Direct Radioimmunoassay of Nuclear 3,5,3' Triiodothyronine in Rat Anterior Pituitary

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ABSTRACT Previous tracer studies have suggested that 5'-monodeiodination of L-thyroxine (T_4) in anterior pituitary may contribute a substantial portion of specifically bound nuclear 3,5,3' L-triiodothyronine (T_3) in this tissue in rats. To evaluate this possibility, a radioimmunoassay for nuclear T_3 in individual anterior pituitaries was developed. Animals received [^{125} I]- T_3 60 min before removal of the anterior pituitary and isolation of the nuclei by differential centrifugation. This allowed calculation of the nuclear:serum T_3 ratio and comparison of expected with measured T_3 . T_3 was extracted in ethanol, dried, and reconstituted in assay buffer. In untreated hypothyroid rats, anterior pituitary nuclear T_3 was 0.18 ± 0.06 pg/ μ g DNA which was 0.13 pg/ μ g DNA greater than expected from the serum T_3 concentration and the pituitary nuclear:serum [^{125} I] T_3 ratio. In 10 hypothyroid rats given a single bolus of 400 ng T_3 /100 g body wt., the nuclear T_3 by radioimmunoassay was 1.0 ± 0.06 pg/ μ g DNA, whereas that expected from the T_3 specific activity calculations was 0.85 pg/ μ g DNA ($P < 0.025$). Serum T_4 concentrations in these rats were < 0.25 μ g/dl but the nuclear T_3 derived from as little as 0.2 μ g/dl T_4 could explain a large portion of these small discrepancies between observed and measured nuclear T_3 . In 29 normal rats, anterior pituitary nuclear T_3 was 0.63 ± 0.04 pg/ μ g DNA, whereas that expected from the serum T_3 concentration (55 ± 2 ng/dl) was 0.23 ± 0.02 pg/ μ g DNA ($P < 0.001$). Total pituitary T_3 based on this measurement was 92 ± 6 pg. Because the maximal nuclear binding capacity for T_3 in rat anterior pituitary is 0.77 pg/ μ g DNA, these results suggest there is 82% occupancy of these nuclear receptors. The requirement for normal serum concentrations of both T_4 and T_3 to achieve normal nuclear T_3 saturation in anterior

pituitary is in marked contrast to the situation in liver, kidney, and heart muscle which appear to require only a normal serum T_3 . As a consequence, the anterior pituitary can monitor both serum T_4 and T_3 and respond appropriately to changes in their concentrations.

INTRODUCTION

Recent *in vivo* and *in vitro* studies in hypothyroid rats have demonstrated that there is rapid intrapituitary monodeiodination of L-thyroxine (T_4)¹ to 3,5,3' L-triiodothyronine (T_3) and that this conversion is required for the rapid inhibition of thyroid-stimulating hormone (TSH) release found after infusion of physiological quantities of T_4 (1-5). Conversion of T_4 to T_3 also occurs in pituitaries of euthyroid rats (4-7). Tracer studies in these animals have suggested that the contribution of intrapituitary T_4 5'-monodeiodination to pituitary nuclear T_3 is equal to the quantity of nuclear T_3 derived directly from the serum (6). As a result, we estimated that the anterior pituitary nuclear T_3 receptors were ~80% saturated due to this additional source of T_3 whereas nuclear receptors in liver and kidney, where this reaction contributes less to nuclear T_3 , were only 50% saturated. If this is the case, it has important physiological implications regarding the feedback regulation of thyrotropin (TSH) secretion by thyroid hormones. It also raises the possibility that individual tissues might have the capacity to regulate the local intracellular T_3 concentration instead of depending entirely on the circulation for this hormone.

Comparison of the radioimmunoassayable nuclear T_3 in single anterior pituitaries with that expected from the serum T_3 in the same rats has not been reported. If our previous studies using tracer T_3 and T_4 are

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¹Abbreviations used in this paper: BSA, bovine serum albumin; GAB, glycine acetate buffer; RIA, radioimmunoassay; t_m , equilibrium time; TSH, thyroid-stimulating hormone; T_4 , L-thyroxine; T_3 , 3,5,3' L-triiodothyronine.

valid, substantially greater quantities of radioimmunoassayable nuclear T_3 should be present in the anterior pituitary than can be accounted for by the concentration of serum T_3 and the nuclear:serum T_3 ratio. Our earlier attempts at these analyses were hampered by the presence of " T_3 -like" material that eluted from the resin used to extract T_3 from the pituitary nuclei (8). This background material was present in sufficiently high quantities that the estimated pituitary nuclear T_3 concentrations in normal rats were highly variable. Using the present methodology, this problem has been eliminated and reproducible results have been obtained.

METHODS

Injections and isolation of anterior pituitary nuclei. Sprague-Dawley rats were obtained from Zivic-Miller Laboratories, Allison Park, Pa. Some rats were thyroidectomized by the supplier and parathyroid glands reimplanted in the neck. They received Purina Rat Chow (Ralston Purina Co., St. Louis, Mo.) and tap water with no added calcium. Thyroidectomized animals were kept for a period of at least 2 mon until their weight gain had plateaued before being used in these experiments.

Pituitary nuclear:serum [^{125} I] T_3 ratios and pituitary nuclear T_3 radioimmunoassay (RIA) measurements were obtained for each pituitary. Intravenous injections of [^{125} I] T_3 ± unlabeled T_3 were given via the jugular vein under light ether anesthesia 60 min before killing the rats. About 1 ml whole blood was removed for base-line T_3 , T_4 and TSH measurements before tracer T_3 injection. For tracer studies, 10–20 μ Ci of [^{125} I] T_3 (3,300 μ Ci/ μ g) were given per rat. This was dissolved in phosphate-buffered saline (pH 7.4) with 10% normal rat serum as carrier protein. Rats received 0.2 ml of this solution/100 g body wt. Animals were killed by exsanguination through the aorta under ether anesthesia and anterior pituitaries removed and nuclei isolated by the method of Samuels and Tsai, (9) slightly modified. The procedure consisted of an initial homogenization of pituitary tissue in 2.5 ml cold 0.25 M sucrose, 1.1 mM $MgCl_2$, 0.02 M Tris, pH 7.85. The pituitaries were centrifuged at 1,000 g for 10 min and the nuclear pellet washed twice with 2.5 ml cold 0.25 M sucrose, 1.1 mM $MgCl_2$, 0.02 M Tris, pH 7.85 containing 0.5% Triton X-100 (Rohm and Haas Co., Philadelphia, Pa.) After this, the pellet was washed two times with 2.5 ml 0.15 M NaCl at 4° C to remove residual Triton. Only isolated nuclei were seen by phase-contrast microscopy of nuclei prepared in this fashion. In normal rats, 25–35% of the total pituitary tracer T_3 was recovered in the nuclear pellet. Nonspecific nuclear binding was $2.4 \pm 0.1\%$ (SE) of total pituitary T_3 in rats receiving 20 μ g T_3 . After the saline washes, 1 ml of absolute ethanol was added to the nuclear pellet which was vortexed and incubated at 37°C water bath. A mean of $80 \pm 0.09\%$ of specifically bound nuclear T_3 was recovered from the isolated nuclei. RIA results were corrected for this loss which was documented for each pituitary. The DNA content of the extracted pellet was measured by the method of Burton (10) as modified by Giles and Myers (11). Recovery of pituitary DNA was 85–90%.

RIA of T_3 in the nuclear extract. The T_3 in the dried extract was dissolved in 0.1 ml of 0.1 N NaOH. Then 0.44 ml of glycine-acetate buffer (GAB, 0.2 M glycine, 0.13 M sodium acetate, pH 8.6) was added followed by 0.1 ml of a 32% vol/vol T_3 -free human serum solution in GAB. Recovery of [^{125} I] T_3 from the dried extract was 100%. The following solu-

tions were also prepared: sodium salicylate, 27.4 mg/ml in GAB containing 0.01% bovine serum albumin (GAB-BSA); rabbit anti- T_3 antibody diluted $1:3.6 \times 10^{-5}$ in GAB-BSA as described (12); tracer T_3 , 1,500–2,500 cpm (2–4 pg)/50 μ l of GAB-BSA. Each 200 μ l of diluted standard contained 10 μ l of T_3 -free human serum in GAB in addition to T_3 (0.3–20 pg). The assay was prepared as follows: 200 μ l of standard or unknown were added to glass tubes followed by 100 μ l of sodium salicylate solution and 150 μ l of antibody solution. Nonspecific binding was assessed for each pituitary nuclear sample and for the assay as a whole by substitution of GAB-BSA for the antibody solution. After 2 d incubation at 4° C, 50 μ l of [^{125} I] T_3 solution was added and after a further 2 d, 1 ml of dextran-charcoal solution in GAB. The tubes were then incubated for 40 min in an ice-water bath after which they were centrifuged at 2,000 g for 15 min and the supernate decanted and counted. The charcoal solution was a 1:64 dilution in GAB of a suspension containing 1.2 g dextran and 12.5 g decolorizing norit charcoal per liter GAB. Sufficient counts were collected to achieve >95% counting accuracy. The [^{125} I] T_3 present in the nuclei did not contribute significantly to the counts added for the RIA.

In Fig. 1 is shown a typical displacement curve for T_3 . Specifically bound T_3 ranged from 22 to 30%. The minimum detectable T_3 concentration as defined by that dose resulting in [^{125} I] T_3 displacement from antibody greater than two standard deviations from the "zero" binding varied from 0.6 to 2.5 pg T_3 /tube. In most assays, it was 1.25 pg. The quantity of T_3 that caused a 50% decrease from zero binding was between 5 and 8 pg/tube.

Determination of serum [^{125} I] T_3 concentrations. We have previously reported that trichloroacetic acid precipitation of serum from tracer T_3 -injected rats results in artifactually high values for T_3 recovery. This is caused by the presence

FIGURE 1 Displacement of antibody bound [^{125} I] T_3 by increasing quantities of unlabeled T_3 . This displacement curve is compared with that obtained by increasing quantities of resuspended anterior pituitary nuclear extract from euthyroid, male Sprague-Dawley rats. In this experiment, the total binding of [^{125} I] T_3 alone was 31.7% and nonspecific binding was 10%. Nonspecific binding in the pituitary nuclear extracts was 11.7%. A volume of 100 μ l is approximately equivalent to the quantity of nuclear extract used in the standard RIA of pituitary nuclear T_3 . B_0 , zero binding.

of non- T_3 , protein-bound, labeled compounds that are generated or concentrated relative to T_3 after intravenous injection (3, 6). Therefore, [^{125}I] T_3 from sera of injected rats was isolated using specific T_3 antibody coupled to agarose as we have described (6, 13). Recovery of authentic (uninjected) [^{131}I] T_3 from 0.1 ml rat serum in this system varied from 40 to 60%. The quantities of tracer [^{125}I] T_3 were corrected for this simultaneously determined recovery and the results were expressed as a fraction of the trichloroacetic acid-precipitable injected [^{125}I] T_3 dose. Trichloroacetic acid precipitation is a valid technique for assessing the fraction of uninjected tracer that is not I^- .

Miscellaneous methods. [^{125}I] T_3 and [^{131}I] T_3 were synthesized in the laboratory according to the method of Weeke and Orskov (14). The specific activity was 3,200–3,400 $\mu Ci/\mu g$. Serum T_3 and T_4 were assayed by methods described (2, 15). Serum TSH in rats was measured using materials kindly provided by the National Pituitary Agency using reported modifications of the methods that are provided with these materials (16). T_3 and T_4 were the free acid and were obtained from Sigma Chemical Co., St. Louis, Mo. All results are mean $\pm$ SE. Statistical comparison of means was performed by either paired or unpaired t tests as appropriate.

RESULTS

RIA methodology. Shown in Fig. 1 is the displacement of [^{125}I] T_3 from antibody by increasing aliquots of pituitary nuclear extract. For this experiment, the nuclear extract from six anterior pituitaries of euthyroid rats were pooled and redissolved in one-half the customary buffer volume. In Fig. 1, 100 μl corresponds roughly to the quantity of nuclear extract customarily present per tube. As shown, the displacement caused by increasing quantities of nuclear extract is parallel to the displacement curve for authentic T_3 . Nonspecifically bound T_3 in the standard tubes varied from 7 to 11%. As previously discussed, a variable portion (one-third to one-half) of this "nonspecifically" bound label is $^{125}I^-$ which does not bind to charcoal (12). The remainder is a result of residual protein- T_3 binding. The 640- μl vol in which the nuclear extract was redissolved allowed two samples of 200 μl for T_3 assay and a single 200 μl for evaluation of nonspecific bonding. In each assay, 6–12 nuclear extracts were measured and the mean of the nonspecific binding values was determined for both the standard curve and the pituitary nuclear extracts. The nonspecific T_3 binding in the nuclear extracts was consistently 0.5–1.5% higher than in the standard curve samples but varied <1.5% between individual samples. The observed percent bound for each unknown was corrected for this difference.

The quantity of T_3 per tube in the assay depended upon the thyroid status of the rat and on the recovery of nuclear T_3 throughout the isolation procedure. The major losses occurred in the isolation of the nuclei (10–15%) and in the ethanol extraction of T_3 from nuclei (20%). Therefore, because the total pituitary nuclear extract was divided into 3.2 aliquots, roughly

21% of the pituitary nuclear T_3 was assayed in each tube. For normal rats and hypothyroid rats injected with T_3 (see below), this resulted in roughly 5–6 pg of T_3 per tube. In hypothyroid rats not given T_3 , <1–2 pg of T_3 were present per tube.

Anterior pituitary nuclear T_3 in chronically hypothyroid rats with and without injected T_3 . In Table I, part A, are shown anterior pituitary nuclear T_3 measurements in seven hypothyroid rats. The serum T_3 in these rats was 16 ± 3.6 ng/dl and serum T_4 was <0.25 $\mu g/dl$ confirming the hypothyroid state. This was further verified by measurements of TSH that were >1,000 $\mu U/ml$ with a normal range for our assay of 50–250 $\mu U/ml$. Rats prepared in this fashion also have extremely low hepatic mitochondrial α -glycerophosphate dehydrogenase activities in addition to cessation of weight gain and are then physiologically hypothyroid in spite of clearly detectable serum T_3 concentrations (17, 18). The nuclear T_3 by RIA in these rats was 9.1 pg/pituitary or 0.18 pg/ μg DNA. In the last two columns of Table I are shown the expected T_3 values based on the nuclear:serum ratio for [^{125}I] T_3 in each animal and the serum T_3 measured in a sample obtained just before the tracer injection. The expected nuclear T_3 was 2.9 pg or 0.05 pg/ μg DNA which was significantly less than was measured ($P < 0.05$). The mean discrepancy was 6 pg of nuclear T_3 /pituitary.

In part B of Table I are shown nuclear T_3 measurements in chronically hypothyroid animals given 400 ng of T_3 /100 g body wt containing $^{125}I/T_3$. This dose was chosen because our previous studies suggested it would nearly saturate the anterior pituitary nuclear receptors for T_3 (2). The gravimetric concentration of the injected T_3 was further verified by assay of suitable dilutions of the dose in the same assay with the nuclear extracts. Because the specific activity of the administered T_3 was known, the accuracy of the method could be assessed by comparison of the measured nuclear T_3 with that expected from the specific activity. The calculation included the small contribution to injected T_3 of the endogenous T_3 present in the serum before T_3 injection (<10% of the injected T_3). The expected quantity of T_3 was calculated from the counts present in each ethanol extract. A mean of 1.87×10^{-4} of the T_3 dose was present per pituitary 60 min after injection and 15% of this T_3 was found in the nuclear extract. The mean nuclear T_3 measured was 34 ± 2.5 (SE) pg or 1 pg $T_3/\mu g$ DNA. This was not significantly different from the value of 0.96 per pg T_3 μg DNA for the maximal binding capacity of anterior pituitary nuclei of hypothyroid rats determined by saturation analysis (2). The expected nuclear T_3 based on specific activity calculations was 20 ± 1.5 pg T_3 or 0.85 ± 0.03 pg T_3 per μg DNA. The 5-pg discrepancy between the measured and the expected nuclear T_3 was significant ($P < 0.025$).

Nuclear and total anterior pituitary T_3 in normal

TABLE I
Anterior Pituitary Nuclear T₃ Measured by RIA

Animal wt	Pit wet wt	Pit DNA	Serum T ₃	Serum T ₄	Nuclear T ₃			
					Measured		Expected from serum T ₃ and the nuclear:serum ratio	
g	mg	μg	ng/dl	μg/dl	pg	pg/μg DNA	pg	pg/μg DNA
A Chronically hypothyroid rats (n = 7)								
238	10.1	50	16	<0.25	9.1	0.18 (<0.05)*	2.9	0.05
±8	±0.6	±3.1	±3.6		±2.9	±0.06	±0.8	±0.01
B Chronically hypothyroid rats given 400 ng T ₃ /100 g body wt 60 min previously (n = 10)								
					Measured		Expected from serum T ₃ and the T ₃ -specific activity†	
256	9.2	35	—	<0.25	34	1.00 (<0.025)*	29	0.85
±12	±0.44	±1.9			±2.5	±0.6	±1.5	±0.03
C Euthyroid male Sprague-Dawley rats (n = 29)								
					Measured		Expected from serum T ₃ and the nuclear:serum ratio‡	
372	8.8	44	55	4.0	28	0.63 (<0.001)*	9.8	0.23
±11	±0.25	±1.7	±2.0	±0.21	±1.9	±0.04	±0.7	±0.02

* Figures in parentheses are *P* values for the statistical significance (by paired *t* test) of the difference between the measured value and that expected.

† The pituitaries of these rats contained 1.87 ± 0.13 (SE) $\times 10^{-2}\%$ of the T₃ dose per anterior pituitary. Tracer T₃ in the assayed nuclear extract was 15 ± 1 (SE)% of the total anterior pituitary T₃.

‡ Assayed nuclear T₃ was $30 \pm 1\%$ (SE) of the total anterior pituitary tracer T₃.

rats. In part C of Table I are shown analyses of anterior pituitary nuclear T₃ in 29 euthyroid animals. Serum T₃ concentrations in these rats were 55 ± 2 (SE) ng/dl and serum T₄ concentrations 4.0 ± 0.21 μg/dl. The pituitary glands of these animals were smaller relative to body weight than were those of the hypothyroid rats, but the quantity of DNA/μg wet wt of pituitary was not significantly different. Nuclear T₃ 28 ± 1.9 pg corresponding to 0.63 pg of T₃/μg DNA. The mean nuclear T₃ calculated from the product of the 60-min nuclear:serum ratio (0.42 ± 0.02) and the radioimmunoassayable serum T₃ was only 9.8 pg of nuclear T₃ or 0.23 pg/μg DNA. The difference between these two values was substantial (18 pg/pituitary) and highly significant (*P* < 0.001). In previous studies, the nuclear T₃ receptor binding capacity in the anterior pituitary of the euthyroid rat by saturation analysis was estimated to be 0.77 (12) to 0.79 (19) pg/μg DNA. The value of 0.63 pg T₃/μg DNA represents 82% saturation of these nuclear T₃ receptors. Because we also determined that the T₃ isolated in these nuclei represented $30 \pm 1\%$ of the total anterior pituitary T₃ in these animals, it was possible

to calculate total pituitary T₃ which was 92 ± 6 pg, an average of 10.5 pg T₃/wet wt.

Since the quantities of nuclear T₃ measured in the hypothyroid animals given T₃ and in the normal animals were quite similar, and because the nuclear:serum ratio for tracer T₃ in the hypothyroid animals was also known, we compared the calculated nuclear:serum T₃ ratios for tracer T₃ with those of radioimmunoassayable T₃ in both experiments. The serum T₃ concentrations in the hypothyroid rats were the sum of the injected T₃ determined from the serum counts and the specific activity and the preinjection radioimmunoassayable T₃. These data are summarized in Table II. In the 10 hypothyroid rats given 400 ng T₃/100 g body wt, the nuclear:serum ratio for tracer T₃ was 0.14 ± 0.1 , whereas that for radioimmunoassayable T₃ was 0.18 ± 0.1 . These values were not significantly different. In the 29 normal rats, the nuclear:serum ratio for tracer T₃ was 0.42 ± 0.02 , whereas that for radioimmunoassayable T₃ was 1.2 ± 0.08 . The difference between these two ratios was highly significant (*P* < 0.001). The fraction of nuclear T₃ derived from

TABLE II
Comparison of Nuclear:Serum Ratios for Tracer T_3 and T_3 by RIA (Mean $\pm$ SE)

Nuclear T_3 /Serum T_3	Hypothyroid rats	Normal rats
400 ng T_3 /100 g body wt		
<i>n</i>	10	29
cpm T_3 /μg DNA/cpm T_3 /μl serum	0.14 $\pm$ 0.01	0.42 $\pm$ 0.02
pg T_3 /μg DNA/pg T_3 /μl serum	0.18 $\pm$ 0.02 (NS)*	1.19 $\pm$ 0.08 ($P < 0.001$)*

* Statistical comparisons performed by paired *t* test.

plasma T_3 in normal rats can be obtained by dividing the nuclear:serum ratio for tracer T_3 by that for radioimmunoassayable T_3 . The quotient is 0.35 indicating that, as assessed by this technique, 35% of pituitary nuclear T_3 derives from plasma T_3 , whereas 65% derives from intrapituitary T_4 to T_3 conversion.

DISCUSSION

Methodology. The data in Fig. 1 and in Table I, indicate that the recovery of T_3 from pituitary nuclei is adequate and that the material extracted from the nuclei gives a parallel displacement curve to that obtained with authentic T_3 . However, it appears from Table I, part B, that the recovery of known quantities of T_3 from the nuclei is ~17% (or 5 pg) above that expected based on specific activity calculations. A similar discrepancy of 6 pg was observed in pituitary nuclei from hypothyroid rats though this might be attributed in part to the small quantities of T_3 being assayed. Control studies have shown that there is no T_3 -like material either in the ethanol or in the solutions used to isolate nuclei or reconstitute the dried nuclear extracts. There remain several possible explanations for the discrepancy. One is that some pituitary nuclear T_3 could be attributed to the presence of quantities of T_4 in the serum that are below the limit of detectability using the T_4 assay described (0.25 μg/dl). A rough estimate of this potential contribution can be made as follows. We have previously reported that in euthyroid rats, the nuclear T_3 derived from serum T_4 via intrapituitary conversion is $1.3 \pm 0.2 \times 10^{-2} \times \text{ng } T_4/\text{ml serum per mg DNA}$ (6). In a euthyroid rat with a plasma T_4 of 40 ng/ml, this source would contribute 0.52 ng T_3 /mg DNA (or pg T_3 /μg DNA). T_4 5'-monodeiodination is approximately threefold more active in vitro in anterior pituitary fragments from hypothyroid rats than in those from euthyroid animals (4). This difference is supported by comparison of the ratios of pituitary nuclear [^{125}I] T_3 to serum [^{125}I] T_4 determined in vivo which are also higher in hypothyroid rats (2, 6). Therefore, a serum T_4 of 2 ng/ml in a hypothyroid rat could result in $1.3 \times 10^{-2} \times 3 \times 2 \text{ ng}$ or 0.08 pg T_3 /μg DNA in anterior pituitary nuclei. Because 50 μg of DNA are recovered in the

pituitary nuclear pellet, this source might account for as much as 4 pg T_3 /pituitary.

A second consideration is in the accuracy of the determination of the nuclear per serum T_3 ratio and consequently the expected nuclear T_3 . It has been previously determined that the only point at which the specific activity of T_3 in the serum is the same as that of the portion of the intracellular T_3 pool derived from serum is at the time of peak nuclear occupancy (the "equilibrium time point" or t_m) (20–22). We have shown that there does not appear to be a demonstrable t_m for pituitary nuclear T_3 but rather a period of maximum nuclear T_3 that may last from 30 to 120 min after T_3 injection (1, 6). During this time, the tracer T_3 in the serum is decreasing. Because the nuclear T_3 remains constant as serum T_3 falls, the nuclear:serum ratio is rising over the t_m . The difference in the nuclear:serum ratio between 30 min and 2 h can be as much as 50% (6). Because we presume there is an actual t_m but recognize that this moment is impossible to detect given the limits of the experimental methods, we have chosen a time of 60 min for these studies which both we and Oppenheimer et al. (19) have estimated to be the best approximation of the midpoint of the t_m for the euthyroid pituitary (6). An underestimate of the nuclear:serum ratio could explain a relatively small portion of the above discrepancy because the increment of 50% in expected nuclear T_3 would apply only to that quantity of nuclear T_3 derived from the endogenous T_3 present in the serum. Lastly, it is possible that there are cross-reacting T_3 -like substances in the nuclear extract. This possibility cannot be rigorously excluded on the basis of the present information.

Despite the above, the present method would appear to have satisfactory accuracy for the purposes of the present study, especially in light of the 18-pg T_3 /pituitary discrepancy between observed and expected nuclear T_3 in anterior pituitary nuclei from euthyroid rats. This large difference can be attributed to the T_3 derived from intrapituitary T_4 -5'-monodeiodination and therefore confirms our earlier tracer studies (6). The discrepancy of 0.40 pg T_3 /μg DNA is quite similar to the predicted contribution of serum T_4 to anterior pituitary nuclear T_3 (0.52 pg T_3 /μg DNA) as calculated

above for rats with a serum T_3 of 40 ng/ml based on our previous report (6). The estimation of the quantity of nuclear T_3 derived from intrapituitary T_4 -5'-monodeiodination depends on both the RIA of total nuclear T_3 and the estimate of the contribution of serum T_3 to nuclear T_3 via the nuclear:serum T_3 ratio. The limitations of the latter with respect to the pituitary have been discussed above. Even if the appropriate nuclear:serum T_3 ratio were as much as 50% higher, the measured nuclear T_3 of 28 pg would still be substantially more than the 14 pg which could then be attributed to serum T_3 . However, the estimate of the fraction of the nuclear T_3 derived from T_4 in this case would decrease from 65 to 50%. Another way of evaluating these results is presented in Table II. The similarity of the tracer and RIA nuclear:serum T_3 ratios in the T_3 -injected hypothyroid rats is in marked contrast to the discrepancy between these two nuclear:serum T_3 ratios in the normal rats where serum T_4 is present.

Comparison of these results with previous studies.

We are not aware of previous RIA measurements of pituitary nuclear T_3 , but two reports of total pituitary T_3 measurements have been published. Gordon and Spira (23) measured anterior pituitary T_3 in a pool of 100 rat pituitaries and obtained a result of 6.8 pg/mg wet wt, a value which is approximately two-thirds of that which we have observed. In a more recent report, Obregon et al. (24) measured total pituitary T_3 in female Wistar rats with unspecified body and pituitary weights (24). They found a total pituitary T_3 of 70 ± 4 (SE) pg per anterior pituitary gland. Because it is likely that these female rats were smaller than the males used in this study, the value calculated for total pituitary T_3 would appear to be in good agreement with that of these authors, though somewhat higher than that of Gordon and Spira.

Physiological implications. We have previously shown that in both hypothyroid and euthyroid rats, the apparent contribution of intracellular T_4 to T_3 conversion to nuclear T_3 was much greater in the pituitary gland than it was in the liver, kidney, or heart (2, 6). The results suggested that this source of nuclear T_3 might be quantitatively significant in pituitary and less so in other tissues. The substantial excess of radioimmunoassayable pituitary nuclear T_3 over that predicted from serum T_3 measurements is in marked contrast to the general agreement between these two methods for estimating T_3 in liver and kidney nuclei reported by Surks and Oppenheimer (25).

An important consequence of the intrapituitary T_3 derived from T_4 is that it results in a greater saturation of the nuclear T_3 receptors in this tissue than can be obtained from serum T_3 alone at its endogenous concentration. The 0.63-pg $T_3/\mu\text{g}$ DNA in the present studies would result in 82% saturation of anterior pituitary nuclear receptors which is quite similar to our

previous estimate of 78% saturation based on pulse-injection tracer methodology (6). The latter studies also suggested that <10% of pituitary nuclear iodothyronine is T_4 . If the anterior pituitary nuclear iodothyronine receptor does bear a casual, inverse relationship to TSH production as has been suggested from our data (1, 2) and from studies of pituitary thyrotrophs in culture (26), and if the nuclear receptor saturation in thyrotrophs is similar to the average pituitary value of 82%, then TSH secretion could increase and remain increased when serum T_4 is reduced even if serum T_3 remains or returns to normal. This would have an important role in the compensatory response of the thyrotroph to early hypothyroidism or iodine deficiency. It differs importantly from the situation in most other tissues of the rat that would appear to depend largely if not entirely on serum T_3 alone for nuclear T_3 . A high degree of endogenous nuclear T_3 receptor saturation in the somatotrophs of euthyroid rats might also explain the failure of growth hormone secretion to increase after thyroid hormone administration as reported by Couloumbe et al. (27). The physiological explanation for the unique importance of local T_4 -5'-monodeiodination as a source of intracellular T_3 in this critically important endocrine tissue is not understood at the present time.

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