Triiodothyronine and Thyroxine in the Serum and Thyroid Glands of Iodine-Deficient Rats

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ABSTRACT Triiodothyronine (T_3) and thyroxine (T_4) were measured by immunoassay in the serum and thyroid hydrolysates of control (group A), mildly iodine-deficient (group B), and severely iodine-deficient rats (group C). These results were correlated with changes in thyroidal weight, ^{131}I uptake and ^{127}I content as well as with the distribution of ^{131}I in Pronase digests of the thyroid. There was a progressive increase in thyroid weight and ^{131}I uptake at 24 h with decrease in iodine intake. The ^{127}I content of the thyroids of the group B animals was 44% and that of the group C animals 2% of that in group A. The mean labeled monoiodotyrosine/diiodotyrosine (MIT/DIT) and T_3/T_4 ratios in group A were 0.42±0.07 (SD) and 0.12±0.01, 0.59±0.06 and 0.11±0.03 in group B, and 2.0±0.3 and 1.8±0.9 in the group thyroid digests.

Mean serum T_4 concentration in the control rats was 4.2±0.6 (SD) μg T_4/100 ml, 4.5±0.3 μg/100 ml in group B animals, and undectectable (<0.5 μ_4/100 ml) in group C animals. There was no effect of iodine deficiency on serum T_3 concentrations, which were 44± 9 (Mean±SD) ng/100 ml in A animals, 48±6 ng/100 ml .n B animals, and 43±6 ng/100 ml in the C group. Thyroidal digest T_3 and T_4 concentrations were 39 and 400 ng/mg in group A animals and were reduced to 5 and 1% of this, respectively, in group C. The molar ratio of T_3/T_4 in the thyroid digests of the groups A and B animals was identical to the ratio of labeled T_3/T_4 and was slightly less (1.0±0.9) than the labeled T_3/T_4 ratio in the group C animals.

The mean ratio of labeled T_4 to labeled T_3 in the serum of the severely iodine-deficient animals 24 h after isotope injection was 11±1 (SEM). With previously published values, it was possible to correlate the ratio of labeled T_4/T_3 in the thyroid digest with the labeled T_4/T_3 ratio in the serum of each iodine-deficient animal. This analysis suggested that the labeled thyroid hormones in the severely iodine-deficient rat were secreted in the ratio in which they are present in the gland.

Kinetic analysis of total iodothyronine turnover indicated that two-thirds of the T_3 utilized per day by the iodine-sufficient rat arises from T_4. If the T_4–T_3 conversion ratio remains the same in iodine deficiency, then the analysis suggests that about 90% of the T_3 arises directly from the thyroid. Therefore, it would appear that absolute T_3 secretion by the thyroid increases severalfold during iodine deficiency. The fact that serum T_3 remains constant and T_4 decreases to extremely low levels, combined with previous observations that iodine-deficient animals appear to be euthyroid, is compatible with the hypothesis that T_4 in the normal rat serves primarily as a precursor of T_3.

INTRODUCTION

The thyroidal response to iodine deficiency has been an area of active investigation for many years. Previous studies in the rat reviewed by Studer and Greer indicate that when iodine intake is severely restricted there is an increase in thyroid weight, a decrease in protein-bound iodine (PBI)[1] and an altered pattern of tracer iodine distribution in the thyroid gland (1). The last-mentioned changes include an increase in labeled monoiodotyrosine (MIT) and decrease in labeled diiodotyrosine (DIT) as well as a progressive increase in the ratio of labeled triiodothyronine (T_3) to labeled thyroxine (T_4). Although Studer and Greer, and Greer, Grimm, and Studer have demonstrated that serum PBI decreases to very low levels within 1 mo of initiation of a low-iodine diet, rats maintained on this regimen for even 1 yr re-

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[1] *Abbreviations used in this paper:* DIT, diiodotyrosine; MIT, monoiodotyrosine; PBI, protein-bound iodine; T_3, triiodothyronine; T_4, thyroxine; TAA, tertiary-amyl-alcohol/hexane/ammonia; TSH, thyroid-stimulating hormone.

main healthy and grow at normal rates (2, 3). Recent studies by Silva have shown that O_2 consumption and body temperature regulation of rats under severe iodine restriction for 120 days is not different from that of control animals (4).

The shift of the predominant intrathyroidal-labeled iodothyronine from T_4 to T_3 in response to iodine restriction has led to speculation that the iodine-deficient rat maintains its apparent euthyroid status by preferential synthesis and release of T_3 (3). Since it has not been possible previously to measure serum T_3 directly under these circumstances, this hypothesis has not been substantiated. While this study was in progress, Volpert and Werner presented data suggesting that there was a decrease in the immunoassayable serum T_3 in the iodine-deficient rat though the ratio of T_3 to PBI was increased (5). However, the small group of animals examined necessarily limited the scope of their conclusions. In the following study data are presented that correlate the changes in serum immunoassayable T_3 and T_4 with changes in thyroidal T_3, T_4, and iodine content in mildly and severely iodine-deficient rats.

METHODS

Animals and diets. Sprague-Dawley male rats weighing 150–200 g were maintained on Remington low-iodine test diet (Diet I, Nutritional Biochemicals Corporation, Cleveland, Ohio) for a period of 3 mo. Half of this group received distilled water and the other half distilled water containing 1.3 μg iodide/ml (estimated intake 20–30 ml H_2O/day). After 3 mo the supplier of the low-iodine diet was changed (Diet II, General Biochemicals Div., Mogul Corp., Chagrin Falls, Ohio) since the anticipated elevation in MIT/DIT ratios was not observed up to this time. The level of iodine supplementation in the control animals remained the same. However, the substitution of a different commercial low-iodine diet after 3 mo of this study made it impossible to examine the longitudinal effects of sustained iodine restriction under constant conditions. Therefore, we have not attempted an analysis of the data as related to duration of iodine deprivation. The diets were analyzed by the Boston Medical Laboratory, and both contained nondetectable amounts of iodine (< 0.165 μg/g diet [6]). The fact that the thyroidal changes in animals receiving diet II were substantially greater than those in animals receiving diet I is best explained by the assumption that it contained less iodine. However, without verification of this by actual measurement, the theoretical possibility that diet II contains a goitrogen that accelerated the appearance of the changes in the low-iodine animals cannot be entirely discounted.

Administration of ^{131}I and calculation of uptake. 5–40 μCi of carrier-free ^{131}I was injected intraperitoneally 24 h before sacrifice. Groups of control and iodine deficient-rats were killed at various intervals after starting the diets and thyroids were dissected from the trachea and weighed. Radioactivity in thyroids and suitable dilutions of the injected dose were measured with an end window Geiger counter under conditions of constant geometry. Blood was obtained from each animal by cardiac puncture.

Digestion and extraction. One or two thyroid glands were digested with Pronase (Calbiochem, Los Angeles, Calif.), by the method of Inoue and Taurog (7). This procedure was slightly modified as we have previously described (8). Labeled [^{125}I]T_3 and T_4 (Abbott Laboratories, North Chicago, Ill.) were added at the start of digestion to monitor recovery and identify [^{131}I]iodothyronines. The labeled iodothyronines contained less than 3% I^- and no other significant contaminants. In addition, after digestion the hydrolysate was extracted twice with 0.4 ml of methanol in 7.4 N ammonia (1:1, vol:vol). Residual ^{131}I and ^{125}I in the pellet after this procedure were 6% or less of the total. Analysis of the pellet ^{127}I in the animals on the high-iodine intake showed similar losses of ^{127}I. Digestion of extracts was nearly complete as estimated by chromatography of the extracts in collidine—2 N ammonia (3:1, vol:vol). The amount of ^{127}I and ^{131}I remaining at the origin was less than 4%. The identification of ^{131}I-labeled I-*,[2] MIT* and DIT* was carried out as previously described by chromatography in butanol/acetic acid (8). Separation of T_4* and T_3* was obtained in tertiary-amyl-alcohol hexane/ammonia (TAA) also as previously described (8). Briefly, 25–75 μl of the extract was chromatographed in both systems, and 1–2 cm segments of the strips were subsequently counted for both ^{125}I and ^{131}I with suitable correction. In the TAA system, the percent T_3* was corrected for the 0.34% artifactual deiodination of T_4* during the chromatography as we have previously described (9). T_3* and T_4* were corrected for losses during the procedure by reference to the original amounts of [^{125}I]T_3 and T_4 added. Results were expressed as a fraction of the total ^{131}I in the extract.

^{127}I content in 0.1 ml of the extract (about 0.8 ml total volume) was determined by Boston Medical Laboratory (6). This was then corrected for the total extraction volume as well as the small losses occurring during extraction of the Pronase hydrolysate. Results were expressed as ng ^{127}I per mg wet weight of thyroid.

Serum and thyroidal T_3 and T_4 content. T_3 and T_4 content in the thyroid hydrolyates and serum were determined by radioimmunoassays as previously described (10, 11). All assays included samples from paired control and iodine-deficient rats and were performed in duplicate at two dilutions. In analyzing the extract for iodothyronines, suitable dilutions were made in MeOH/NH_4OH (99:1, vol:vol). 5 or 10 μl of this dilution was then added to tubes containing either T_3-free or T_4-free human serum in 1 ml total volume. This concentration of MeOH/NH_4OH does not affect the assays. In measuring T_4 and T_3 in thyroid hydrolysates from iodine sufficient rats, the MeOH/NH_4OH dilutions used were 1/100 to 1/500. In both assays, curves parallel to the standard were obtained with increasing quantities of extract. Thus, the duplicate determinations at two dilutions were in excellent agreement. In the extracts of thyroids from the group C animals, dilutions as low as $\frac{1}{2}$ and $\frac{1}{5}$ were sometimes necessary for accurate quantitation, indicating that there was no artifactual contribution to either the calculated T_3 or T_4 value from noniodine-containing substances in the thyroid extracts. Again, excellent agreement was obtained in the estimated iodothyronine content with two different dilutions. There is no significant displacement of either labeled iodothyronine from its antibody by 1000-fold excesses (by weight) of I^-, MIT, or DIT. Since there is essentially no cross-reaction of the T_3 antibody with T_4, prior separation of T_3 and T_4 is

[2] An asterisk will be used to denote ^{131}I-labeled compounds present in thyroid glands or in serum.

not required for this method. Total thyroid T_3 and T_4 were corrected for losses in extraction and expressed as ng T_3 or T_4 per mg wet weight of thyroid.

Analysis of serum T_3 was performed by using a modification of the T_3-free human serum system that employs sodium salicylate to block T_3-protein binding (10). Preliminary studies indicated that as much as 100 μl of rat serum could be substituted for human serum in the assay without disturbing the parallelism of the rat serum curve with the standard curve or changing the percentage of tracer bound in the absence of antibody. Thus, unknown samples contained either 100 or 50 μl unknown rat serum plus 100 or 150 μl of T_3-free human serum added to make the serum concentration 20% in all tubes. Duplicates of the two dilutions were in excellent agreement. T_3-free rat serum prepared as we have previously described for human serum contained no T_3 (10). Recovery of T_3 from pooled rat sera was 96±8% (mean±SD) for 100 ng T_3/100 ml and 99 ±8% (mean±SD) for 200 ng T_3/100 ml. Similar recoveries were obtained using serum from iodine-deficient rats in a smaller series of studies. Likewise, the T_4 determinations could be performed in the system containing T_4-free human serum as long as the quantity of rat serum per tube did not exceed 5 μl (total serum per tube, 10 μl).

Distribution of labeled compounds in serum 24 h after ^{131}I injection. In the severely iodine-deficient animals the distribution of radioactivity in the serum was analyzed. The value for I^{-}* was determined as the percent radioactivity migrating from the origin during 90-min electrophoresis of whole serum in glycine-acetate buffer, pH 8.6, at 150 V. T_3* and T_4* were determined by the method of Sterling, Dellabarba, Newman, and Brenner, as modified in our laboratory (9, 12). [^{125}I]T_3 and T_4 were added before chromatography to allow correction for losses. T_3* was corrected for the artifactual increases due to deiodination of T_4* as described above. Results were expressed as a fraction of the total serum radioactivity.

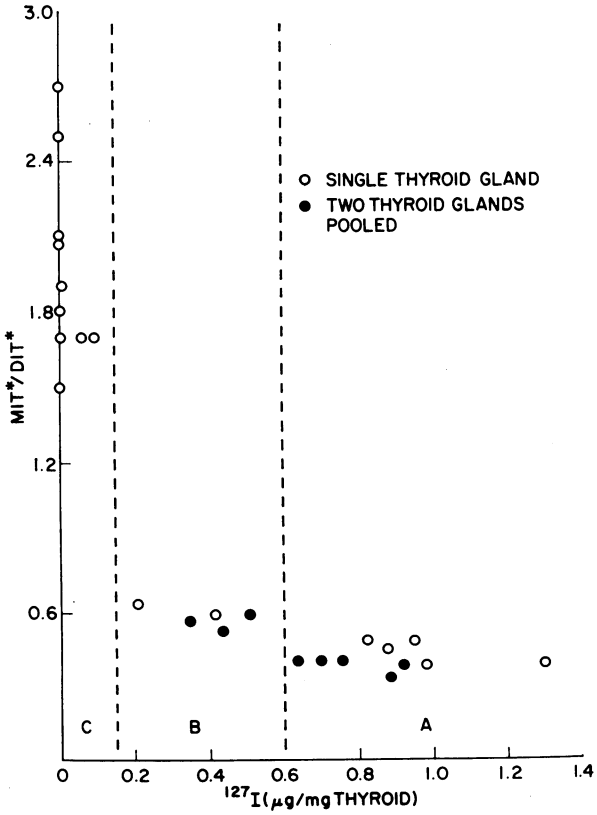

FIGURE 1 Relationship between iodine content and the ratio of ^{131}I-labeled MIT to DIT in the pronase hydrolysates of rat thyroid glands. The letters A, B, and C denote the grouping of the animals: A, control; B, mild iodine deficiency; and C, severe iodine deficiency.

RESULTS

Effect of iodine deprivation on thyroidal weight, ^{127}I content, ^{131}I uptake and the distribution of ^{131}I in thyroidal iodoamino acids. Since previous pulse-labeling studies have shown that the ratio MIT*/DIT* is a sensitive index of iodine deficiency (13, 14), animals were grouped on the basis of this parameter. The animals were classified as follows: A, control (MIT*/DIT*, < 0.5); B, mildly iodine-deficient (MIT*/DIT*, 0.51–0.75); and C, severely iodine-deficient (MIT*/DIT*, > 1.5). All animals that received iodine supplementation had MIT*/DIT* ratios less than 0.5, regardless of diet, and were in group A. Group B consisted of animals that had received diet I and no supplement for 4–8 wk. All group C animals had received Diet II for 4 wk (two animals), 8 wk (four animals), or 12 wk (four animals). The body weights of control and iodine-deficient animals studied at each interval were not different.

In Fig. 1 the correlation between the MIT*/DIT* and thyroidal iodine content is illustrated. There is a progressive increase in the MIT/DIT ratio as the mean iodine content of the thyroid gland decreases. The mean thyroidal ^{127}I in the thyroids of the various groups is presented in Table I, and for group A was 880 ng/mg; group B, 390 ng/mg; and group C, 21 ng/mg. Thus, the iodine concentration of the thyroids of the group C animals was reduced to about 2% of the iodine present in the A group.

The validity of this classification was further substantiated by the significant differences among the various groups with respect to two other characteristics of the thyroidal response to iodine deficiency. The thyroid weight was moderately increased in the B group, 5.1 mg/100 g body wt as opposed to 4.1 mg in controls ($P < 0.005$). It was increased to 12 mg/100 g body wt in the C group, approximately three times the weight in the control group ($P < 0.001$). The thyroidal uptake of ^{131}I at 24 h increased progressively with iodine deficiency. However, since the maximal uptake of ^{131}I occurs at time intervals earlier than 24 h in the severely iodine-deficient rat, the increase from control values of 15% in group A to 42% in group C ($P < 0.001$) may underestimate the difference in maximal ^{131}I uptake.

TABLE I

^{127}I Content, Gland Weight, and Uptake and Distribution of ^{131}I in Pronase Hydrolysates of Thyroid Glands from Control and Iodine-Deficient Rats (Mean±SD)

Group	n	^{127}I	Thyroid weight	RAIU‡	I^-*	MIT*	DIT*	T_3*	T_4*	MIT*/DIT*	T_3*/T_4*
		ng/mg thyroid	*mg/100 g body wt*								
A	12	880±180	4.1±0.7	15±10	4.8±1.0	19±3	46±3	2.6±0.3	21±1	0.42±0.07	0.12±0.01
B	9	390±100	5.1±0.6	27±11	3.8±1.1	26±3	44±3	2.5±0.8	23±3	0.59±0.06	0.11±0.03
C‖	1	7.7	10	55	5.0	44	17	20	7.3	2.7	2.7
	2	4.4	13	49	3.5	41	16	19	5.2	2.5	3.5
	3	6.9	12	36	3.3	38	18	17	6.7	2.1	2.4
	4	6.4	14	43	5.5	41	20	16	12	2.1	1.3
	5	6.1	13	37	6.0	38	20	16	14	1.9	1.1
	6	9.5	7	48	5.1	36	21	23	17	1.8	1.4
	7	7.7	12	37	4.3	33	19	22	17	1.7	1.3
	8	7.2	16	54	2.6	32	21	23	17	1.5	1.4
	9§	55	11	26	3.6	37	22	18	12	1.7	1.5
	10§	97	7.1	33	2.7	38	23	18	14	1.7	1.3
Mean ±SD		21±30	12±3	42±9	4.2±1.2	38±3	20±3	19±3	12±3	2.0±0.3	1.8±0.9

‡ Uptake of ^{131}I in thyroid 24 h after isotope injection.
§ Animals on iodine restriction for 4 wk.
‖ In group C, data are provided for individual animals.

The distribution of radioactivity in the Pronase hydrolysates of the thyroids is presented in Table I. The I^-* percentage was not different in the three groups. The percent of MIT* increases progressively as the iodine supply is further restricted, the mean being 19 in group A, 26 in group B, and 38 in group C. There is no difference in the DIT* percentage in groups A and B, but the value of 20% in group C is significantly lower than that observed in the control group ($P < 0.001$). In addition, there is also no significant difference between the A and the B animals with regard to the relative distribution of isotope in T_3 and T_4, while in group C there is a significant increase in the percent T_3* from a mean of 2.6 in groups A and B to 19 in group C ($P < 0.001$). In addition, there is a substantial decrease in the percent T_4* from a mean of 22 in groups A and B, to 12 in group C ($P < 0.001$). Approximately 90–100% of the label present in the thyroid hydrolysate is accounted for by these five components. The remainder of the labeled material appeared to be distributed evenly along chromatographic paper strips between the identifiable peaks.

As previously mentioned, there is a progressive increase in the ratio of MIT*/DIT* with increasing severity of iodine restriction. The ratio of T_3*/T_4* is 0.12 in group A, and not significantly different (0.11) in group B. However in group C the ratio is reversed, T_3* being 1.8 times T_4*. The distribution of the isotope in these normal and iodine-deficient glands is substantially the same as that observed by previous investigators (1).

Serum T_3 and T_4 levels in control and iodine-deficient rats. Mean levels of serum T_3 and T_4 are presented in Table II. Where thyroid glands from two animals were pooled, the mean of the values for serum T_3 and T_4 were used. Serum T_3 levels in the control animals ranged from 29 to 54 ng/100 ml with a mean of 44. T_3 levels were not significantly different in group B with a range of from 42 to 61 ng/ml with the mean 48 ng/100 ml. In group C, the mean serum T_3 concentration was unchanged from the group A animals, being 43 ng/100 ml. Thus there was no change in the serum T_3 concentration in the iodine-deficient rat.

In the second column are given the concentrations of immunoassayable T_4. In control animals, the mean was 4.2 μg/100 with a range of 3.6–5.3 μg/100 ml. Again there was no difference in the values obtained in the group B animals, where the mean was 4.5±0.1 (mean ±SD). However, in the group C animals, T_4 was undetectable. With current methods, we were able to quantitate T_4 in rat serum to a minimum of 0.5 μg/100 ml. In parenthesis the values for PBI are shown, as determined by Boston Medical Laboratory in the serum of most of the group C animals. While it is not known precisely what quantities of noniodothyronine iodine are present in severely iodine-deficient rats, these low PBI values confirm the low serum T_4 estimates obtained by immunoassay. Inspection of the group C

animals shows that in animals 9 and 10, which had been on the diet for a period of only 4 wk, T_4 had already disappeared. These animals were examined at 10 days after institution of the low-iodine diet and still had normal T_4 levels at that time.

T_3 and T_4 in the pronase hydrolysates of control and iodine-deficient thyroid glands. The T_3 and T_4 concentrations of the rat thyroid hydrolysates are also shown in Table II. In the thyroid glands of the control rats, the T_3 concentration varied from 27 to 58 ng/mg wet wt with a mean of 39 ng/mg. In mildly deficient animals, T_3 content was moderately reduced to a mean of 26 ng/mg ($P < 0.005$). An even more substantial reduction, to less than 5% of the control group, was observed in the severely iodine-deficient thyroids. The mean T_3 content in this group was 1.7 ng/mg. While the T_3 concentration was markedly reduced in the group C animals, the larger size of the thyroid gland resulted in an overall decrease of the gland T_3 content of approximately 85%, from a mean of 0.64 μg to about 0.10 μg/total gland.

T_4 content of the normal rat thyroid gland was 400 ng/mg wet wt. Like T_3, the concentration of this iodothyronine was reduced in mild iodine deficiency to a mean of 270 ng/mg, significantly less than that of the A group ($P < 0.001$). The mean thyroidal T_4 content in the C animals was 3.2 ng/mg wet wt, less than 1% of the control T_4 concentration. This is similar in magnitude to the reduction in the ^{127}I content of the gland. The total T_4 content of the goitrous glands was only 3% of the control value.

Of considerable interest is the fact that the molar ratio of T_3 to T_4 in these thyroid glands is similar to the ratio of T_3^*/T_4^* given in Table I. This ratio is precisely the same for groups A and B. However, in group C there appears to be a slight difference, the mean molar ratio of T_3/T_4 of 1.0 being less than the ratio of 1.8 of the labeled hormones. However, the ratio is markedly increased over the control value by both methods. Thus, it would appear that the T_3^*/T_4^* ratio even as soon as 24 h after labeling is an accurate reflection of the absolute ratio of T_3 to T_4 present in the iodine-sufficient gland. This agreement occurs despite the fact that prolonged periods are required for complete equilibration of labeled iodine in the thyroid gland, and it will be discussed at greater length below.

Distribution of ^{131}I in the serum of severely iodine-deficient rats. Analysis of the labeled components in the serum in iodine-deficient rats was performed 24 h after isotope injection. Accurate estimates of T_3^* and T_4^* were possible only in this group, due to the high ratio of T_4^* to T_3^* present in normal animals. Since there is artifactual deiodination of a variable fraction of T_4^* during chromatographic separation, it is difficult to determine accurately how much T_3^* present in normal serum arises in vivo.

Direct chromatography of serum in collidine/NH_4OH indicated that the only labeled components in serum of iodine-deficient rats were I^-*, T_3*, and T_4*, as has been previously reported (3). As can be seen in Table III, the quantity of I^-* present at 24 h was quite small, in all cases 6% or less of the total. The percentage of

TABLE II

T3 and T4 Concentrations in Serum and Thyroids of Control and Iodine-Deficient Rats (Mean±SD)

Group	n	Serum		Thyroid				Molar ratio T_3/T_4
		T_3	T_4	T_3		T_4		
		ng/100 ml	*μg/100 ml*	*ng/mg*	*μg/gland*	*ng/mg*	*μg/gland*	
A	12	44±9	4.2±0.6	39±10	0.64±0.27	400±100	6.7±3.0	0.12±0.03
B	9	48±6	4.5±0.3	26±6	0.44±0.14	270±85	4.4±0.8	0.12±0.03
C§	1	41	<0.50 (0.4)‡	0.79	0.042	0.82	0.044	1.1
	2	45	" (0.6)	0.55	0.039	0.18	0.013	3.6
	3	40	"	0.88	0.065	1.9	0.14	0.55
	4	48	" (0.6)	0.97	0.081	2.2	0.19	0.53
	5	41	"	0.89	0.079	1.1	0.097	0.97
	6	33	" (0.6)	2.9	0.14	3.7	0.18	0.93
	7	35	" (0.6)	2.0	0.16	2.8	0.22	0.85
	8	58	" (0.6)	2.9	0.30	2.5	0.25	0.83
	9‖	40	" (0.8)	1.8	0.070	6.4	0.38	0.33
	10‖	53	" (0.6)	3.6	0.14	9.9	0.38	0.43
Mean±SD		43±6	<0.50 (0.6±0.1)	1.7±0.9	0.099±0.078	3.2±2.8	0.19±0.12	1.01±0.90

‡ PBI, μg I/100 ml.
§ In Group c, data are provided for individual animals.
‖ Animals on iodine restriction for 4 wk.

label as T_3 varied from 5 up to 17% at this time, whereas T_4* ranged from 70 to 102% of the total. Since T_3* and T_4* content of the original serum was determined independently by ^{125}I-labeled recovery standards, it was possible that the total percent recovery could be greater than 100. The mean recovery of labeled compounds totaled 96.5%, quite close to the anticipated figure of 100%. The ratio of T_4*/T_3* in the serum of the iodine-deficient animals varied from a low of 4.1 to as high as 18 with a mean of 11. In the last column, the predicted ratio of T_4*/T_3* is presented based on the gland T_4*/T_3*. The derivation of these ratios is discussed subsequently.

Estimation of the respective contributions of the thyroid and peripheral T_4-to-T_3 conversion to the peripheral T_3 pool in the rat. It has been previously shown that peripheral T_4-to-T_3 conversion contributes substantially to the circulating T_3 pool in man (15). A similar pathway for T_4 metabolism has been shown in the rat by Schwartz, Surks, and Oppenheimer (16). If the clearances of T_3 and T_4 do not change in the iodine-deficient rat, it should be possible to calculate the relative contributions of the thyroid and peripheral T_4-to-T_3 conversion to the serum T_3 pool in this situation. If plasma T_3 or T_4 clearance did change in iodine deficiency, then the knowledge of the serum concentrations alone would not suffice to calculate the total production rate of each hormone. Silva has recently demonstrated that there are no changes in the clearance of labeled T_3 and T_4 in rats deprived of iodine for 3 mo (4) Using the kinetic data for T_3 and T_4 clearance in the rat, previously published by Oppenheimer et al. (17), and Silva's conclusions (4), it is possible to calculate total T_3 and T_4 utilization in the control and iodine-deficient rats by using our values for serum T_3 and T_4. It is then possible to estimate the relative proportion of T_3 coming from T_4 and that coming from the thyroid gland with the T_4-to-T_3 conversion ratio of 0.17 determined by Schwartz et al. in the rat (16). The estimates of the quantities of T_3 and T_4 metabolized by these rats are presented in Table IV (see Appendix for formulae). Total T_4 degradation is 710 and 760 ng/100 g body wt/day in groups A and B. Total T_3 metabolism is 150 and 160 ng/100 g/day with approximately 67% of this T_3 arising from T_4 to T_3 deiodination. In group C, the total T_4 metabolism is reduced to approximately 11% of that present in the A and B groups even if the maximum T_4 value of 0.5 μg/100 ml is used as the estimate of the T_4 level. Nevertheless, T_3 utilization is essentially unchanged. Since there is little T_3 arising from T_4, over 90% of this T_3 must originate from the thyroid gland if the conversion rate of 0.17 remains constant. While these data are obviously approximations, it would appear that a two-to-threefold increase in the absolute thyroidal T_3 secretion rate must occur in the iodine-deficient rat. Even if the T_4 to T_3 conversion rate increased to 100% in these animals, a maximum of 71 ng of T_3/100 g body wt/day could be generated. This is still less than one-half of the T_3 utilized per day.

TABLE III

Labeled Compounds in the Serum of Severely Iodine-Deficient Rats 24 h After ^{131}I Administration

	% Total				
	I^-*	T_3*	T_4*	T_4*/T_3*	Predicted‡ T_4*/T_3*
1	4.5	10	74	7.4	7.0
2	6.3	17	70	4.1	5.5
3	4.4	10	74	7.4	7.7
4	2.6	5.6	84	15	13
5	2.4	5.0	92	18	16
6	4.1	5.6	89	16	13
7	3.2	7.6	83	11	14
8	4.0	7.5	90	12	12
9	4.4	8.6	83	9.7	12
10	4.6	7.4	102	14	13
Mean	4.1	8.4	84	11	11
SEM	0.4	1.1	3	1	1

‡ Predicted on the basis of the gland T_4*/T_3* (see Discussion).

TABLE IV

Analysis of the Quantity and Origin of T_3 and T_4 Utilized by the Rat‡

Group	T_4	T_3	Source of T_3			
			T_4		Thyroid	
	ng/100 g day^{-1}	*ng/100 g day^{-1}*	*ng*	*% Total*	*ng*	*% Total*
A	710	150	101	67	49	33
B	760	160	108	68	52	32
C	85§	150	12	8	138	92

‡ Values based on mean serum iodothyronine concentrations in the three groups.
§ Calculated if maximum serum T_4 (0.5 μg/100 ml) is assumed.

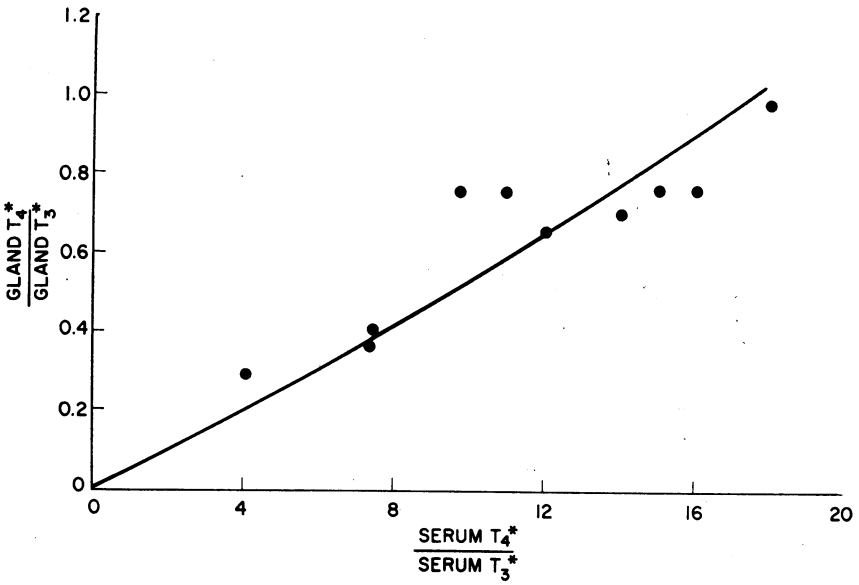

FIGURE 2 Correlation of predicted and observed ratios of ^{131}I-labeled T_4^*/T_3^* in serum and thyroid glands of severely iodine-deficient rats based on the assumption that the labeled hormones are secreted in the same ratio present in the gland. The solid line is constructed from the equation:

$$\frac{\text{Serum } T_4^*}{\text{Serum } T_3^*} = \frac{20.2 \text{ Gland } T_4^*}{\text{Gland } T_3^* + 0.17 \text{ Gland } T_4^*}.$$

The points indicate the observed ratios in severely iodine-deficient animals.

Inspection of Table IV also reveals that the calculated molar ratio of secreted T_3 to secreted T_4 in the group C animals is about 2:1, similar to the ratio of T_3^*/T_4^* (1.8) in the thyroid glands of this group. The similarity of these ratios in the iodine-deficient rat suggested that these hormones might be secreted in the ratio in which they exist in the gland. Previous investigators have made similar speculations (3). Since both gland and serum T_4^*/T_3^* ratios were determined in the iodine-deficient animals, it was possible to test this hypothesis rigorously by attempting to predict the serum T_4^*/T_3^* ratio based on the gland T_4^*/T_3^*. This requires the assumption that there is relative equilibrium of the T_3^*/T_4^* ratios in the gland and serum at 24 h. This has been verified experimentally for the gland T_3^*/T_4^* by previous investigators (1). Since the half-lives of T_3 and T_4 in the rat are 6 and 12 h., respectively, it seems likely that the various differences in the distribution volume and fractional clearance rates of T_3 and T_4 have already taken place 1 day after injection.

Based on these assumptions, the ratio of secreted hormones can be calculated by substituting the percent label in gland T_3 and T_4 in the appropriate equations, which are derived in the Appendix. In Fig. 2, the ideal line for this relationship has been constructed. The equation yields a hyperbolic curve, though at the lower extreme it approximates a straight line. Also plotted in this figure are the actual ratios of gland T_4^*/T_3^* and serum T_4^*/T_3^* in the group C animals where accurate estimates of serum T_3^* are possible. There is an excellent agreement of these points with the predicted curve over a fourfold range of gland T_4^*/T_3^* ratios. Furthermore, in the last column of Table III, the individual predicted ratios of serum T_4^* to T_3^* are given, based on these assumptions. It is apparent that there is excellent correlation between these figures when these animals are examined individually. The mean predicted T_4^*/T_3^* ratio of 11 is precisely that observed.

DISCUSSION

The changes in the pattern of radioiodine distribution in the thyroid glands of the mildly and severely iodine-deficient rats in these studies are similar to those changes observed by others (1). In addition, the decrease of serum T_4 to undetectable levels within 4 wk of iodine restriction is similar to the effect of iodine deficiency on the PBI (2). Despite the profound decrease in serum T_4, no changes were detected in the levels of serum immunoassayable T_3 at either 1, 2, or 3 mo after restriction of iodine intake.

These data appear to be at variance with the results of a previous systematic study of this problem. Heninger and Albright calculated serum T_3 and T_4 values in control and iodine-deficient rats using equilibrium labeling and iodine specific activity (18). In normal rats, the serum T_3 was estimated to be 43 ng/100 ml, similar to our control values. In animals receiving iodine-deficient diet for 2 mo, a serum T_3 concentration of 101 ng/100 ml was calculated. However, determination of the iodine specific activity in the low-iodine animals depended on accurate estimates of iodine at very low levels in the diet. Whether or not the different results obtained might be due to an overestimation of the dietary iodine remains to be determined.

Volpert and Werner reported values of 19–41 ng/100 ml in seven samples of serum from rats on low iodine diet for 4–5 wk as opposed to levels of 55 to 60 ng/100 ml in animals receiving a normal diet (5). Nejad, Bollinger, Mitnick, and Reichlin have recently reported no significant change in T_3 values in rats fed a low iodine diet for an unspecified period of time while T_4 levels decreased to less than 25% of control (19). However, the estimates of normal T_3 concentrations in rat serum in these studies (16 ng/100 ml) were substantially lower than those we have obtained, for reasons not immediately apparent.

It has been previously speculated by Gross and Pitt-Rivers that T_4 might have to undergo deiodination to T_3 in order to be metabolically active and that, therefore, T_4 might act primarily as a precursor of the active thyroid hormone, T_3 (20). Oppenheimer, Schwartz, and Surks have demonstrated that T_4-to-T_3 conversion occurs in the rat and that this process can be inhibited by propylthiouracil (16, 21). In order to maintain hepatic mitochondrial alpha glycerophosphate dehydrogenase at normal levels in the propylthiouracil-treated animals, 2.5 times the usual replacement dose of T_4 had to be given. This also restored the net T_3 production from T_4 to normal levels. If, as these studies suggest, T_4 must be converted to T_3 to exert its metabolic effect, feedback regulation of the thyroid-pituitary-hypothalamic axis would most likely be dependent on tissue (pituitary) T_3 levels. The presence of specific binding sites for T_3 but not for T_4 in the nuclei of pituitary cells provides further support for this concept (22). The T_3 present in the pituitary could be derived either from T_4 or from the thyroid gland via direct secretion. One would anticipate under these circumstances that the normal homeostatic mechanism in the rat would then operate to maintain T_3 production constant regardless of the source of the hormone.

Analysis of data in Tables III and IV suggests that these animals compensate for low iodine uptake by a shift from T_4 as the primary source of T_3, to the thyroid gland itself. Our data further indicate that this marked change in the ratio of secreted hormone results from increases in the molar ratio of T_3/T_4 in iodine-deficient thyroglobulin. Whether this change in thyroglobulin composition is a result of increased thyroid-stimulating hormone (TSH) stimulation or decreased iodination, or both, remains to be determined. However, our analysis of the labeled hormone ratios in the serum is consistent with the simple assumption that they are secreted in the proportion in which they exist in the gland. A similar process may also occur in the normal rat, though the calculated molar ratio of secreted T_4 to T_3 is 12:1 (Table IV) and the ratio in the thyroid gland is 8-9/1.

As indicated above, iodine-deficient rats have elevated TSH levels that lead to the goiter and increased radioactive iodine uptake characteristic of this condition. It may be argued that such animals are, by definition, hypothyroid. Nevertheless, as previously pointed out, they appear to be "clinically" euthyroid (2–4). Whether, in fact, these animals are euthyroid in all respects awaits the results of more careful in vitro studies of tissue thyroid status. If it can be demonstrated that such animals are euthyroid and T_3 levels remain normal with markedly decreased serum T_4, the results would be consistent with the interpretation that the TSH elevation in these animals is a compensatory response designed to maintain the animal in a euthyroid state. In addition, it would support the above-mentioned hypothesis that T_4, in the normal rat, is primarily a precursor of T_3. Other investigators, as well as ourselves, have observed that normal serum T_3 concentrations may be associated with substantial TSH elevations and low serum T_4 levels in patients with primary thyroid disease or after radioactive iodine treatment for hyperthyroidism (23–25). It is possible that some of these patients may have "intrathyroidal" iodine deficiency due to acquired defects in iodide trapping or organification with a similar compensatory response.

Loewenstein and Wollman have shown that prolonged periods ($>$ 99 days) are required for complete equilibration of isotopic iodine into the total body iodine pool in the rat (26). For this reason accurate measurement of thyroidal and serum T_3 and T_4 by equilibrium labeling techniques is tedious. Inspection of Tables I and II shows that in groups A and B, multiplication of the fraction of T_4* by the ^{127}I content results in calculated T_4 concentrations that are considerably less than the immunoassayable estimates. This presumably results largely from the incomplete equilibration of the isotope with the iodine pool and is in agreement with earlier studies (14). Nevertheless, immunoassayable thyroid T_4 content in the control group agrees well with the values of about 350 ng T_4/mg wet wt in rat thyroid

that can be calculated from recent [^{127}I]T_4 measurements (14). The fact that acute labeling with ^{131}I results in the same T_3/T_4 ratios as the immunoassay determination indicates that, in spite of the functional heterogeneity of thyroid gland iodine previously shown by many investigators, the T_3/T_4 ratio of acutely formed hormone and total thyroidal iodothyronine are essentially the same. Since the immunoassay does not require separation of T_3 and T_4 with its attendant artifacts, it has obvious advantages in studies of this type and even more in the study of human thyroid tissue that cannot be chronically labeled.

Whether or not the rat has a mechanism for conservation of iodine efficient enough to allow a normal T_3 to be maintained indefinitely is not known. Our preliminary evidence suggests that after prolonged restriction of iodine intake, T_3 levels begin to decrease. More extensive studies are currently under way to confirm this observation.

APPENDIX

The T_4 or T_3 metabolized in 24 h can be expressed as follows:

$$\text{Total } Tx = [Tx] \times V_{Tx} \times K_{Tx}$$

where $[Tx]$ is the concentration of iodothyronine (moles per milliliter), V_{Tx} is the volume of distribution per 100 g body wt and K_{Tx} is the mean daily fractional removal rate of T_3 or T_4.

Under steady-state conditions, the T_4 cleared daily equals the T_4 secreted by the thyroid. It has been shown in the rat that approximately 17% of the T_4 is metabolized via conversion to T_3 (16). Thus, thyroidal T_3 secretion can be expressed as the difference between the metabolized T_3 and the quantity derived from T_4 or:

$$\text{Secreted } T_3 = ([T_3] \times V_{T_3} \times K_{T_3}) - 0.17([T_4] \times V_{T_4} \times K_{T4})$$

Substitution of experimentally measured values for kinetic constants gives (17):

$$\text{Secreted } T_4/100 \text{ g/day} = [T_4] \times 16.4 \times 1.032$$

$$\text{Secreted } T_3/100 \text{ g/day} = ([T_3] \times 165 \times 2.07) - 0.17([T_4] \times 16.4 \times 1.032)$$

If T_4* and T_3* are secreted in the same ratio as they exist within the gland and the peripheral pool is subsequently labeled with ^{131}I, then when the ratios of T_4* to T_3* in the gland and in the serum are in relative equilibrium:

$$\frac{\text{Secreted } T_4^*}{\text{Secreted } T_3^*} = \frac{\text{Gland } T_4^*}{\text{Gland } T_3^*} = \frac{(T_4^* \times 16.4 \times 1.032)}{T_3^* \times 165 \times 2.07 - 0.17 \times (T_4^* \times 16.4 \times 1.032)}. \quad (1)$$

Eq. (1) reduces to

$$\frac{\text{Gland } T_4^*}{\text{Gland } T_3^*} = \frac{16.9\ T_4^*}{340.96\ T_3^* - 2.88\ T_4^*}. \quad (2)$$

Eq. (2) can be solved for the ratio of labeled iodothyronines in the serum

$$\frac{T_4^*}{T_3^*} = \frac{20.2 \text{ Gland } T_4^*}{\text{Gland } T_3^* + 0.17 \text{ Gland } T_3^*}. \quad (3)$$

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