Physiological and Pharmacological Influences on Thyroxine to 3,5,3'-Triiodothyronine Conversion and Nuclear 3,5,3'-Triiodothyronine Binding in Rat Anterior Pituitary

R. G. CHERON, M. M. KAPLAN, and P. R. LARSEN, Howard Hughes Medical Institute Laboratory, Thyroid Unit, Department of Medicine, Peter Bent Brigham Hospital, and Harvard Medical School, Boston, Massachusetts 02115

ABSTRACT Our recent in vivo studies have suggested that intrapituitary L-thyroxine (T_4) to 3,5,3'triiodo-L-thyronine (T₃) conversion with subsequent nuclear binding of T₃ is an important pathway by which circulating T₄ can inhibit thyrotropin release. The present studies were performed to evaluate various physiological and pharmacological influences on these two processes in rat anterior pituitary tissue. Intact pituitary fragments were incubated in buffer-1% bovine serum albumin containing 0.14 ng/ml [¹³¹I]T₃ and 3.8 ng/ml [125I]T4. Nuclei were isolated after 3 h of incubation and the bound iodothyronines identified by paper chromatography. There was 0.3-1% [¹²⁵I]T₃ contaminating the medium [125I]T4, and this did not change during incubation. Nuclear [125I]T4 was not decreased by 650-fold excesses of medium T_3 or T_4 , suggesting that it was nonspecifically bound. The ratio of nuclear to medium [¹³¹I]- and [¹²⁵I]T₃ were expressed as nuclear counts per minute per milligram wet weight of tissue:counts per minute per microliter medium. Intrapituitary T_4 to T_3 conversion was evidenced by the fact that the nuclear:medium (N:M) ratio for [¹³¹I]T₃ was 0.45±0.21, whereas that for $[^{125}I]T_3$ was 2.23 ± 1.28 $(\text{mean}\pm\text{SD}, n = 51)$. A ratio (R), the N:M [¹²⁵I]T₃ divided by the N:M [131]T3, was used as an index of intrapituitary T_4 to T_3 conversion. Increasing medium T₃ concentrations up to 50 ng/ml caused a progressive decrease in the N:M ratio for both T₃ isotopes, but no change in the value for R, indicating that both competed for the same limited-capacity nuclear receptors. Increasing concentrations of medium T₄ caused no change in the N:M [131]T₃ but did cause a significant decrease in R in three of four experiments. These results suggest saturation of T₄-5'-monodeiodination occurred at lower T₄ concentrations than saturation of nuclear T₃ binding sites. In hypothyroid rats, the N:M ratios for both [131]T₃ and [125]T₃ were increased (P < 0.005), but R was three-fold higher than in controls (P < 0.005). Animals given 10 μ g T₄/100 g body wt per d for 5 d had significantly decreased N:M ratios for both [131]T3 and [125]T3, as well as a decreased value for R. In fasted rats, neither N:M ratio was depressed, although hepatic T_4 to T_3 conversion in the same animals was 50% of control (P < 0.005). Iopanoic acid (13 μ M), but not 6-*n*-propylthiouracil (29 μ M), decreased the N:M $[^{125}I]T_3$ with a significant decrease in the value for R (P < 0.025 or less). Neither sodium iodide (6 μ M) nor thyrotropin-releasing hormone (7-700 nM) affected the T₃ N:M ratios. These results indicate that intrapituitary T₄ to T₃ conversion is stimulated in hypothyroidism and depressed in T4-treated animals, whereas opposite changes occur in hepatic T₄-5'monodeiodination. Unlike liver, anterior pituitary T₄-5'-monodeiodination is not affected by fasting or incubation with 6-n-propyl-2-thiouracil, but T4 to T3 conversion is inhibited in both by iopanoic acid. These results indicate that there are important differences between anterior pituitary and other tissues in the regulation of T₄-5'-monodeiodination.

INTRODUCTION

Recent in vivo studies from our laboratory have demonstrated a quantitative and chronological relationship between the occupancy of the nuclear iodothyronine receptor of the anterior pituitary tissue

This material was presented in part at the 52nd Annual Meeting of the American Thyroid Association, 13–16 September 1978, Portland, Oregon.

Received for publication 9 February 1979 and in revised form 22 June 1979.

by 3.5.3'-triiodo-L-thyronine $(T_3)^1$ and the acute suppression of thyrotropin (TSH) release in hypothyroid rats (1, 2). Moreover, the acute TSH suppression observed after infusion of L-thyroxine (T_4) was accompanied by intrapituitary conversion of T_4 to T_3 that was so rapid, virtually all of the acute effect of T₄ on suppression of TSH release could be explained on the basis of the T_3 produced (1, 2). In more recent studies, we have demonstrated that in the euthyroid rat, \approx 50% of specifically bound pituitary nuclear T₃ derives from plasma T_3 , and the remaining 50% appears to originate from intrapituitary T_4 to T_3 conversion (3). The total saturation of available nuclear T3 receptors in the euthyroid rat anterior pituitary was estimated to be 78%, a saturation significantly higher than that of hepatic and kidney nuclear T3 receptors in the same animals (\cong 50%) (3). This provides a possible explanation for the increased secretion of TSH in response to a decrease in plasma T₄ concentration even when plasma T₃ remains constant. This combination of developments is often observed in patients in the early phases of thyroid gland failure and in those with endemic goiter. If this hypothesis is correct, then the regulation of intrapituitary T₄ to T₃ conversion and control of the binding of the T_3 produced to the specific nuclear thyroid hormone receptor constitute an important means for modulation of the thyroid hormone feedback on the pituitary thyrothroph. The following studies were undertaken to evaluate the uptake of T_4 , its conversion to T_3 , and the subsequent nuclear binding of the T_3 produced in anterior pituitary tissue in vitro. In a recent preliminary report, we have described the in vitro system used in the present study and provided data indicating that in vitro T_4 to T_3 conversion is readily detectable using this method (4).

METHODS

Preparation and incubation of pituitary tissue. Anterior pituitaries from 300 to 450-g male, Sprague-Dawley rats (Zivic-Miller Laboratories, Allison Park, Pa.) were removed, cut into quarters, and incubated in modified Gey and Gey buffer containing 1 mg/ml D-glucose, and 10 mg/ml bovine serum albumin (BSA) as previously described (4). About 30 min preincubation time was required for the collection and randomization of tissue fragments. The 400-µl incubation volume contained 5–11 mg anterior pituitary tissue, 2 µCi of [¹²³I]T₄ (specific activity 1,200–1,400 µCi/µg), and 0.2 µCi [¹³¹I]T₃ (specific activity 3,500 µCi/µg). The gravimetric iodothyronine concentrations were \approx 3.8 ng T₄/ml and 0.14 ng T₉/ml, based on these specific activities, except where otherwise specified. The free fraction of T₄ in the 1% BSA-buffer solution was 0.32±0.02% (mean±SE) and the free fraction of T_3 was $2.3 \pm 0.15\%$ as determined by equilibrium dialysis (5). Neither free fraction was altered in the presence of 50 ng/ml T_3 or 75 ng/ml T_4 .

Isolation of nuclei, identification of isotopes, and calculations. After a customary 3-h incubation at 37°C, the pituitary fragments were homogenized in 2.5 ml 0.3 M sucrose/1.1 mM MgCl₂ in a glass homogenizer and nuclei were isolated by centrifugation at 1,000 g. Nuclei were washed twice in 2.5 ml sucrose-MgCl₂ containing 0.5% Triton X-100 (Rohm and Haas Co., Philadelphia, Pa.) and once in 2.5 ml 0.15 M NaCl. Tubes were kept at 4°C throughout. In early studies, nuclear iodothyronines were extracted in 2 ml butanol:2 N HCl (9:1) (37°C for 30 min). The acid:butanol was dried under N₂ at 37°C and redissolved in 100 μl of ethanol:concentrated NH₄OH (99:1), which contained 100 μ g of T₃, T₄, and NaI. These ethanol extracts were applied directly to Whatman 3 MM chromatography strips (Fisher Scientific Co., Pittsburgh, Pa.), which were then developed for 24 h in tertiary amyl alcohol:hexane:NH₄OH as previously described (4, 6). This procedure does not result in the formation of the butyl ester of T_4 as shown by control studies performed with [¹²⁵I] T_4 added to isolated nuclei. In later experiments (about two-thirds of those reported here), the acid-butanol step was omitted, and the nuclear iodothyronines were extracted directly into the ethanol:NH₄OH-containing T₃, T₄, and NaI. These two methods gave identical results with 75–90% recovery of [¹³¹I]T₃. No correction was made for these losses. We previously demonstrated that this chromatographic procedure results in the appearance of <0.4% of $[^{125}I]T_4$ as $[^{125}I]T_3$ (6). In some experiments (Table I), $[^{125}I]T_3$ and $[^{131}I]T_3$ were identified in the extranuclear fractions by adding 1.8 ml of the 2.5 ml supernate of the first 1000 g centrifugation to the T₃ antibody-Sepharose (Pharmacia Fine Chemicals, Piscataway, N. J.) conjugate (7). After overnight incubation, the conjugates were washed as previously described, and the labeled hormones were eluted in MeOH:2 N NH4OH (99:1, vol/vol) and chromatographed as described above (7). The chromatographs of nuclear and extranuclear fractions were counted at the same time to facilitate calculation of the nuclear:extranuclear ratio for each T₃ isotope.

The percent contamination of [¹²⁵I]T₄ with [¹²⁵I]T₃ was quantitated by methods previously described, employing affinity chromatography with specific anti-T₃-Sepharose conjugates as a preliminary purification step (7). The percentage of [¹²⁵I]T₃ in the [¹²⁵I]T₄ ranged from 0.3 to 1% of the total radioactivity and did not change significantly during the incubation as estimated in quadruplicate in four separate experiments (4). Therefore, the [¹²⁵I]T₃ present at the start of the incubation in the calculation of the nuclear:medium ratios for [¹²⁵I]T₃. However, in those four experiments in which [¹²⁵I]T₃ was determined in the medium after incubation, that result was used (see Appendix).

To facilitate discussion of the results, the following terminology has been employed: nuclear $T_3(T_3)$ denotes nuclear $[^{13}I]T_3$ derived from medium $[^{13}I]T_3$; nuclear $T_3(T_4)$ is nuclear $[^{125}I]T_3$ derived either from $[^{125}I]T_3$ contaminating the $[^{125}I]T_4$ in the incubation medium or from $[^{125}I]T_4$ 5'-monodeiodination in the anterior pituitary tissue. The nuclear:medium (N:M) ratio for both T_3 isotopes is expressed as counts per minute nuclear T_3 per milligram wet weight of tissue divided by counts per minute medium T_3 per microliter. In our previous report, these ratios were calculated in terms of nuclear DNA rather than wet weight to be quite consistent between experiments (7.1±0.48 µg DNA per milligram wet weight, mean±SD, n = 26), the pres-

¹Abbreviations used in this paper: BSA, bovine serum albumin; N:M, nuclear medium ratio; PTU, 6-n-propyl-2-thiouracil; R, N:M $T_3(T_4) \div$ N:M $T_3(T_3)$; T_3 , 3,5,3'-triiodo-L-thyronine; T₄, L-thyroxine; TRH, thyrotropin-releasing hormone; TSH, thyrotropin.

ent results are expressed on a weight basis. 75–83% of pituitary DNA is recovered using this technique. No correction was made for this loss. The N:M ratios, expressed as milligrams wet weight were not different in fragments of different sizes within the ranges used in these experiments.

To demonstrate that significant T_4 5'-monode odination has occurred within the pituitary tissue, the N:M ratio for $T_3(T_4)$ must exceed that for $T_3(T_3)$. This can be quantitated from the ratio: N:M $T_3(T_4) \div N/M T_3(T_3)$. This ratio has been abbreviated as R. The inverse of R is the fraction of the nuclear $T_3(T_4)$ that has derived from cellular uptake and nuclear binding of the [¹²⁵I]T₃ contaminating the [¹²⁵I]T₄ in the medium.

Miscellaneous. Hepatic T₄ 5'-monodeiodination was guantitated as previously described (8), and serum T_4 and T_3 were measured by radioimmunoassay (9-11). Chemicals were obtained from the following sources: iopanoic acid was kindly donated by Dr. F. C. Nachod of Sterling-Winthrop Research Institute, Rensselaer, N. Y.; 6-n-propyl-2-thiouracil (PTU) was obtained from Aldrich Chemical Co., Inc., Milwaukee, Wis.; BSA (fraction V) from Arnel Products, Brooklyn, N. Y.; [125I]T₄ from New England Nuclear, Boston, Mass.; and T₃ (free acid) and thyrotropin-releasing hormone (TRH) from Sigma Chemical Co., St. Louis, Mo. PTU and iopanoic acid were dissolved in warm (37°C) H₂O at concentrations of 0.2 mg/ml. T_4 and T_3 were initially dissolved in 0.01 M NaOH (1 mg/ml) before dilution and addition to the experimental buffers. [131]T3 was synthesized by the method of Weeke and Orskov (12). All statistical analyses were by unpaired Student's t test.

RESULTS

Methodological considerations. In Table I are shown data indicating that there is no alteration in the extranuclear (cytosol):medium ratio for $[^{13}I]T_3$ resulting from increasing the medium T_3 concentration to as high as 1,000 ng/ml. This indicates that there is no saturation of T_3 transport into the cytosol at the concentrations of T_3 used in these studies. Therefore, the reduction in the nuclear:extranuclear ratio for $[^{13}I]T_3$ found when the medium T_3 concentration is increased from 0.14 to 12.5 ng/ml (right-hand portion

of Table I) would be identical to the relative reduction in the N:M ratio for [131]T₃. Similar dose-independent and rapid equilibration between plasma and hepatic extranuclear T₃ in rats has been demonstrated in vivo by Oppenheimer et al. (13). The data in the last two columns of Table I also indicate that both T₃ isotopes are distributed in the same proportion between nuclear and extranuclear compartments within the tissue. Thus, the [¹³¹I]T₃ derived from the medium and the [125I]T3 derived both from the medium and from intracellular T_4 5'-monodeiodination mix completely inside the cell. We have also demonstrated that this is the case in anterior pituitary in vivo (2). These two observations provide the theoretical basis for the use of the N:M ratios of these two T₃ isotopes in the evaluation of pituitary T_4 5'-monodeiodination in vitro. In this experiment (Table I) in which each flask contained 7-9 mg of tissue, 8.5±0.4% (mean±SE) of the total [131]T3 in the flask was in the tissue. These results are typical of this experimental system.

In Table II are shown results of typical studies designed to evaluate the specificity of nuclear binding of both labeled iodothyronines in this system. The results in the upper portion of Table II indicate that as medium T₄ concentrations were increased from 1.3 to 2,500 ng/ml, there was a marked decrease in the quantity of both [131]T₃(T₃) and [125I]T₃(T₄) bound to the pituitary nuclei. In the presence of 2,500 ng T_4/ml , the counts of each were <20% of those present at the lowest T₄ concentration. There was no significant change in the quantity of nuclear [125I]T4 over this concentration range. These results indicate that T₃ is specifically bound to limited-capacity nuclear binding sites, whereas the binding of T₄ in this system is largely nonspecific. In the lower portion of Table I is shown the effect of increasing concentrations of medium T_3 on the same parameters. As before, the quan-

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Distribution of [¹³¹]T₃ and [¹²⁵I]T₃ between Tissue and Medium and between Nuclear and Extranuclear Compartments in Pituitary Fragments Incubated for 3 h In Vitro

Condi	Number of			Extranuclear (1311)T.*	Ext	Nuclear T ₃ ranuclear T ₃ *
tions	incubation vessels	Medium T ₃	Medium T ₄	$\frac{\text{Extraindenteal } [1]T_3}{\text{Medium } [^{131}I]T_3}$	[¹³¹ I]T ₃	[¹²⁵ I]T ₃
		ng/ml	ng/ml	cpm/mg wet weight cpm/µl medium		
Α	4	0.14	3.8	2.54 ± 0.17	0.16 ± 0.01	0.16±0.03 (NS)‡
В	4	12.5	3.8	2.77±0.24 (NS)§	0.025 ± 0.003	0.032±0.002 (NS)‡
С	4	1,000	3.8	2.78±0.31 (NS)§		

* The extranuclear [¹³¹I]T₃ is that in the supernate of the initial 1,000 g centrifugation of the tissue homogenate in 0.32 M sucrose. The T₃ in this fraction is 70–80% of the total extranuclear T₃. The remainder is found in the second and third sucrose-Triton-MgCl₂ washes.

 \ddagger Not significantly different from the ratio for $[^{131}I]T_3$ by paired t test.

Not significantly different from the results for A by unpaired t test.

	Nuclear Iodothyro	nine*	
		cp5m	
Experiment A			
Medium T ₄ , ng/ml‡	1.25	75	2,500
$[^{131}I]T_3(T_3)$	$4,586 \pm 218$	$2,716 \pm 318$	795 ± 133
[¹²⁵ I]T ₄	$4,237\pm621$	$4,109 \pm 203$	$3,709 \pm 491$
$[^{125}I]T_3(T_4)$	$1,147 \pm 220$	606 ± 445	88 ± 7
Experiment B			
Medium T3, ng/ml§	0.14	12.5	2,500
$[^{131}I]T_3(T_3)$	$7,157 \pm 928$	$2,155 \pm 226$	367 ± 40
[¹²⁵ I]T ₄	$8,597 \pm 1,937$	$7,359 \pm 1,306$	$6,818 \pm 799$
$[^{125}I]T_3(T_4)$	$3,268 \pm 296$	$1,220 \pm 125$	109 ± 24

 TABLE II

 Specificity of Pituitary Nuclear Binding of T₄ and T₃ Derived Either

 from the Medium or from Intrapituitary T₄ Monodeiodination

* Mean±SEM of triplicate samples incubated 3 h.

 \ddagger Medium T₃ constant, 0.14 ng/ml.

§ Medium T₄ constant, 3.8 ng/ml.

tities of $T_3(T_3)$ and $T_3(T_4)$ bound to the nuclei diminished as medium T₃ concentration was increased. The fraction of T₃ bound in the presence of 2,500 ng/ml concentrations of T_3 is $\cong 5\%$ of that in the presence of 0.14 ng T₃/ml, and similar results were obtained for $T_3(T_4)$. Increasing T_3 had little, if any, effect on the quantities of [125I]T4 bound to the nucleus, again suggesting that it was not bound to the same limitedcapacity sites as was T₃. The ratio of the nuclear counts of [125I]T4 to [125I]T3 in both sets of experiments is generally <10:1, except at extremely high T₃ or T₄ concentrations. Thus, under the conditions used in these studies, there was no significant contribution of artifactual T₄ 5'-monodeiodination during the paper chromatographic separation of the extracted nuclear iodothyronines to the estimated $[^{125}I]T_3$ (4, 6, 7).

These results suggest that the bulk of the [125I]T₄ found in the nuclear fraction was present as a result of contamination of nuclei with incubation medium and/or cytosol. To evaluate this, pituitaries were incubated in the presence of ¹³¹I-labeled BSA and [¹²⁵I]T₄ in the presence of 2,500 ng T₄/ml. No ¹³¹I-BSA was found in the nuclei, indicating that the [125I]T₄ present must have come from cytosol. Because of the large fraction of T₄ that is "non-specifically" bound, it was not possible to determine whether there was specifically bound nuclear T₄ in this system, and this will not be discussed further. To calculate specifically bound nuclear T_3 , nonspecifically bound T_3 should be deducted from the total. However, because this was <10% of that bound in the presence of 0.14 ng T₃/ml (Table II), this correction was not performed for either [¹³¹I]T₃ or [¹²⁵I]T₃ N:M ratios.

In Table III are presented studies of the relationship between the duration of incubation and the N:M ratios for $T_3(T_3)$ and $T_3(T_4)$. In experiment A the N:M ratio for $T_3(T_3)$ increases rapidly to an apparent plateau by 4 h. However, in experiments B and C there was a further increase of 25–50% in the N:M $T_3(T_3)$ ratio over the subsequent 3- to 5-h period. The N:M ratio of $T_3(T_4)$ showed a somewhat different pattern. In experiment A this ratio was not significantly different from that for $T_3(T_3)$ until 2 h of incubation, and the ratio continued to increase up to the 4th h. The increase in the N:M $T_3(T_4)$ relative to the N:M ($T_3(T_3)$) is reflected in a rising value for R. In experiments B and C there were further increases in R with longer incubations up to 8 h. We attempted to evaluate the effects of incubation for longer periods, but the tissue did not

TABLE IIIRelationship of the Duration of Incubation to the N:MRatios for $T_g(T_3)$ and $T_g(T_4)$

	Incuba-		N:M ratios*	
Exper- iment	tion	T ₃ (T ₃)	T ₃ (T ₄)	R
	h			<u></u>
Α	1	0.16 ± 0.006	0.18 ± 0.009	1.11 ± 0.04
	2	0.31 ± 0.01	0.79 ± 0.041	2.51 ± 0.1
	3	0.33 ± 0.022	1.17 ± 0.171	3.51 ± 0.40
	4	0.34 ± 0.027	1.37 ± 0.081	4.01 ± 0.16
В	3	0.66 ± 0.025	2.96 ± 0.240	4.51 ± 0.34
	6	0.95 ± 0.069	6.54 ± 0.591	6.95 ± 0.51
	8	1.08 ± 0.047	9.94 ± 0.750	9.19 ± 0.30
С	3	0.84 ± 0.108	6.29 ± 0.454	7.64 ± 0.49
	6	1.17 ± 0.193	14.27 ± 1.564	12.35 ± 0.62

* Mean±SEM of triplicate incubations.

appear to be viable, and results were inconclusive. As there did not appear to be qualitative differences in the ratios with longer incubation times, we generally used a 3-h interval, recognizing that this was not a true equilibrium value, at least not for the N:M $T_3(T_4)$.

It is apparent from Tables II and III that there was considerable variation from experiment to experiment in the N:M ratios. The N:M $T_3(T_3)$ was 0.45 ± 0.21 (mean±SD), the N:M $T_3(T_4)$ 2.23 ± 1.28 , and R was 5.42 ± 3.46 in 51 individual control flasks. However, the reproducibility within a given experiment was quite satisfactory (see below). Accordingly, control pituitaries from euthyroid rats were included in all experiments described. The fraction of cellular [¹³¹I]T₃ that was bound to nuclei was quantitated in three experiments at medium T₃ concentrations of 0.14 ng T₃/ml. The specifically bound nuclear [¹³¹I]T₃ was 18 ± 1 , 18 ± 3 , and $24\pm2\%$ (all results mean±SD of triplicates) of the total homogenate [¹³¹I]T₃. The mean of the results of the three experiments was 20%.

Inasmuch as anterior pituitary tissue contains both T_3 and T_4 , it was of interest to determine the rate at which these endogenous hormones were released into the incubation medium. Depending on the total quantities involved, this could alter the estimated specific activity of the iodothyronines. To evaluate this, rats were given tracer T₃ or T₄ in vivo, and their anterior pituitaries were removed 1 h later. The exit of T₃ and T₄ into the medium was quantitated under the standard experimental conditions used. During the tissue collection and preincubation period ($\approx 30 \text{ min}$), $\approx 20\%$ of tracer T_3 was released. Over the subsequent 3 h incubation, a further 60-80% of the total pituitary T_3 appeared in the medium. About 50% of tracer T₄ exited during the preincubation period, and the remainder during the subsequent 3 h. Current estimates of total pituitary iodothyronines suggest that there are about 23 pg of T_4 and about 45 pg of $T_3/7$ mg of pituitary tissue (14, 15). Assuming complete mixing of pituitary iodothyronines with those in the medium, the decrease in the specific activity is negligible for T_4 and $\approx 25\%$ for T₃ for euthyroid rats.

Effects of altering concentrations of medium iodothyronines on N:M ratios for $T_3(T_3)$ and $T_3(T_4)$. Results of studies of the effects of increasing concentrations of medium iodothyronines are shown in Tables IV and V. Since the specific nuclear receptor sites have limited binding capacity for T_3 in vivo and in vitro (Table II), one would anticipate that increasing medium T_3 concentrations would lead to decreases in both N:M ($T_3(T_3)$ and N:M $T_3(T_4)$ if these two species are competing for the same nuclear receptor site. This prediction is borne out in the experiments shown in Table IV. A significant reduction in the N:M $T_3(T_3)$ and N:M $T_3(T_4)$ was achieved in experiment A at 2.5 ng T₃/ml and in experiments C and D at 12.5 ng T₃/ml. Although there are also apparent decreases in both N:M ratios in experiments B and E, these differences did not achieve statistical significance in the triplicate samples. In no experiment was the R value different from that at the lowest T₃ concentration of 0.14 ng T₃/ml.

The effects of increasing medium T₄ concentrations were considerably different (Table V). In none of the four experiments were significant differences found in the N:M $T_3(T_3)$ ratio with medium T_4 concentrations up to 75 ng/ml. Changes in the N:M ratio for $T_3(T_4)$ were also not significant at any of these T₄ concentrations. However, there was a numerical decrease in the N:M $T_3(T_4)$ at the highest concentration of T_4 used in all experiments. This change is reflected in a decrease in the value for R that was statistically significant in three of the four experiments. These results suggest that there is saturation of the pathway for generating nuclear $T_3(T_4)$ before saturation of the nuclear receptor sites by the T₃ present in the T₄ or by that generated from it. The more ready saturation of the nuclear receptors with T₃, as opposed to T₄, is reflected in the fact that there is little, if any, decrease in the N:M $T_3(T_3)$ at medium T_4 concentrations which are fiveand sixfold in excess of those at which a T₃ effect was usually observed.

Effects of alterations in thyroid status on pituitary T_4 5'-monodeiodination and nuclear binding. Hypothyroidism has been shown to reduce and T4 administration to increase, the rate of 5'-monodeiodination in rat liver (16). To examine the effects of altered thyroid status on T_4 to T_3 conversion in the anterior pituitary, animals were given 10 μ g T₄/100 g body wt per d for 5 d subcutaneously or were thyroidectomized 2-3 mo before the study (Table VI). In T₄-treated animals, the N:M ratio for T₃(T₃) was slightly but significantly reduced from that present in euthyroid control animals from the same group. An even greater reduction was observed in the N:M ratios for $T_3(T_4)$, which were <50% of those observed in simultaneously studied controls. As a result, the value for R in T₄treated rat pituitaries was roughly half that obtained in euthyroid rats.

In chronically hypothyroid rats, the N:M ratios for both $T_3(T_3)$ and $T_3(T_4)$ were significantly higher than those in controls in the same experiment. The N:M ratio for $T_3(T_3)$ was nearly twice that of normal animals, and the N:M ratio for $T_3(T_4)$ was approximately five times that of the controls. The ratio R was increased two- to threefold over that in the euthyroid rats. When compared with the results for all the euthyroid controls (see above), the N:M for $T_3(T_4)$ and R were also significantly greater in pituitaries from hypothyroid rats, P < 0.001 and P < 0.025, respectively. However,

Medium T ₃ , ng/ml	0.14	0.75	2.5	12.5	50
Experiment A*					
$N:M T_3(T_3)$	0.39 ± 0.02	_	0.23±0.02‡	0.11 ± 0.01 §	—
N:M $T_3(T_4)$	3.06 ± 0.20	_	2.01±0.12‡	1.11±0.02§	_
R	7.93 ± 0.92		8.77 ± 0.27	11.18 ± 1.59	—
Experiment B					
$N:M T_3(T_3)$	0.36 ± 0.06	0.33 ± 0.04	0.26 ± 0.03	0.21 ± 0.05	
N:M $T_3(T_4)$	1.14 ± 0.27	0.96 ± 0.21	0.78 ± 0.08	0.58 ± 0.12	_
R	3.12 ± 0.14	2.79 ± 0.35	2.95 ± 0.10	2.86 ± 0.50	
Experiment C					
$N:M T_3(T_3)$	0.53 ± 0.05	_	_	0.14±0.01 [∥]	0.09±0.01
N:M $T_3(T_4)$	1.73 ± 0.09	_	_	0.51 ± 0.06 §	0.29±0.03§
R	3.14 ± 0.32	_	—	3.79±0.20	3.48±0.27
Experiment D					
$N:M T_3(T_3)$	0.51 ± 0.03	-		0.11±0.01§	0.08±0.02§
N:M $T_3(T_4)$	3.21 ± 0.26	_		0.65±0.06§	0.34±0.08§
R	6.28 ± 0.21	—	-	5.92 ± 0.58	4.46±0.25
Experiment E					
$\hat{N}:M T_3(T_3)$	0.23 ± 0.02	0.27 ± 0.03	0.21 ± 0.06	0.19 ± 0.05	_
N:M $T_3(T_4)$	1.16 ± 0.12	1.13 ± 0.03	0.84 ± 0.36	0.72 ± 0.20	
R	5.18 ± 0.87	4.22 ± 0.44	3.74 ± 0.65	3.86±0.26	_

TABLE IVEffect of Alterations in Medium T_3 Concentrations on the N:M Ratios for $T_4(T_3)$ and $T_4(T_4)$ in Rat Anterior Pituitary Tissue

Mean±SE of triplicates.

* Incubation times were 3 h for experiments A-C; 6 h for experiments D and E. Medium T₄, 3.8 ng/ml.

 $\ddagger P$ values < 0.01.

§ P < 0.001 for differences of N:M ratios from those present at medium T₃ concentrations

of 0.14 ng/ml (unpaired t test). "P < 0.005.

1 < 0.000.

the N:M for $T_3(T_3)$ was not significantly higher in the hypothyroid pituitaries than in the total control population. Thus, T_4 treatment decreased and hypothyroidism increased the capacity of the anterior pituitary to deiodinate T_4 to T_3 , effects opposite to those reported for hepatic tissue in similar circumstances.

Intrapituitary T_4 to T_3 conversion and nuclear binding in fasted rats. Caloric deprivation results in a reduction in hepatic T_4 5'-monodeiodination in rats (8, 17–19). The data in Table VII indicate that neither the N:M ratio for $T_3(T_3)$ nor that for $T_3(T_4)$ were significantly different from control after three night's fasting in 230-g rats. Hepatic T_4 5'-monodeiodination was also quantitated in the same animals from which these anterior pituitaries were obtained (Table VII). Hepatic T_4 5'-monodeiodination was reduced to <50% of that in controls (P < 0.005).

Effects of iopanoic acid, PTU, TRH and NaI on intrapituitary T_4 to T_3 conversion and nuclear binding. Iopanoic acid and PTU inhibit hepatic 5'monodeiodination (8, 20-22). Incubation of pituitary fragments with 13 μ M iopanoic acid results in no alteration in the N:M ratio for T₃(T₃) but does result in a marked decrease in the N:M ratio for T₃(T₄) (Table VIII). For example, in experiment D the amount of T₃(T₄) in the nucleus is reduced to that which would be expected on the basis of [¹²⁵I]T₃ contamination alone. The R values for all experiments were <50% of the respective controls. There was no reduction in tissue [¹²⁵I]T₄ uptake by iopanoic acid as judged by nuclear [¹²⁵I]T₄ in these experiments.

Incubation with 29 μ M PTU had no demonstrable effect on the N:M for T₃(T₃) and did not reduce the N:M T₃(T₄) ratio. As a result, R was not lower, and in one case it was statistically higher than in controls from the same pool of pituitary fragments. To investigate the possibility that in vivo treatment of animals with PTU might be required to inhibit T₄ to T₃ conversion, four normal animals were given 2 mg PTU/100 g body wt i.p. twice daily for 2 d before measuring body pituitary and hepatic T₄ to T₃ conversion. As is shown in Table VIII, there is no effect of PTU pretreatment

Medium T ₄ , ng/ml	1.25	3.8	7.5	25	75
Experiment A*					
$N:M T_3(T_3)$	_	0.25 ± 0.03	0.29 ± 0.01	0.26 ± 0.01	0.25 ± 0.01
N:M $T_3(T_4)$	_	0.68 ± 0.09	0.97 ± 0.04	0.65 ± 0.03	0.47 ± 0.04
R	_	2.68 ± 0.11	$3.32 \pm 0.09 \ddagger$	2.49 ± 0.09	$1.87 \pm 0.17 \ddagger$
Experiment B		· .			
N:M $T_3(T_3)$	0.13 ± 0.01	0.17 ± 0.05	0.18 ± 0.04	0.16 ± 0.03	_
N:M $T_3(T_4)$	2.0 ± 0.39	2.10 ± 0.69	2.36 ± 0.82	1.16 ± 0.11	_
R	16.09 ± 2.30	12.88 ± 5.07	14.73 ± 7.33	$7.48 \pm 0.80 \ddagger$	
Experiment C					
$\hat{N}:M T_3(T_3)$	0.21 ± 0.06	0.28 ± 0.04	0.25 ± 0.05		
N:M $T_3(T_4)$	3.27 ± 0.36	3.16 ± 0.14	2.75 ± 0.22		
R	20.23 ± 4.81	12.23 ± 1.87	11.95 ± 1.85	-	_
Experiment D					
$N:M T_3(T_3)$	0.37 ± 0.07	0.36 ± 0.08	_	0.32 ± 0.03	0.23 ± 0.03
N:M $T_3(T_4)$	3.70 ± 0.57	2.11 ± 0.71		0.87 ± 0.07 §	0.50 ± 0.22 §
R	10.22 ± 1.29	5.64 ± 0.74	_	2.72±0.31"	2.31 ± 1.22 ‡

TABLE V		
Effect of Alterations in Medium T ₄ Concentrations on the N:M	Ratios for	$T_3(T_3)$
and $T_{3}(T_{4})$ in Rat Anterior Pituitary Tissue		

Mean±SE of triplicates.

* Incubation times were 3 h for experiments A-C and 6 h for experiment D.

P < 0.02.

P < 0.01.

P = 0.005 for differences of results from those at the lowest medium T₄ concentrations employed in that experiment (unpaired t test).

on pituitary T_4 to T_3 conversion. In these same animals, the hepatic T_4 5'-deiodination rate was reduced to 12% of the results in controls (P < 0.01, data not shown).

Neither NaI (6 μ M) nor TRH had a significant effect on the N:M T₃(T₃) or T₃(T₄) ratios. The concentrations of TRH used and the observed values for R were as follows: for 7 nM, R was 3.24 ± 1.87 (mean±SE of triplicates); for 70 nM, R was 1.98 ± 0.22 ; and for 700 nM, R was 3.33 ± 0.38 . The control value for R in this pool of euthyroid pituitaries was 3.81 ± 0.51 .

 TABLE VI

 Comparison of Intrapituitary Monodeiodination in Euthyroid, T₄-treated and Hypothyroid Rats

					N:M ratio				
		Euthyroid			T ₄ -treated*			Hypothyroid‡	
Exper- iment	T ₃ (T ₃)	T ₃ (T ₄)	R	T ₃ (T ₃)	T ₃ (T ₄)	R	T ₃ (T ₃)	T ₃ (T ₄)	R
A	0.66 ± 0.08	2.47 ± 0.58	3.65 ± 0.50	_			1.05±0.03§	10.12±0.53§	9.66±0.48§
В	0.34 ± 0.02	1.20 ± 0.15	3.49 ± 0.35	0.26 ± 0.01 §	$0.52 \pm 0.06^{\circ}$	$2.01 \pm 0.25^{\parallel}$	0.62 ± 0.01 §	6.11±0.45§	9.84±0.79§
С	0.42 ± 0.04	1.88 ± 0.20	4.50 ± 0.59	$0.26 \pm 0.01^{\parallel}$	0.53 ± 0.02 §	$2.02 \pm 0.05^{\mu}$		—	—

Mean±SEM of triplicate determinations.

* 10 μ g T₄/100 g body wt per d × 5 d subcutaneously. Serum T₄ was 8.9±1.1 (exp. B) and 11.3±1.1 (exp. C) μ g/dl. Mean±SEM. Pituitary quarters from three rats were pooled in each experiment and randomly combined (four/flask). Controls were from the same group of rats.

‡ At least 2 mo postsurgical thyroidectomy and parathyroid implantation. Serum T₄ was $<0.2 \ \mu g/dl$ for all rats. Pituitary quarters from three rats were pooled in each experiment and randomly combined (four/flask). Control rats were of the same size but not from the same group.

§ P < 0.005 for difference of results from control by unpaired t test.

||P < 0.025.

		Control			Fasted*	
	N:M	ratio		N:M	ratio	
Flask	T ₃ (T ₃)	T ₃ (T ₄)	R	T ₃ (T ₃)	T3(T4)	R
Anterior pituitary						
1	0.18	1.17	6.62	0.20	1.10	5.39
2	0.21	1.27	5.95	0.28	1.34	4.76
3	0.26	1.61	6.15	0.24	1.29	5.29
4	0.26	1.40	5.42	0.24	1.16	4.82
5	0.22	1.12	5.06	0.14	0.79	<u>5.49</u>
Mean	0.23	1.31	5.84	0.22	1.14	5.15
SEM	0.01	0.01	0.27	0.02	0.01	0.15
Liver, fmol T ₃ /min/						
mg protein		n = 8			n = 8	
Mean		46.8			21.9‡	
SEM		5.7			2.3	

 TABLE VII

 Comparison of T₄ to T₃ Conversion in Anterior Pituitary and Liver of Fasted Rats

* Eight rats were fasted for three nights with eight others from the same shipment as controls. Mean starting weights were 232 and 227 g in fasted and control rats, respectively. Mean weights after 3 d were 182 (fasted) and 254 g (control). Anterior pituitaries were pooled and six quarters placed into each flask. Hepatic T₄ 5'-monodeiodination was studied in each individual animal as previously described (8).

 $\ddagger P < 0.005$ for difference from control.

		Control				Experimental	
	N:M	ratio		Medium	N:M	1 ratio	
	T ₃ (T ₃)	T ₃ (T ₄)	R	concen- tration	T ₃ (T ₃)	T ₃ (T ₄)	R
				μΜ			
Iopanoic acid Experiment							
Ā	0.34 ± 0.02	1.20 ± 0.15	3.49 ± 0.35	13	0.46 ± 0.11	0.69 ± 0.15	1.52±0.03*
В	0.42 ± 0.04	1.88 ± 0.20	4.50 ± 0.59	13	0.40 ± 0.04	0.78 ± 0.06	1.99±0.31*
С	0.49 ± 0.02	3.10 ± 0.67	6.54 ± 1.49	13	$0.55 \pm 0.02*$	0.92±0.07‡	1.69±0.14‡
D	0.83 ± 0.08	5.81 ± 0.93	6.94 ± 0.41	13	0.93 ± 0.03	0.90±0.07‡	0.96±0.05‡
PTU							
Experiment							
Â	0.86 ± 0.08	4.08 ± 0.34	4.78 ± 0.19	29	0.91 ± 0.02	3.98 ± 0.28	4.36 ± 0.32
В	0.30 ± 0.01	0.66 ± 0.08	2.15 ± 0.18	29	0.40 ± 0.05	1.14±0.13*	2.90±0.02*
С	0.83 ± 0.09	5.81 ± 0.93	6.94 ± 0.41	29	0.89 ± 0.05	6.90 ± 0.99	7.73 ± 0.71
D	0.24 ± 0.03	0.64 ± 0.09	2.72 ± 0.26	In vivo§	0.18 ± 0.02	0.49 ± 0.05	2.81±0.09

 TABLE VIII

 Effects of Iopanoic Acid and PTU on Intrapituitary T4 to T3 Conversion and Nuclear T3 Binding

Mean±SEM of triplicate determinations.

* P < 0.025.

 $\ddagger P < 0.005$; statistical significance of differences of experimental samples from controls.

§ PTU, 2 mg/100 g body wt i.p., twice daily for 2 d.

DISCUSSION

Methodological considerations and results of basal studies. The principal advantage of the system described in this report is its capacity to measure small quantities of [125I]T₃ generated from [125I]T₄ in anterior pituitary tissue. Since the distribution of [131]T₃ and [125I]T₃ is not different within the tissue (Table I), the net T₄ to T₃ conversion in an experiment can be determined from a comparison of the N:M ratios for [131]T₃ and [125I]T₃. The value R is a reflection of the difference between these two ratios. In the experiment for which detailed data are provided in the Appendix, R is 4.8 and the [125I]T₃ is 0.8% of the T₄ after the incubation. (Preincubation data were not available for this experiment.) The fraction of the total flask [131]T3 in the nuclei is 1.9%. Thus, the fraction of [125I]T4 as nuclear $[^{125}I]T_3$ contamination alone is $1.9 \times 0.8\%$, or 0.015% of the total [125I]T4 in the flask. Since R is 4.8, the observed $[^{125}I]T_3$ in the nuclei is $4.8 \times 0.015\%$, or 0.072%of the total [125I]T₄. The net increment in nuclear $[^{125}I]T_3$ above that expected from nuclear binding of contaminating [125I]T₃ is 0.072% less 0.015%, or 0.057% of the T₄. Because we observed that only 20% of the tissue [¹³¹I]T₃ is bound to the nuclei in this in vitro system (see above), and because the subcellular distribution of [125I]T₃ is not different from that of [131I]T₃ (Table I), this figure must be multiplied by five to estimate the increment in total tissue [125I]T₃ and by two to correct for the loss of specific activity during T_4 5'-monodeiodination. Thus, the net increment in total T_3 from T_4 in the system is 0.57% of the T_4 present. Virtually all of this [125I]T3 is still within the tissue after 3 h of incubation. It is apparent that the $[125I]T_3$ formed in the tissue has not equilibrated with the medium because a 4.8-fold increment in medium [125I]T₃ would be quite simple to recognize, and no significant increment of [125I]T₃ is observed. Although it might be theoretically desirable to determine the net [125I]T₃ produced for each experiment, the data would have no more validity than is achieved more simply by the use of the R value which automatically corrects for any experimentally induced or endogenous differences in nuclear binding and for the modest losses in the isolation and identification of nuclear T3. It should be kept in mind that the extranuclear:medium T₃ ratios were tested and found to be the same only for basal conditions and during incubations with 12.5 ng/ml T₃ (Table I). If any of the other manipulations (altered thyroid function or incubation with PTU or iopanoic acid) altered this ratio without affecting [125I]T4 uptake and conversion, then comparisons with the R value in control tissues would not be valid. The similarity of the N:M ratios for [131]T₃ under all circumstances makes this possibility seem remote.

It is presumably the small quantities of T_3 generated that account for the failure of previous investigators using either tissue fragments (23) or homogenates (24) to demonstrate conversion in rat anterior pituitary tissue in vitro. The technical aspects of the present system that permit the required sensitivity are that: (a) the nuclear receptor acts as a high affinity sink for the [125I]T₃ formed in the tissue, segregating it from $[^{125}I]T_4$; (b) the simultaneous incubation with [131]T₃ allows corrections for transfer of [125I]T₃ from the medium to the nucleus and for identification of any nonspecific effects of the experimental variables on nuclear T₃ binding for each individual tissue; and (c) the sensitivity and precision of affinity chromatography followed by paper chromatography allows quantitation of the amount of [125I]T₃ in the medium, even though it represents only 0.3-1% of the [125I]T₄.

Data supporting the technical aspects of this method have been discussed (4, 7). Physiological substantiation is provided by the following aspects of the present report. Our previous in vivo experiments have shown that pretreatment of rats with amounts of PTU adequate to block \cong 70% of total body T₄ to T₃ production neither decreased intrapituitary T₄ to T₃ conversion nor prevented the acute suppression of TSH release by T₄ in chronically hypothyroid rats (2, 25). In the present studies, incubation of pituitary fragments with doses of PTU 10-fold higher than those that block hepatic T_4 5'-monodeiodination did not affect intrapituitary T4 to T_3 conversion (8). Treatment of rats with quantities of PTU leading to 88% inhibition of T_4 to T_3 conversion in livers subsequently studied in vitro also did not affect pituitary T_4 to T_3 conversion in the in vitro system. On the other hand, iopanoic acid—a potent inhibitor of hepatic T_4 to T_3 conversion (8)—can readily block pituitary T₄ to T₃ conversion, even suppressing the R ratio to unity in some experiments (Table VIII). We have recently been able to demonstrate that iopanoic acid treatment of rats also blocks in vivo generation of T_3 from T_4 in the anterior pituitary and prevents the decrease in TSH associated with acute T₄ administration (26). Thus, with respect to both pharmacological agents, the response measured in this in vitro system is the same as that observed in vivo, and the physiological relevance of these results is supported.

There are, however, several aspects of the system which do not appear to be physiological. The data in Table II indicate that substantial quantities of $[^{125}I]T_4$ are nonspecifically bound to the nucleus, which is not the case in vivo (1–3). This may be caused by the high concentrations of cytosolic $[^{125}I]T_4$ in the in vitro incubation system. Equilibrium is also not achieved in these studies, at least with respect to the N:M ratio for $T_3(T_4)$ at 3 h (Table III), although this does not appear to change the qualitative aspects of the results.

About 16 h is required for equilibrium to be achieved in vivo between nuclear $T_3(T_4)$ and plasma T_4 in euthyroid rats (3). The estimated free T₄ and T₃ concentrations in the medium were 12 and 3 pg/ml at T₄ and T₃ concentrations of 3.8 and 0.14 ng/ml. The free hormone fractions obtained with this fraction V BSA preparation are lower than those usually obtained with crystalline BSA because it is a cruder preparation. The free fractions in the medium do not change at the T₃ and T₄ concentrations we have employed. Our studies in normal rats suggest that free T₄ and T₃ concentrations are about 15-25 and 1.1-2.0 pg/ml, respectively (27). The data in Table V suggest that increasing the medium-free T₄ concentration to the upper limits of the normal range might decrease the N:M ratio for $T_3(T_4)$, though it would still remain substantially higher than that for $T_3(T_3)$.

We have not been able to determine the reason for the relatively wide range of results in euthyroid rats in different experiments. Small differences in the quantities of pituitary iodothyronines would not appear to be sufficient to influence the N:M ratios based on the evidence from Tables IV and V. Furthermore, because the replication within a given experiment is quite satisfactory (e.g., Table VII), the factor(s) responsible for the variation seems to be common to all flasks of a given experiment. We are continuing to investigate this question, but the present results indicate that use of normal controls for each experiment are sufficient for the present conclusions. The absence of an effect of NaI indicates that artifactual labeling of T_3 with ${}^{125}I^-$ does not play a role in the generation of $[^{125}I]T_3$ in the anterior pituitary, nor does I⁻ inhibit the conversion process per se.

Effects of T_3 and T_4 on pituitary T_4 monodeiodination and nuclear T_3 binding. Since the nuclear T_3 receptor binding capacity is limited, the observation (Table IV) that increasing concentrations of medium T_3 decrease both the $T_3(T_3)$ and $T_3(T_4)$ N:M ratios is not surprising. That R does not change during this process indicates that the nuclear binding of $T_3(T_3)$ and $T_3(T_4)$ are equally affected by increases in tissue T_3 . This suggests that the two T_3 pools, T_3 from the medium and T_3 from intrapituitary T_4 generation, are completely miscible. This is consistent with our previous conclusions based on in vivo studies (2) and with the data in Table I. Secondly, the results also indicate that T_3 itself does not inhibit T_4 to T_3 conversion in anterior pituitary tissue.

The maximum binding capacity for the pituitary nuclear T₃ receptors can be roughly estimated from experiments C and D (Table IV), taking non-specific binding into account. The result is 0.3-0.4 pg T₃/µg DNA, which is about 50% of the in vivo result (3, 28). This difference may be a reflection of the failure to reach equilibrium, as well as of impaired tissue function under in vitro conditions. In vitro only 20% of the tissue T₃ is bound to the nuclei, whereas in vivo this figure is \approx 50% (28).

The data in Table V show that with increasing medium T_4 concentrations, the $T_3(T_4)$ N:M ratio falls before that of $T_3(T_3)$. This suggests that saturation of either the T_4 5'-monodeiodination process or of tissue uptake of T_4 has occurred. Because we have not found substantial differences in tissue [¹²⁵I]T₄ (as reflected by nuclear [¹²⁵I]T₄) with increasing medium T_4 concentrations (Table II), we suspect that it is the conversion mechanism that is saturated. The data are not consistent enough to allow accurate estimation of the T_4 concentration at which saturation occurs, but it could be close to the physiological range for free T_4 . The lack of change in the N:M ratio for $T_3(T_3)$ indicates that the depression of the N:M ratio for $T_3(T_4)$ by T_4 is not caused by saturation of nuclear receptors by T_3 or T_4 .

Physiological alterations in anterior pituitary T_4 5'monodeiodination. Anterior pituitary 5'-monodeiodination appears to be markedly increased in hypothyroid rats. This is in contrast to hepatic T₄ 5'-monodeiodination, which is reduced under similar circumstances (16). This observation is most intriguing and could be explained in several ways. First, it is conceivable that the increased oxygen consumption of the hypothyroid pituitary, possibly a reflection of increased TSH synthesis by the thyrotrophs, is somehow an important factor in the regulation of T_4 5'monodeiodination (29). Increased O₂ consumption in liver is associated with hyperthyroidism and an increased rate of T_4 to T_3 conversion (16). Secondly, Surks and DeFesi (30) have indicated that in chronically hypothyroid rats there is approximately a fourfold increase in the number of thyrotrophs. This increase could explain the increment in T_4 to T_3 conversion if one assumed that this conversion reaction occurred predominantly in the thyrotroph population. At present we have no data on this question. Samuels and Tsai (31) have not observed significant T_4 to T_3 conversion in GH-1 cells grown in culture. Neither has Gershengorn (32) reported T_4 to T_3 conversion in mouse thyrotroph tumor cells in short-term culture. Therefore, both tumors may be inappropriate models for the T_4 to T_3 conversion process in normal pituitary. The increase in activity of T_4 5'-monodeiodination in hypothyroid rats is accompanied by a higher N:M ratio for $T_3(T_3)$ when compared with controls in the same experiment. The differences in the quantities of T₃ in normal and hypothyroid pituitaries would not seem to be great enough in the context of Table IV to produce this change. The N:M T₃(T₃) ratio in hypothyroid pituitaries of 0.84 is not significantly higher than the value of 0.45 in the entire group of normal pituitaries.

This may well be due to the large variation between results of experiments done at different times referred to above. If the N:M $T_3(T_3)$ ratios are systematically higher than normal in hypothyroid rats, as is suggested by the data in Table VI, this could be explained by an increase in nuclear T₃ receptor caused by lack of tonic suppression of T_3 receptor synthesis or assembly. Samuels et al. (33) have reported that about one-half of the nuclear T₃ receptors in GH-1 cells are depletable by incubation with T₃, presumably caused by a decreased synthetic rate of receptor. This hypothesis finds further support in the fact that the N:M ratio for $T_3(T_3)$ in the T₄-treated animals was significantly less than that in euthyroid rats (Table VI). Another possible explanation is that there are different amounts of nuclear T₃ receptor per milligram DNA in different populations of pituitary cells (e.g., thyrotrophs as opposed to somatotrophs), and that the alteration in the cell population of the pituitary gland under hypothyroid conditions is sufficient to result in a change in the N:M $T_3(T_3)$. Further investigation will be required to resolve these questions.

Previous studies by Kaplan and Utiger (16) demonstrate that injection of 10 μ g T₄/d for 5 d causes a two- to threefold increase in hepatic 5'-monodeiodination. In anterior pituitary tissue, the effect is opposite. T_4 5'-monodeiodination is suppressed under these circumstances, and the short time required for this change suggests that this is not due to depletion of thyrotrophs but to an alteration in pituitary cell function. Despite the fact that serum T₄ and T₃ concentrations are higher in T₄-treated animals, the data in Tables IV and V suggest that this cannot explain the phenomenon because pituitary T_4 is only a small fraction of the T_4 in the system. Both of these physiological observations, namely, stimulation of T_4 5'-monodeiodination in hypothyroidism and its suppression in hyperthyroidism, have been observed by Kaplan in pituitary homogenates (34).

A third physiological situation in which hepatic and pituitary T_4 5'-monodeiodination differ is that of fasting. Hepatic T_4 5'-monodeiodination in the rat is inhibited to about 50% of control during fasting (8, 19, 22), whereas there appears to be no effect on pituitary 5'monodeiodination in vitro (35).² It has been suggested that the suppression of hepatic T_4 5'-monodeiodination in fasted rats is caused by depletion of a required cytosolic cofactor, probably GSH (19, 36, 37). Thus, even though GSH concentrations are reportedly lower in pituitary than in liver (38), depletion may occur less readily (37). The maintenance of normal pituitary T_4 5'-monodeiodination in the fasting state in man could partially explain why TSH concentrations do not rise when T_4 to T_3 conversion is reduced and plasma T_3 falls (39-43). However, in the rat, the predominant effect of fasting on the thyroid axis is to suppress TSH, suggesting that a major alteration in thyroid status is achieved through the hypothalamus as well as through alterations in peripheral T_4 to T_3 conversion (8, 44).

Effect of TRH on T_4 5'-monodeiodination. Early studies by Reichlin et al. (45) demonstrate that transplantation of pituitary tissue into the anterior chamber of the eye results in a decrease in the amount of T_3 derived from injected T_4 in that tissue. They suggest that a hypothalamic factor may be required to stimulate T_4 5'-monodeiodination. Because TRH is a likely candidate for such a hormone, it is of interest that this compound does not appear to affect T_4 to T_3 conversion or nuclear T_3 binding in this system in amounts known to affect TSH release. Therefore, even though there may be mechanisms by which hypothalamic hormones can alter T_4 to T_3 conversion, it would not appear that this is a direct and immediate effect of TRH.

In summary, these results extend our earlier in vivo studies and demonstrate clear effects of thyroid status and iopanoic acid on pituitary T_4 to T_3 conversion. The ability to study both T_4 to T_3 conversion and nuclear T₃ binding simultaneously in vitro without the complex techniques and assumptions required for in vivo studies provides obvious advantages for the investigation of the variables involved in this process in this tissue. Qualitative differences are found in the regulation of T_4 to T_3 conversion in this organ when compared with the liver. These differences may have an important physiological role in TSH regulation. Because the thyrotroph is responsible for maintenance of the euthyroid state, it would be appropriate for this cell to be the most active of those in the pituitary with respect to T₄ to T₃ conversion. This is one conclusion that can be drawn from Table VI. Further studies will be required to resolve the uncertainties regarding the cellular regulation of T_4 to T_3 conversion in this unique tissue which appears to derive at least one-half of its nuclear T₃ from local T₄ 5'-monodeiodination.

APPENDIX

The following results of a typical experiment are provided to allow the reader to assess the precision and accuracy of the primary data (4). The results are the mean \pm SD of the individual results of triplicate samples after a 3-h incubation of normal rat anterior pituitary fragments with 0.14 ng/ml medium T₃ and 3.8 ng/ml medium T₄. The medium volume is 400 μ l.

- 1. Pituitary wet weight 8.9±0.7 mg/flask
- 2. Medium [¹³¹I]T₃ (TCA precipitable)
- 3. Medium [¹²⁵I]T₄ (TCA precipitable)

 $\begin{array}{c} 1,267\pm87\\ {\rm cp5m}/\mu{\rm l}\\ 22,694\pm1,499\\ {\rm cp5m}/\mu{\rm l} \end{array}$

² Note added in proof. Kaplan has found a small but statistically significant reduction in T_4 5'-monode iodination rates in anterior pituitary homogenates from fasted rats (45).

4.	Medium [¹²⁵ I]T ₃	
	(a) Total ¹²⁵ I in the " T_3 spot" of the paper	
	chromatograph of the Sepharose	
	eluate of 200 μ l of medium as	
	and [31] and country (postingubation)	10 103+603
	and [1]13 counts (postificultation)	$r_{19,195\pm095}$
	(b) Counts of 125 I in the T ₂ spot due to	cp011/200 µ1
	crossover (16% of the $[^{131}I]T_3$	
	counts). These counts are higher	
	than actually observed on the strip	
	because they have been corrected	
	for decay to zero time. (I his portion	
	until 1-2 wk had elansed.)	10.764 + 256
		cp5m/200 μl
	(c) Net $[^{125}I]T_3$ (line $4a$ -line $4b$)	9,774±864
		cp5m/200 μ l
5.	Recovery of $[^{131}I]T_3$ in the T_3 spot of line $4a$	$27 \pm 1\%$
6.	Total $[^{125}I]T_3$ in the medium (line $4c$	
	\div line 5 \div 200 μ l)	181 ± 16
-	[125]]T /[125]]T /[125]]T	$cp5m/\mu I$
1.	$[1301]1_3/[1301]1_4$ ($[1301]1_3$ contamination)	0.79±0.02%
	Nuclear T ₃ (counts corrected for paper b	ackground)
8.	[¹³¹ I]T ₃	9,597±1,478
		cp5m
9.	[¹³¹ I]T ₃	$1,083 \pm 175$
10	(1951)T. (cp5m/mg
10.	[¹⁰]] ¹ ₃ (corrected for ¹⁰ 1 crossover)	0,525±905
11	[125]]T.	735+99
	[-]-3	cp5m/mg
	N.M. mation	
	N:M ratios	0.00.010
12.	N:M ratios [¹³¹ I]T ₃ (line 9 ÷ line 2)	0.86±0.13
12.	N:M ratios [¹³¹ I]T ₃ (line 9 ÷ line 2)	0.86±0.13 cpm/mg
12.	N:M ratios $[^{131}I]T_3$ (line 9 ÷ line 2) $(^{125}I)T_2$ (line 11 ÷ line 6)	0.86±0.13 cpm/mg cpm/µl medium
12. 13.	N:M ratios [¹³¹ I]T ₃ (line 9 ÷ line 2) [¹²⁵ I]T ₃ (line 11 ÷ line 6)	0.86±0.13 cpm/mg cpm/µl medium 4.08±0.58 cpm/mg
12. 13.	N:M ratios [¹³¹ I]T ₃ (line 9 ÷ line 2) [¹²⁵ I]T ₃ (line 11 ÷ line 6)	$\begin{array}{c} 0.86 \pm 0.13 \\ cpm/mg \\ \hline cpm/\mu l \ medium \\ 4.08 \pm 0.58 \\ cpm/mg \\ \hline cpm/\mu l \ medium \end{array}$
12. 13. 14.	N:M ratios [¹³¹ I]T ₃ (line 9 ÷ line 2) [¹²⁵ I]T ₃ (line 11 ÷ line 6) R (N:M [¹²⁵ I]T ₃ :N:M [¹³¹ I]T ₃) (line 13	0.86±0.13 cpm/mg cpm/μl medium 4.08±0.58 cpm/mg cpm/μl medium
12. 13. 14.	N:M ratios [¹³¹ I]T ₃ (line 9 ÷ line 2) [¹²⁵ I]T ₃ (line 11 ÷ line 6) R (N:M [¹²⁵ I]T ₃ :N:M [¹³¹ I]T ₃) (line 13 ÷ line 12)	0.86±0.13 cpm/mg cpm/μl medium 4.08±0.58 cpm/mg cpm/μl medium 4.78±0.32
12. 13. 14.	N:M ratios $[^{131}I]T_3$ (line 9 ÷ line 2) $[^{125}I]T_3$ (line 11 ÷ line 6) R (N:M $[^{125}I]T_3$:N:M $[^{131}I]T_3$) (line 13 ÷ line 12) Other calculations of interest	0.86±0.13 cpm/mg cpm/µl medium 4.08±0.58 cpm/mg cpm/µl medium 4.78±0.32
12. 13. 14.	N:M ratios $[^{13}I]T_3$ (line 9 ÷ line 2) $[^{125}I]T_3$ (line 11 ÷ line 6) R (N:M $[^{125}I]T_3$:N:M $[^{13}I]T_3$) (line 13 ÷ line 12) Other calculations of interest Exection of total $[^{13}I]T_3$ in the flack	$\begin{array}{c} 0.86 \pm 0.13 \\ \text{cpm/mg} \\ \hline \\ \text{cpm/} \mu \text{l medium} \\ 4.08 \pm 0.58 \\ \text{cpm/mg} \\ \hline \\ \text{cpm/} \mu \text{l medium} \\ 4.78 \pm 0.32 \end{array}$
12. 13. 14. 15.	N:M ratios $[^{131}I]T_3$ (line 9 ÷ line 2) $[^{125}I]T_3$ (line 11 ÷ line 6) R (N:M $[^{125}I]T_3$:N:M $[^{131}I]T_3$) (line 13 ÷ line 12) Other calculations of interest Fraction of total $[^{131}I]T_3$ in the flask found in the nuclei (line 8 ÷ line 2)	0.86±0.13 cpm/mg cpm/µl medium 4.08±0.58 cpm/mg cpm/µl medium 4.78±0.32
12. 13. 14. 15.	N:M ratios $[^{131}I]T_3 (line 9 \div line 2)$ $[^{125}I]T_3 (line 11 \div line 6)$ R (N:M [^{125}I]T_3:N:M [^{131}I]T_3) (line 13 \div line 12) Other calculations of interest Fraction of total [^{131}I]T_3 in the flask found in the nuclei (line 8 ÷ line 2 \div 400 µl)	0.86±0.13 cpm/mg cpm/µl medium 4.08±0.58 cpm/mg cpm/µl medium 4.78±0.32
 12. 13. 14. 15. 16. 	N:M ratios [¹³¹ I]T ₃ (line 9 ÷ line 2) [¹²⁵ I]T ₃ (line 11 ÷ line 6) R (N:M [¹²⁵ I]T ₃ :N:M [¹³¹ I]T ₃) (line 13 ÷ line 12) Other calculations of interest Fraction of total [¹³¹ I]T ₃ in the flask found in the nuclei (line 8 ÷ line 2 ÷ 400 μ l) Counts of [¹²⁵ I]T ₃ expected in the nuclei	0.86±0.13 cpm/mg cpm/µl medium 4.08±0.58 cpm/mg cpm/µl medium 4.78±0.32
 12. 13. 14. 15. 16. 	N:M ratios [¹³¹ I]T ₃ (line 9 ÷ line 2) [¹²⁵ I]T ₃ (line 11 ÷ line 6) R (N:M [¹²⁵ I]T ₃ :N:M [¹³¹ I]T ₃) (line 13 ÷ line 12) Other calculations of interest Fraction of total [¹³¹ I]T ₃ in the flask found in the nuclei (line 8 ÷ line 2 ÷ 400 μ l) Counts of [¹²⁵ I]T ₃ expected in the nuclei in the absence of [¹²⁵ I]T ₄ 5'-mono-	0.86±0.13 cpm/mg cpm/µl medium 4.08±0.58 cpm/mg cpm/µl medium 4.78±0.32
 12. 13. 14. 15. 16. 	N:M ratios [¹³¹ I]T ₃ (line 9 ÷ line 2) [¹²⁵ I]T ₃ (line 11 ÷ line 6) R (N:M [¹²⁵ I]T ₃ :N:M [¹³¹ I]T ₃) (line 13 ÷ line 12) Other calculations of interest Fraction of total [¹³¹ I]T ₃ in the flask found in the nuclei (line 8 ÷ line 2 ÷ 400 μ l) Counts of [¹²⁵ I]T ₃ expected in the nuclei in the absence of [¹²⁵ I]T ₄ 5'-mono- deiodination (line 15 × line 7 × line	0.86±0.13 cpm/mg cpm/µl medium 4.08±0.58 cpm/mg cpm/µl medium 4.78±0.32
 12. 13. 14. 15. 16. 17 	N:M ratios [¹³¹ I]T ₃ (line 9 ÷ line 2) [¹²⁵ I]T ₃ (line 11 ÷ line 6) R (N:M [¹²⁵ I]T ₃ :N:M [¹³¹ I]T ₃) (line 13 ÷ line 12) Other calculations of interest Fraction of total [¹³¹ I]T ₃ in the flask found in the nuclei (line 8 ÷ line 2 ÷ 400 μ l) Counts of [¹²⁵ I]T ₃ expected in the nuclei in the absence of [¹²⁵ I]T ₄ 5'-mono- deiodination (line 15 × line 7 × line 3 × 400 μ l) Evenes of counts of [¹²⁵ I]T ₄ chapter d curve	0.86±0.13 cpm/mg cpm/µl medium 4.08±0.58 cpm/mg cpm/µl medium 4.78±0.32 1.9%
 12. 13. 14. 15. 16. 17. 	N:M ratios [¹³¹ I]T ₃ (line 9 ÷ line 2) [¹²⁵ I]T ₃ (line 11 ÷ line 6) R (N:M [¹²⁵ I]T ₃ :N:M [¹³¹ I]T ₃) (line 13 ÷ line 12) Other calculations of interest Fraction of total [¹³¹ I]T ₃ in the flask found in the nuclei (line 8 ÷ line 2 ÷ 400 μ l) Counts of [¹²⁵ I]T ₃ expected in the nuclei in the absence of [¹²⁵ I]T ₄ 5'-mono- deiodination (line 15 × line 7 × line 3 × 400 μ l) Excess of counts of [¹²⁵ I]T ₃ observed over that derived from [¹²⁵ I]T ₃ contamination	0.86±0.13 cpm/mg cpm/µl medium 4.08±0.58 cpm/mg cpm/µl medium 4.78±0.32 1.9% 1,363 cp5m
 12. 13. 14. 15. 16. 17. 	N:M ratios [¹³¹ I]T ₃ (line 9 ÷ line 2) [¹²⁵ I]T ₃ (line 11 ÷ line 6) R (N:M [¹²⁵ I]T ₃ :N:M [¹³¹ I]T ₃) (line 13 ÷ line 12) Other calculations of interest Fraction of total [¹³¹ I]T ₃ in the flask found in the nuclei (line 8 ÷ line 2 ÷ 400 μ l) Counts of [¹²⁵ I]T ₃ expected in the nuclei in the absence of [¹²⁵ I]T ₄ 5'-mono- deiodination (line 15 × line 7 × line 3 × 400 μ l) Excess of counts of [¹²⁵ I]T ₃ observed over that derived from [¹²⁵ I]T ₃ contamination of the medium (lines 10–16)	$\begin{array}{c} 0.86 \pm 0.13 \\ \text{cpm/mg} \\ \hline \end{array}$
 12. 13. 14. 15. 16. 17. 18. 	N:M ratios [¹³¹ I]T ₃ (line 9 ÷ line 2) [¹²⁵ I]T ₃ (line 11 ÷ line 6) R (N:M [¹²⁵ I]T ₃ :N:M [¹³¹ I]T ₃) (line 13 ÷ line 12) Other calculations of interest Fraction of total [¹³¹ I]T ₃ in the flask found in the nuclei (line 8 ÷ line 2 ÷ 400 μ l) Counts of [¹²⁵ I]T ₃ expected in the nuclei in the absence of [¹²⁵ I]T ₄ 5'-mono- deiodination (line 15 × line 7 × line 3 × 400 μ l) Excess of counts of [¹²⁵ I]T ₃ observed over that derived from [¹²⁵ I]T ₃ contamination of the medium (lines 10–16) Nuclear [¹²⁵ I]T ₄	$\begin{array}{c} 0.86 \pm 0.13 \\ \text{cpm/mg} \\ \hline \end{array}$
 12. 13. 14. 15. 16. 17. 18. 	N:M ratios [¹³¹ I]T ₃ (line 9 ÷ line 2) [¹²⁵ I]T ₃ (line 11 ÷ line 6) R (N:M [¹²⁵ I]T ₃ :N:M [¹³¹ I]T ₃) (line 13 ÷ line 12) Other calculations of interest Fraction of total [¹³¹ I]T ₃ in the flask found in the nuclei (line 8 ÷ line 2 ÷ 400 μ l) Counts of [¹²⁵ I]T ₃ expected in the nuclei in the absence of [¹²⁵ I]T ₄ 5'-mono- deiodination (line 15 × line 7 × line 3 × 400 μ l) Excess of counts of [¹²⁵ I]T ₃ observed over that derived from [¹²⁵ I]T ₃ contamination of the medium (lines 10–16) Nuclear [¹²⁵ I]T ₄	$\begin{array}{c} 0.86 \pm 0.13 \\ \text{cpm/mg} \\ \hline \\ \textbf{cpm/} \mu l \text{ medium} \\ 4.08 \pm 0.58 \\ \text{cpm/mg} \\ \hline \\ \textbf{cpm/} \mu l \text{ medium} \\ 4.78 \pm 0.32 \\ \hline \\ 1.9\% \\ \hline \\ 1.363 \text{ cp5m} \\ \hline \\ 5,160 \text{ cp5m} \\ 5,949 \pm 1,091 \\ \text{cp5m} \\ \end{array}$
 12. 13. 14. 15. 16. 17. 18. 19. 	N:M ratios [¹³¹ I]T ₃ (line 9 ÷ line 2) [¹²⁵ I]T ₃ (line 11 ÷ line 6) R (N:M [¹²⁵ I]T ₃ :N:M [¹³¹ I]T ₃) (line 13 ÷ line 12) Other calculations of interest Fraction of total [¹³¹ I]T ₃ in the flask found in the nuclei (line 8 ÷ line 2 ÷ 400 μ]) Counts of [¹²⁵ I]T ₃ expected in the nuclei in the absence of [¹²⁵ I]T ₄ 5'-mono- deiodination (line 15 × line 7 × line 3 × 400 μ]) Excess of counts of [¹²⁵ I]T ₃ observed over that derived from [¹²⁵ I]T ₃ contamination of the medium (lines 10–16) Nuclear [¹²⁵ I]T ₄	$\begin{array}{c} 0.86 \pm 0.13 \\ \text{cpm/mg} \\ \hline \\ \textbf{cpm/} \mu l \text{ medium} \\ 4.08 \pm 0.58 \\ \text{cpm/mg} \\ \hline \\ \textbf{cpm/} \mu l \text{ medium} \\ 4.78 \pm 0.32 \\ \hline \\ 1.9\% \\ \hline \\ 1,363 \text{ cp5m} \\ \hline \\ 5,160 \text{ cp5m} \\ 5,949 \pm 1,091 \\ \text{cp5m} \\ \end{array}$
 12. 13. 14. 15. 16. 17. 18. 19. 	N:M ratios [¹³¹ I]T ₃ (line 9 ÷ line 2) [¹²⁵ I]T ₃ (line 11 ÷ line 6) R (N:M [¹²⁵ I]T ₃ :N:M [¹³¹ I]T ₃) (line 13 ÷ line 12) Other calculations of interest Fraction of total [¹³¹ I]T ₃ in the flask found in the nuclei (line 8 ÷ line 2 ÷ 400 μ l) Counts of [¹²⁵ I]T ₃ expected in the nuclei in the absence of [¹²⁵ I]T ₄ 5'-mono- deiodination (line 15 × line 7 × line 3 × 400 μ l) Excess of counts of [¹²⁵ I]T ₃ observed over that derived from [¹²⁵ I]T ₃ contamination of the medium (lines 10–16) Nuclear [¹²⁵ I]T ₄	$\begin{array}{c} 0.86 \pm 0.13 \\ \text{cpm/mg} \\ \hline \\ \textbf{cpm/} \mu l \text{ medium} \\ 4.08 \pm 0.58 \\ \text{cpm/mg} \\ \hline \\ \textbf{cpm/} \mu l \text{ medium} \\ 4.78 \pm 0.32 \\ \hline \\ 1.9\% \\ \hline \\ 1,363 \text{ cp5m} \\ \hline \\ 5,160 \text{ cp5m} \\ 5,949 \pm 1,091 \\ \text{cp5m} \\ \end{array}$
 12. 13. 14. 15. 16. 17. 18. 19. 	N:M ratios [¹³¹ I]T ₃ (line 9 ÷ line 2) [¹²⁵ I]T ₃ (line 11 ÷ line 6) R (N:M [¹²⁵ I]T ₃ :N:M [¹³¹ I]T ₃) (line 13 ÷ line 12) Other calculations of interest Fraction of total [¹³¹ I]T ₃ in the flask found in the nuclei (line 8 ÷ line 2 ÷ 400 μ l) Counts of [¹²⁵ I]T ₃ expected in the nuclei in the absence of [¹²⁵ I]T ₄ 5'-mono- deiodination (line 15 × line 7 × line 3 × 400 μ l) Excess of counts of [¹²⁵ I]T ₃ observed over that derived from [¹²⁵ I]T ₃ contamination of the medium (lines 10–16) Nuclear [¹²⁵ I]T ₄ Estimated maximum nuclear [¹²⁵ I]T ₃ due to artifactual deiodination of nuclear [¹²⁵ I]T ₄ during paper chromatography (0.4% of line 18, see reference 6). (Line	$\begin{array}{c} 0.86 \pm 0.13 \\ \text{cpm/mg} \\ \hline \\ \textbf{cpm/} \mu l \text{ medium} \\ 4.08 \pm 0.58 \\ \text{cpm/mg} \\ \hline \\ \textbf{cpm/} \mu l \text{ medium} \\ 4.78 \pm 0.32 \\ \hline \\ 1.9\% \\ \hline \\ 1,363 \text{ cp5m} \\ \hline \\ 5,160 \text{ cp5m} \\ 5,949 \pm 1,091 \\ \text{cp5m} \\ \end{array}$
 12. 13. 14. 15. 16. 17. 18. 19. 	N:M ratios [¹³¹ I]T ₃ (line 9 ÷ line 2) [¹²⁵ I]T ₃ (line 11 ÷ line 6) R (N:M [¹²⁵ I]T ₃ :N:M [¹³¹ I]T ₃) (line 13 ÷ line 12) Other calculations of interest Fraction of total [¹³¹ I]T ₃ in the flask found in the nuclei (line 8 ÷ line 2 ÷ 400 μ l) Counts of [¹²⁵ I]T ₃ expected in the nuclei in the absence of [¹²⁵ I]T ₄ 5'-mono- deiodination (line 15 × line 7 × line 3 × 400 μ l) Excess of counts of [¹²⁵ I]T ₃ observed over that derived from [¹²⁵ I]T ₃ contamination of the medium (lines 10–16) Nuclear [¹²⁵ I]T ₄ Estimated maximum nuclear [¹²⁵ I]T ₃ due to artifactual deiodination of nuclear [¹²⁵ I]T ₄ during paper chromatography (0.4% of line 18, see reference 6). (Line 10 was not corrected for this small	$\begin{array}{c} 0.86 \pm 0.13 \\ \text{cpm/mg} \\ \hline \\ \textbf{cpm/} \mu l \text{ medium} \\ 4.08 \pm 0.58 \\ \text{cpm/mg} \\ \hline \\ \textbf{cpm/} \mu l \text{ medium} \\ 4.78 \pm 0.32 \\ \hline \\ 1.9\% \\ \hline \\ 1,363 \text{ cp5m} \\ \hline \\ 5,160 \text{ cp5m} \\ 5,949 \pm 1,091 \\ \text{cp5m} \\ \end{array}$
 12. 13. 14. 15. 16. 17. 18. 19. 	N:M ratios [¹³¹ I]T ₃ (line 9 ÷ line 2) [¹²⁵ I]T ₃ (line 11 ÷ line 6) R (N:M [¹²⁵ I]T ₃ :N:M [¹³¹ I]T ₃) (line 13 ÷ line 12) Other calculations of interest Fraction of total [¹³¹ I]T ₃ in the flask found in the nuclei (line 8 ÷ line 2 ÷ 400 μ l) Counts of [¹²⁵ I]T ₃ expected in the nuclei in the absence of [¹²⁵ I]T ₄ 5'-mono- deiodination (line 15 × line 7 × line 3 × 400 μ l) Excess of counts of [¹²⁵ I]T ₃ observed over that derived from [¹²⁵ I]T ₃ contamination of the medium (lines 10–16) Nuclear [¹²⁵ I]T ₄ Estimated maximum nuclear [¹²⁵ I]T ₃ due to artifactual deiodination of nuclear [¹²⁵ I]T ₄ during paper chromatography (0.4% of line 18, see reference 6). (Line 10 was not corrected for this small artifact.)	$\begin{array}{c} 0.86 \pm 0.13 \\ cpm/mg \\ \hline cpm/\mu l medium \\ 4.08 \pm 0.58 \\ cpm/mg \\ \hline cpm/\mu l medium \\ 4.78 \pm 0.32 \\ \hline 1.9\% \\ \hline 1,363 cp5m \\ 5,160 cp5m \\ 5,949 \pm 1,091 \\ cp5m \\ \hline 24 cp5m \end{array}$
 12. 13. 14. 15. 16. 17. 18. 19. 	N:M ratios [¹³¹ I]T ₃ (line 9 ÷ line 2) [¹²⁵ I]T ₃ (line 11 ÷ line 6) R (N:M [¹²⁵ I]T ₃ :N:M [¹³¹ I]T ₃) (line 13 ÷ line 12) Other calculations of interest Fraction of total [¹³¹ I]T ₃ in the flask found in the nuclei (line 8 ÷ line 2 ÷ 400 μ l) Counts of [¹²⁵ I]T ₃ expected in the nuclei in the absence of [¹²⁵ I]T ₄ 5'-mono- deiodination (line 15 × line 7 × line 3 × 400 μ l) Excess of counts of [¹²⁵ I]T ₃ observed over that derived from [¹²⁵ I]T ₃ contamination of the medium (lines 10–16) Nuclear [¹²⁵ I]T ₄ Estimated maximum nuclear [¹²⁵ I]T ₃ due to artifactual deiodination of nuclear [¹²⁵ I]T ₄ during paper chromatography (0.4% of line 18, see reference 6). (Line 10 was not corrected for this small artifact.)	$\begin{array}{c} 0.86 \pm 0.13 \\ cpm/mg \\ \hline cpm/\mu l medium \\ 4.08 \pm 0.58 \\ cpm/mg \\ \hline cpm/\mu l medium \\ 4.78 \pm 0.32 \\ \hline 1.9\% \\ \hline 1,363 cp5m \\ 5,160 cp5m \\ 5,949 \pm 1,091 \\ cp5m \\ \hline 24 cp5m \\ \end{array}$
 12. 13. 14. 15. 16. 17. 18. 19. 	 N:M ratios [¹³¹I]T₃ (line 9 ÷ line 2) [¹²⁵I]T₃ (line 11 ÷ line 6) R (N:M [¹²⁵I]T₃:N:M [¹³¹I]T₃) (line 13 ÷ line 12) Other calculations of interest Fraction of total [¹³¹I]T₃ in the flask found in the nuclei (line 8 ÷ line 2 ÷ 400 µl) Counts of [¹²⁵I]T₃ expected in the nuclei in the absence of [¹²⁵I]T₄ 5'-mono-deiodination (line 15 × line 7 × line 3 × 400 µl) Excess of counts of [¹²⁵I]T₃ contamination of the medium (lines 10–16) Nuclear [¹²⁵I]T₄ Estimated maximum nuclear [¹²⁵I]T₃ due to artifactual deiodination of nuclear [¹²⁵I]T₄ during paper chromatography (0.4% of line 18, see reference 6). (Line 10 was not corrected for this small artifact.) 	$\begin{array}{c} 0.86 \pm 0.13 \\ cpm/mg \\ \hline cpm/\mu l medium \\ 4.08 \pm 0.58 \\ cpm/mg \\ \hline cpm/\mu l medium \\ 4.78 \pm 0.32 \\ \hline 1.9\% \\ \hline 1,363 cp5m \\ 5,160 cp5m \\ 5,949 \pm 1,091 \\ cp5m \\ \hline 24 cp5m \\ \hline \end{array}$

This work was supported by grants AM 18616, 1 F32 AM 05826, and 5-F32-AM 05553 from the National Institute of Arthritis, Metabolism, and Digestive Diseases; Biomedical Research Support grant S07 RR05489-16 from the National Institutes of Health, Division of Research Resources, and a grant from the William F. Milton Fund.

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