. 1. The brackets indicate mean  $\pm$  1 SD. Plasma and nuclear  $T_3$  were calculated from the known specific activity of injected  $^{131}\text{I}-T_3$  or  $^{125}\text{I}-T_4$ .

trations 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  $T_4$  to the nuclear  $T_3$  in hypothyroid animals given 70 ng/100 g body wt  $T_3$ .** When 400 ng/100 g body wt  $^{125}\text{I}-T_4$  was given together with 70 or 210 ng  $^{131}\text{I}-T_3$ , the degree of nuclear saturation obtained for  $T_3$  alone was similar for all of these tissues (bar graph in Fig. 2). When the contribution of  $^{125}\text{I}-T_3$  derived from local  $^{125}\text{I}-T_4$  tissue conversion is considered, it can be appreci-

ated 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  $T_3$ -400 ng  $T_4$  dose this contribution was significant for both tissues (Table I)). The plasma concentrations of  $T_4$  and  $T_3$ , 3 h after intravenous injection of 70 ng  $T_3$  + 400 ng  $T_4$ /100 g body wt were in the physiological range ( $0.45 \pm 0.07$  ng  $T_3$ /ml and  $3.0 \pm 0.4$   $\mu\text{g}$   $T_4$ /dl, mean  $\pm$  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  $T_3$  dose levels,  $^{125}\text{I}-T_4$  contributed more  $T_3$  to the pituitary nuclear  $T_3$  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  $T_4$ , whereas this source of  $T_3$  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  $T_4$  to nuclear  $T_3$  are reduced proportionately when the dose of  $T_3$  given is trebled, suggesting that the  $T_3$  derived from these two sources can mix in an intracellular compartment before binding to the nuclear receptor.

An evaluation of the N/P  $T_3$  ratios in the three tissues is presented in Table II. The pituitary N/P ratio for  $T_3$  derived from plasma alone was  $0.73 \pm 0.22$  but when the contribution of  $T_3$  from  $T_4$  was added, this was significantly higher,  $1.03 \pm 0.26$  ( $P < 0.005$ ). The increase in the N/P for liver nuclei due to  $T_3$  derived from  $T_4$  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  $T_4$  to nuclear  $T_3$  from intracellular monodeiodination is likely to be dependent on the  $T_4$  concentration within the cell, and this in turn is related to plasma  $T_4$  concentration, it was of interest to evaluate the nuclear  $^{125}\text{I}-T_3$  to plasma  $^{125}\text{I}-T_4$  ratio under the same circumstances (Table III). This ratio, although smaller than the N/P  $T_3$  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  $T_3$  ( $P < 0.005$ ). As expected, the nuclear  $T_3$ /plasma  $T_4$  ratio decreased in all three tissues when the dose of  $T_3$  was increased, reflecting the limited binding capacity of the nuclear  $T_3$  receptors.

**Correlation of TSH suppression with nuclear and plasma  $T_3$ .** Severely hypothyroid rats seemed suitable models to evaluate the quantitative relationships between nuclear  $T_3$  and TSH suppression because the component of nuclear  $T_3$  derived from local  $T_4$  to  $T_3$  conversion is negligible. Fig. 3 shows the correlation between  $\Delta\text{TSH}$  and  $\Delta\text{TSH}/[\text{T}_3]$  as discussed in Methods. The maximal theoretical suppression obtained 3 h after the  $T_3$  injection was 95%, and the  $[\text{T}_3]_{50}$  was 0.62 ng/ml ( $r = -0.91$ ,  $P < 0.001$ ).

**TABLE I**  
**Source of Nuclear T<sub>3</sub> in Pituitary, Liver, and Kidney of Hypothyroid Rats 3 h after Intravenous <sup>131</sup>I-T<sub>3</sub> (70 or 210 ng/100 g Body wt) and 400 ng/100 g Body wt <sup>125</sup>I-T<sub>4</sub>**

Dose		Source of nuclear T <sub>3</sub> in pituitary (P), liver (L), and kidney (K) % of MBC																					
		Plasma hormone concentrations 3 h after dose				T <sub>3</sub> from plasma						T <sub>3</sub> from intracellular T <sub>4</sub> to T <sub>3</sub> monodeiodination						Nuclear T <sub>3</sub> from both sources % of MBC					
						P values†			P values†			P values†			P values†								
						P	L	K	P	L	K	P	L	K	P	L	K						
T <sub>3</sub>	T <sub>4</sub>	T <sub>3</sub> *	T <sub>4</sub> *	P	L	K	vs. L	vs. K	vs. L	P	L	K	vs. L	vs. K	vs. L	P	L	K	vs. L	vs. K	vs. L		
ng/100 g body wt		ng/dl μg/dl																					
70	400	Mean	45	3.0	34	27	33	NS	NS	NS	24	8.5	7.8	<0.005	<0.005	NS	58	36	41	<0.005	<0.05	NS	
		SD	7	0.4	7	5	2				4	2.0	0.5				11	8	2				
210	400	Mean	112	2.4	68	46	55	<0.05	NS	<0.025	13	3.5	3.5	<0.001	<0.005	<0.025	81	50	59	<0.025	<0.05	NS	
		SD	34	0.4	15	14	11				1	0.6	0.6				15	13	10				

\* Estimated from the specific activity of the respective injected iodothyronine.  
 † Paired t test.

Fig. 4 was generated by plotting the 3-h ΔTSH/ΔMAX (i.e. the fraction of maximal attainable suppression) and nuclear T<sub>3</sub> as a function of plasma T<sub>3</sub>. The regression lines were obtained from Eqs. 4 and 7 (Figs. 1 and 3). The 95% confidence limit (dotted lines) of nuclear T<sub>3</sub> as a function of plasma T<sub>3</sub> is plotted as an index of the expected experimental variation. It can be appreciated that the plasma T<sub>3</sub> 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 T<sub>3</sub> concentration of approximately 20 ng/ml,

**TABLE II**  
**N/P T<sub>3</sub> Ratios 3 h after Injection of <sup>131</sup>I-T<sub>3</sub> (70 or 210 ng/100 g Body wt) and 400 ng/100 g Body wt <sup>125</sup>I-T<sub>4</sub> into Hypothyroid Rats**

Iodothyronine dose		N/P T <sub>3</sub> ratios (ng T <sub>3</sub> /mg DNA)/(ng T <sub>3</sub> /ml plasma) Mean ± SD		
T <sub>3</sub>	T <sub>4</sub>	Pituitary	Liver	Kidney
ng/100 g body wt				
Nuclear T <sub>3</sub> from plasma T <sub>3</sub> only ( <sup>131</sup> I-T <sub>3</sub> )				
70	400	0.73 ± 0.22	0.25 ± 0.04	0.10 ± 0.01
210	400	0.59 ± 0.05	0.18 ± 0.03	0.07 ± 0.01
Total nuclear T <sub>3</sub> ( <sup>131</sup> I-T <sub>3</sub> + <sup>125</sup> I-T <sub>3</sub> )				
70	400	1.03 ± 0.26*	0.27 ± 0.04‡	0.10 ± 0.01
210	400	0.70 ± 0.07*	0.19 ± 0.03‡	0.07 ± 0.01

\* P < 0.005 or P < 0.025 (‡), for difference from nuclear T<sub>3</sub>/plasma T<sub>3</sub> ratio calculated using the plasma T<sub>3</sub> contribution (<sup>131</sup>I-T<sub>3</sub>) alone.

similar to that which produces 95% inhibition of TSH release.

*Effect of the increment in nuclear T<sub>3</sub> provided by T<sub>4</sub> to the inhibition of pituitary TSH release.* In Table IV are shown the biological effects of administration of 70 ng T<sub>3</sub>/100 g body wt with or without 400 ng/100 g body wt T<sub>4</sub>. The percent saturation of the nuclear T<sub>3</sub> receptors by T<sub>3</sub> from both plasma and intracellular T<sub>4</sub> to T<sub>3</sub> conversion is also presented. 70 ng T<sub>3</sub> alone resulted in 32% saturation of the nuclear T<sub>3</sub> receptors at 3 h after administration, and a 39% decrease in plasma TSH. Injection of T<sub>3</sub>-70 + T<sub>4</sub>-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 T<sub>3</sub> alone). At a dosage level of 210 ng T<sub>3</sub>/100 g body wt, the nuclear T<sub>3</sub> 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 T<sub>4</sub>/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 T<sub>3</sub> and ΔTSH are more difficult to detect.

*Effect of PTU pretreatment on the acute contribution of injected T<sub>4</sub> to pituitary and hepatic nuclear T<sub>3</sub>.* The results of T<sub>4</sub> injections given to control and PTU-treated hypothyroid rats are presented in Table V. In the pituitary, 800 ng T<sub>4</sub>/100 g body wt increased nuclear T<sub>3</sub> 0.42 ng/mg DNA. This was identical to the quantity of nuclear T<sub>3</sub> derived from 800 ng T<sub>4</sub>/100 g body wt in PTU-pretreated rats. Thus, the lack of effect of PTU pretreatment on T<sub>4</sub>-induced TSH suppression was reflected in the absence of effect on nuclear T<sub>3</sub> as well. Under the acute conditions of the

TABLE III

Nuclear <sup>125</sup>I-T<sub>3</sub>/Plasma <sup>125</sup>I-T<sub>4</sub> Ratios in Pituitary, Liver, and Kidney of Hypothyroid Rats after Administration of <sup>131</sup>I-T<sub>3</sub> (70 or 210 ng/100 g Body wt) and 400 ng/100 g Body wt <sup>125</sup>I-T<sub>4</sub>

Iodothyronine dose		Nuclear <sup>125</sup> I-T <sub>3</sub> /plasma <sup>125</sup> I-T <sub>4</sub> ratios (ng/mg DNA)/(ng/ml)			P values*		
		Mean ± SD			P vs. L	P vs. K	L vs. K
T <sub>3</sub>	T <sub>4</sub>	Pituitary × 10 <sup>-3</sup>	Liver × 10 <sup>-3</sup>	Kidney × 10 <sup>-3</sup>			
ng/100 g body wt							
70	400	7.9 ± 1.3	1.2 ± 0.2	0.4 ± 0.1	<0.005	<0.005	<0.01
210	400	5.3 ± 1.0	0.6 ± 0.2	0.2 ± 0.1	<0.005	<0.005	<0.025
P values (unpaired t test)							
70-400 vs. 210-400		<0.025	<0.005	NS			

\* P (unpaired t test).

experiment, PTU pretreatment did not alter the small contribution of local T<sub>4</sub> monodeiodination to T<sub>3</sub> to hepatic nuclear T<sub>3</sub>.

DISCUSSION

To analyze the relationship between nuclear content of T<sub>3</sub> in the pituitary and the resulting suppression of TSH, the maximal T<sub>3</sub>-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 T<sub>3</sub>/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 T<sub>3</sub> specifically bound to the nucleus either coming from plasma or from local T<sub>4</sub> to T<sub>3</sub> conversion can be fully displaced by an excess of cold T<sub>3</sub>, we may conclude that all the nuclear binding sites are available to both sources of T<sub>3</sub>. It follows that the approach of saturation analysis gives a good estimation of the MBC. How-

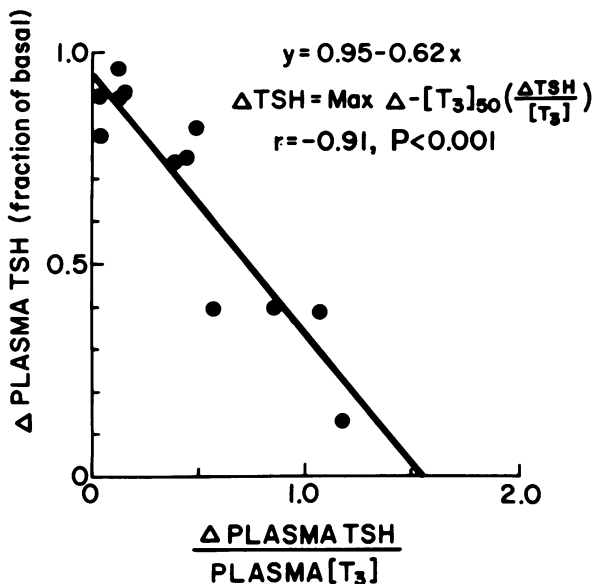


FIGURE 3 Δ Plasma TSH as a function of ΔTSH/[T<sub>3</sub>] ratio in hypothyroid animals given the quantities of T<sub>3</sub> depicted in Fig. 1 3 h before analysis. Each point is the result of the response of a single animal. The maximum ΔTSH in the equation corresponds to the maximal obtainable suppression of TSH, and the [T<sub>3</sub>]<sub>50</sub> is the plasma T<sub>3</sub> concentration associated with the 50% decrease in plasma TSH concentration.

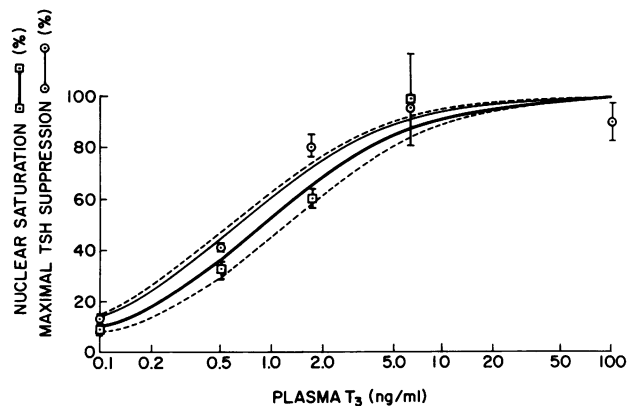


FIGURE 4 Pituitary nuclear saturation and maximal TSH suppression plotted as a function of plasma T<sub>3</sub> concentration in chronically hypothyroid rats injected with various doses of T<sub>3</sub> 3 h before analysis of pituitary T<sub>3</sub> and plasma TSH. The heavy line (□) is the curve describing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



TABLE IV  
Biological Effects of Nuclear Pituitary T<sub>3</sub> Generated Intracellularly from T<sub>4</sub> in Hypothyroid Rats 3 h after Intravenous Administration of Iodothyronine(s)

Dose		n	Nuclear T <sub>3</sub> (% saturation)	Decrease in plasma TSH (% of basal TSH concentration)
T <sub>3</sub>	T <sub>4</sub>			
ng/100 g body wt				
70	0	3	32±3	39±1
70	400	4	58±11	61±10
P			<0.025	<0.025
210	0	3	60±4	77±4
210	400	4	81±15	78±9
P			NS	NS

Mean±SD.

ever, as discussed below, this approach may not give an accurate estimate of the degree of saturation at endogenous levels of plasma T<sub>3</sub> in euthyroid animals.

For reasons stated in the Introduction, we have speculated that a local mechanism for generation of T<sub>3</sub> from T<sub>4</sub> should be quantitatively more important as a source of nuclear T<sub>3</sub> in the pituitary than in the liver of the rat. The results in Fig. 2 and Tables I–III indicate that when T<sub>4</sub> is given together with T<sub>3</sub>, the increment in the pituitary nuclear T<sub>3</sub> 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 T<sub>4</sub> deiodination in these three tissues. Several “artifactual” explanations can be readily excluded for the presence of T<sub>3</sub> in the nuclei of these tissues soon after T<sub>4</sub> injection. The quantity of locally derived T<sub>3</sub> in the nuclei is determined from the net <sup>125</sup>I-T<sub>3</sub> present over that which can be accounted for by

TABLE V  
Lack of Effect of 6-N-Propylthiouracil (PTU) Pretreatment on the Acute Contribution of Tissue T<sub>4</sub> to Pituitary and Hepatic Nuclear T<sub>3</sub>\*

Pituitary			Liver		
Control	PTU	P	Control	PTU	P
Nuclear T <sub>3</sub> , ng/mg DNA					
0.42±0.04	0.42±0.08	NS	0.07±0.02	0.06±0.01	NS
Plasma TSH response, % of basal concentration					
67.5±8.1	59.6±15.3	NS	—	—	—

Mean±SD.

\* Hypothyroid rats received 800 ng/100 g body wt T<sub>4</sub> with or without intraperitoneal injections of 1 mg/100 g PTU 16 and 2 h previously. Animals were killed 2 h after T<sub>4</sub> injection.

the small quantities of <sup>125</sup>I-T<sub>3</sub> present in plasma. Therefore, the accurate determination of plasma <sup>125</sup>I-T<sub>3</sub> is quite critical. Inasmuch as the <sup>125</sup>I-T<sub>3</sub> can be followed throughout the identification and quantitation procedure using the injected <sup>131</sup>I-T<sub>3</sub> (see Appendix for calculations), we are unable to postulate a mechanism by which the recoveries of the two T<sub>3</sub> molecules, labeled with different isotopes, could be different. Because the <sup>131</sup>I-T<sub>3</sub> 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 <sup>125</sup>I-T<sub>3</sub> were underestimated, it would result in an overestimation of locally produced nuclear T<sub>3</sub> from T<sub>4</sub>. 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 <sup>125</sup>I-T<sub>3</sub> from the large quantities of plasma <sup>125</sup>I-T<sub>4</sub> such as the T<sub>3</sub>-antibody-Sepharose conjugate technique used here (18). The possibility of a rapid and disproportionately greater <sup>125</sup>I-T<sub>4</sub> monodeiodination (with a subsequent peak of plasma <sup>125</sup>I-T<sub>3</sub>) on the first passage of T<sub>4</sub> through the tissues can also be eliminated. Because the plasma <sup>125</sup>I-T<sub>3</sub> derived from this reaction would disappear at the same rate as the initially injected <sup>131</sup>I-T<sub>3</sub>, we should have seen fivefold higher quantities of <sup>125</sup>I-T<sub>3</sub> than were actually observed 2–3 h after <sup>125</sup>I-T<sub>4</sub> injection. As the unlabeled T<sub>4</sub> contained even less contaminating T<sub>3</sub> 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 T<sub>4</sub> to T<sub>3</sub> 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 T<sub>4</sub> to T<sub>3</sub> 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 T<sub>4</sub> to T<sub>3</sub> conversion in pituitary homogenates at rates at least similar to the rat liver on a weight basis<sup>2</sup> under certain in vitro circumstances.

Of the total nuclear T<sub>3</sub> in animals receiving 70 ng T<sub>3</sub> and 400 ng T<sub>4</sub>, 41% is derived from local T<sub>4</sub> monodeiodination in pituitary, whereas only 23 and 19% of nuclear T<sub>3</sub>, respectively, is derived from this source in liver and kidney. In rats given 210 ng T<sub>3</sub> plus 400 ng T<sub>4</sub>/100 g body wt, the relative contribution of T<sub>4</sub> is less, but again, much higher in the pituitary than in the liver. Correspondingly, the N/P

<sup>2</sup> Silva, J. E., T. E. Dick, and P. R. Larsen. Manuscript submitted for publication.

ratio for  $T_3$  after  $T_4$  is markedly higher for pituitary nuclei than it is for those of the liver and kidney. Although the biological significance of this  $T_3$  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  $T_3$  on the hepatic or renal tissues to allow early estimates of the possible physiological significance of the  $T_3$  derived from  $T_4$  in these tissues. Thus, despite the *in vitro* capacity of hepatic and renal tissues to produce  $T_3$  from  $T_4$ , the  $T_3$  generated does not appear to contribute substantially to the nuclear  $T_3$  in these tissues.

Because the apparent affinity of nuclear receptors for  $T_3$  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  $T_3$  content after  $T_4$  in the pituitary has to be explained on a different basis. The higher MBC of the pituitary (0.96 ng  $T_3$ /mg DNA) compared to the liver (0.43 ng  $T_3$ /mg DNA) cannot account for the difference either because expressing nuclear  $T_3$  derived from  $T_4$  as a percent of the MBC, i.e., in terms of saturation, the contribution of  $T_4$  to nuclear  $T_3$  in the pituitary is still significantly higher (threefold) than in the liver.

It can also be proposed that local  $T_4$  to  $T_3$  conversion and subsequent binding to the nucleus reaches equilibrium at very different intervals after  $T_4$  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 total tissue  $T_4$  and plasma  $T_4$  in rat pituitary, liver, and kidney (25, 38). We have found that the nuclear  $^{125}\text{I-T}_3$ /plasma  $^{125}\text{I-T}_4$  ratio is about 20% higher at 3 h than at 2 h after injection of  $^{125}\text{I-T}_4$  in both pituitary and liver suggesting that neither tissue has reached equilibrium at 3 h. Therefore, calculation of nuclear  $T_3$  from  $T_4$  at 3 h does not overestimate (but could underestimate) the contribution of this process to the nuclear  $T_3$ . 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  $^{125}\text{I-T}_3$  to plasma  $^{125}\text{I-T}_4$  is not attained until 16–18 h after  $^{125}\text{I-T}_4$  injection.<sup>3</sup>

We have demonstrated that nuclear binding of the  $T_3$  derived from  $T_4$  to  $T_3$  conversion can be completely blocked by 20  $\mu\text{g } T_3/100 \text{ g body wt}$  (14). These results and those of the present study showing a decrease in the fraction of nuclear  $T_3$  derived from  $T_4$  as plasma

$T_3$  concentration increases (Table I) indicate that the nuclear pools of  $T_3$  derived from both sources are miscible. We would speculate that the relative proportions of each source of  $T_3$  in the nuclei of the various tissues would be determined by the plasma concentrations of  $T_4$  and  $T_3$ , the transfer rates of these hormones into the cell, the rate of intracellular  $T_4$  to  $T_3$  conversion in each tissue, and the characteristic subcellular distribution of the  $T_3$  derived from both sources in that particular tissue. The weight of each of these factors in determining the sources of nuclear  $T_3$  is not known and is currently under investigation.<sup>4</sup> Hepatic and renal tissues have been demonstrated to be quite active in  $T_4$  monodeiodination, and yet our studies indicate that relatively small amounts of this locally produced  $T_3$  are bound to the nuclear  $T_3$  receptor. This is certainly in part influenced by the fact that <10% of total tissue  $T_3$  is found in the nucleus in these tissues, whereas more than 40% of pituitary  $T_3$  is nuclear (21).

There is a striking correlation between plasma  $T_3$  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  $T_3$  and the rate of TSH release. First, there is a short delay (<1 h) between nuclear occupancy by  $T_3$  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  $T_3$  at 3 h follows closely the time of the peak nuclear occupancy, and this time is not affected by increasing  $T_3$  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  $T_3$  injection. These considerations provide strong support for a linear relationship between nuclear  $T_3$  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) ( $[T_3]_{50}$  0.62 ng/ml for TSH suppression as opposed to the  $[T_3]_{50}$  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  $\approx 60$  min previously. For the range of  $T_3$  doses examined, this is  $\approx 20\%$  higher at 2 than at 3 h. These data add further support to the concept that  $T_3$  effects on TSH release, like those on growth hormone stimulation and prolactin suppression in pituitary cell cul-

<sup>3</sup> 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  $T_3$  from local  $T_4$  monodeiodination to plasma  $T_4$  had equilibrated in pituitary, liver, and kidney by 16–18 h. At that time, the relative contributions of local  $T_4$  monodeiodination to nuclear  $T_3$  showed the pattern observed in the present experiments.

<sup>4</sup> Joffe, J., J. E. Silva, and P. R. Larsen. Manuscript in preparation.

tures (5, 11), and  $\alpha$ -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  $T_4$  can also be correlated with the quantity of pituitary nuclear  $T_3$ , further supporting this hypothesis. However, as with the data for  $\alpha$ 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  $T_3$  production from  $T_4$ . In euthyroid rats or  $T_4$ -maintained hypothyroid rats, this deiodination process has been shown to be partly sensitive ( $\sim 70\%$ ) 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  $T_4$  to  $T_3$  conversion in PTU-treated animals. The explanation for this is not clear. It is possible that the mechanism for converting  $T_4$  to  $T_3$  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  $T_3$  receptor binding is involved in suppression of TSH release is correct, then the contribution of  $T_4$  to pituitary nuclear  $T_3$  could not be depressed by PTU pretreatment, because this does not affect the acute TSH response to  $T_4$  as we have shown previously (17) and confirmed in the present study. The lack of a significant decrease in hepatic nuclear  $T_3$  due to local  $T_4$  monodeiodination in acutely PTU-treated hypothyroid rats could be explained in a similar way and by the fact that the fraction of the  $T_3$  derived from  $T_4$  that binds to liver nuclei is so small. The often demonstrated inhibition of induction of hepatic  $\alpha$ GPD associated with chronic  $T_4$  + PTU administration to hypothyroid rats is better explained by a decrease in the nuclear  $T_3$  fraction derived from plasma  $T_3$  (substantially depressed under these circumstances) than by a decrease in the nuclear  $T_3$  derived directly from intracellular  $T_4$  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  $T_3$  than by inhibition of  $T_4$  to  $T_3$  conversion in the pituitary (17, 40–42). More data are needed in euthyroid rats to exclude completely a PTU effect on intrapituitary  $T_4$  to  $T_3$  conversion under these circumstances.

If local pituitary  $T_4$  to  $T_3$  conversion is an important source of pituitary nuclear  $T_3$ , if there is a linear relationship between nuclear  $T_3$  receptor occupancy and

the rate of TSH release, and if in the euthyroid rat the TSH secretion rate is only  $\cong 10\%$  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  $^{125}\text{I}$ - $T_3$ . This methodological approach is adequate to calculate the MBC because all the receptor sites are available to plasma  $T_3$  as demonstrated by the full blockade of tracer  $T_3$  binding (from plasma  $T_3$  or tissue  $T_4$ ) by an excess of cold  $T_3$  (14). However, because the intrinsic *in vivo* affinity of these sites is unknown and the degree of the dilution of the  $T_3$  entering the cell from plasma by the  $T_3$  derived from tissue  $T_4$  is also unknown, this method might considerably underestimate nuclear  $T_3$  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  $T_3$  concentrations and the N/P  $T_3$  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  $T_3$  derived locally from tissue  $T_4$ . This significant contribution was found in association with a plasma  $T_4$  concentration in the physiological range for the rat (3  $\mu\text{g}/\text{dl}$ ). Using techniques similar to those of the present study, we have recently estimated that pituitary nuclear receptor saturation is  $\cong 78\%$  in euthyroid rats in agreement with this hypothesis. One-half of this nuclear  $T_3$  was derived from intrapituitary  $T_4$  monodeiodination.<sup>3</sup>

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  $T_3$  derived locally from  $T_4$  would be similarly followed by an increased TSH release from the euthyroid pituitary. Thus, a decrease in plasma  $T_4$  alone could lead to an increase in TSH release. Our present studies suggest that the contribution of  $T_4$  to nuclear  $T_3$  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  $T_4$  alone. This could explain the circumstances in the iodine-deficient rat where an apparently euthyroid state is associated with a low plasma  $T_4$ , normal plasma  $T_3$ , 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  $T_3$  can appear euthyroid as well as why patients with early thyroid dysfunction with reduced plasma  $T_4$ , normal plasma  $T_3$ , 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 T<sub>3</sub> or T<sub>4</sub> 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 T<sub>3</sub> in animals given <sup>131</sup>I-T<sub>3</sub> and <sup>125</sup>I-T<sub>4</sub> is given below. Suitable corrections for <sup>131</sup>I appearing in the <sup>125</sup>I spectrometer window and changes in geometry have been performed where indicated. The nuclear T<sub>3</sub> content was calculated based on the specific activity of injected iodothyronines as follows:

Because the nuclear <sup>131</sup>I was >95% <sup>131</sup>I-T<sub>3</sub>, the nuclear T<sub>3</sub> from injected T<sub>3</sub> was:

$$\frac{\text{Nuclear } ^{131}\text{I counts}}{\text{Total injected } ^{131}\text{I}} \times \frac{1}{\text{mg DNA}} \times \text{dose T}_3 \text{ (ng)} = \text{ng T}_3/\text{mg DNA.} \quad (1)$$

The <sup>125</sup>I-T<sub>3</sub> in the nucleus was determined by paper chromatography using <sup>131</sup>I-T<sub>3</sub> in the nucleus as a recovery and localization standard (Methods).

Nuclear <sup>125</sup>I-T<sub>3</sub> (percent of <sup>125</sup>I-T<sub>4</sub> dose/mg DNA)

$$= \frac{^{125}\text{I-T}_3 \text{ counts selected area}}{^{131}\text{I-T}_3 \text{ counts selected area}} \times \text{total nuclear } ^{131}\text{I-T}_3 \text{ counts} \times \frac{1}{\text{mg DNA}} \times \frac{100}{\text{total injected } ^{125}\text{I}}$$

To deduct from the above <sup>125</sup>I-T<sub>3</sub> that coming from the plasma, quantitation of the plasma <sup>125</sup>I-T<sub>3</sub> content was required. The T<sub>3</sub>-Ab Sepharose conjugate (T<sub>3</sub>-Ab-S) was used to reduce the <sup>125</sup>I-T<sub>4</sub> in plasma as described (14, 18). As an example in one rat:

	<sup>125</sup> I	<sup>131</sup> I
	counts/100 μl plasma	
Trichloroacetic acid precipitable	689,598 counts/min (100%)	10,242 counts/min (100%)
T <sub>3</sub> -AB-S	7,240 counts/5 min (0.21%)	16,203 counts/5 min (31%)
T <sub>3</sub> area on paper	2,870 counts/10 min	28,370 counts/10 min (28%)

Plasma <sup>125</sup>I-T<sub>3</sub> (percent <sup>125</sup>I dose/ml)

$$\times \frac{2,870 \times 10}{0.28} \times \frac{1}{\text{Total } ^{125}\text{I counts injected}} \times 100 = 0.0039\% \text{ } ^{125}\text{I/ml}$$

Since the N/P <sup>131</sup>I ratio was known, the contribution of plasma <sup>125</sup>I-T<sub>3</sub> to the nucleus could be determined. In the above rat,

$$\text{N/P } ^{131}\text{I-T}_3 = \frac{3,379 \text{ (counts/10 min per nucleus)}}{0.054 \text{ (mg DNA)}} \div 102,420 \text{ (counts/10 min per ml)} = 0.61.$$

The N/P for the 20 μg/100 g-injected rat was 0.016±0.003 (nonspecific binding). Therefore, the nuclear <sup>125</sup>I-T<sub>3</sub> from plasma <sup>125</sup>I-T<sub>3</sub> was:

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

In this example, the total nuclear <sup>125</sup>I-T<sub>3</sub> was 0.018% dose/mg DNA. Therefore the <sup>125</sup>I-T<sub>3</sub> derived from local T<sub>4</sub> monodeiodination was:

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

To convert to nanograms T<sub>3</sub>:

$$0.0157 \times \frac{1}{100} \times 2 \times \frac{651}{777} \times 400 \text{ (ng) T}_4 \times \frac{315 \text{ (g)}}{100} = 0.33 \text{ (ng T}_3/\text{mg DNA).}$$

To allow estimates of the dispersion of the data, the detailed calculation of pituitary nuclear T<sub>3</sub> in the experiment in which 70 ng <sup>131</sup>I-T<sub>3</sub> plus 400 ng <sup>125</sup>I-T<sub>4</sub> was given is presented as follows: (results are the mean (±SD) of four rats): Injected <sup>125</sup>I counts, 61,688,400 cpm/100 g body wt; Injected <sup>131</sup>I counts, 657,800 cpm/100 g body wt; Animal weight, 340±17 g; Pituitary nuclear DNA, 0.047±0.009 mg; Total N <sup>125</sup>I-T<sub>3</sub>, 11,063±2,210 (counts/10 min); Total N <sup>131</sup>I-T<sub>3</sub>, 1,470±372 (counts/10 min); Total plasma trichloroacetic acid-precipitable counts at 3 h: <sup>125</sup>I, 45,199,090±1,852,365 counts/10 min per ml; <sup>131</sup>I, 61,326±9,015 counts/10 min per ml; Plasma <sup>125</sup>I-T<sub>3</sub>: (a) from contamination, 63,446±10,881 counts/10 min per ml (1% of <sup>125</sup>I-T<sub>4</sub> was <sup>125</sup>I-T<sub>3</sub>), (b) from T<sub>4</sub>→T<sub>3</sub> 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. <sup>131</sup>I-T<sub>3</sub> N/P ratio, 0.51±0.12; Total nuclear <sup>125</sup>I-T<sub>3</sub>, 283,150±50,337 counts/10 min per mg DNA; From plasma, 82,847±26,996 counts/10 min per mg DNA; Local T<sub>4</sub>→T<sub>3</sub> conversion, 201,350±41,948 counts/10 min per mg DNA, (9.6±2) × 10<sup>-3</sup>% <sup>125</sup>I dose/mg DNA, 0.23±0.05 ng T<sub>3</sub>/mg DNA; From injected T<sub>3</sub>, 0.33±0.08 ng T<sub>3</sub>/mg DNA.

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