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

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ABSTRACT Triiodothyronine (T₃) and thyroxine (T₄) 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, ¹³¹I uptake and ¹³¹I content as well as with the distribution of ¹³¹I in Pronase digests of the thyroid. There was a progressive increase in thyroid weight and ¹³¹I uptake at 24 h with decrease in iodine intake. The ¹³¹I 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 moniodotyrosine/diiodotyrosine (MIT/DIT) and T₃/T₄ 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 T₃ concentration in the control rats was 4.2±0.6 (SD) μg T₃/100 ml, 4.5±0.3 μg/100 ml in group B animals, and undetectable (<0.5 μg/100 ml) in group C animals. There was no effect of iodine deficiency on serum T₃ concentrations, which were 44±9 (Mean±SD) ng/100 ml in A animals, 48±6 ng/100 ml in B animals, and 43±6 ng/100 ml in the C group. Thyroidal digest T₃ and T₄ 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 T₃/T₄ in the thyroid digests of the groups A and B animals was identical to the ratio of labeled T₃/T₄ and was slightly less (1.0±0.9) than the labeled T₃/T₄ ratio in the group C animals.

The mean ratio of labeled T₃ to labeled T₄ 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 T₃/T₄ in the thyroid digest with the labeled T₃/T₄ 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 T₄ utilized per day by the iodine-sufficient rat arises from T₃. If the T₃/T₄ conversion ratio remains the same in iodine deficiency, then the analysis suggests that about 90% of the T₃ arises directly from the thyroid. Therefore, it would appear that absolute T₃ secretion by the thyroid increases severalfold during iodine deficiency. The fact that serum T₃ remains constant and T₄ decreases to extremely low levels, combined with previous observations that iodine-deficient animals appear to be euthyroid, is compatible with the hypothesis that T₃ in the normal rat serves primarily as a precursor of T₄.

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 moniodotyrosine (MIT) and decrease in labeled diiodotyrosine (DIT) as well as a progressive increase in the ratio of labeled triiodothyronine (T₃) to labeled thyroxine (T₄). 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-

¹ Abbreviations used in this paper: DIT, diiodotyrosine; MIT, moniodotyrosine; PBI, protein-bound iodine; T₃, triiodothyronine; T₄, thyroxine; TAA, tertiary-amyyl-alcohol/hexane/ammonia; TSH, thyroid-stimulating hormone.
main healthy and grow at normal rates (2, 3). Recent studies by Silva have shown that O2 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 T3 to T4 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 T4 (3). Since it has not been possible previously to measure serum T3 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 T3 in the iodine-deficient rat though the ratio of T3 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 T3 and T4 with changes in thyroidal T3, T4, 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 H2O/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 131I and calculation of uptake. 5-40 μCi of carrier-free 131I 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 [125I]T3 and T4 (Abbott Laboratories, North Chicago, Ill.) were added at the start of digestion to monitor recovery and identify [125I]iodothyronines. The labeled iodothyronines contained less than 3% 131I 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 131I and 125I in the pellet after this procedure were 6% or less of the total. Analysis of the pellet 131I in the animals on the high-iodine intake showed similar losses of 131I. 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 131I and 125I remaining at the origin was less than 4%. The identification of 131I-labeled I*-125I, T3* and DIT* was carried out as previously described by chromatography in butanol/acetic acid (8). Separation of T* and T3* 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 131I and 125I with suitable correction. In the TAA system, the percent T4* was corrected for the 0.34% artifactual deiodination of T4* during the chromatography as we have previously described (9). T3* and T4* were corrected for losses during the procedure by reference to the original amounts of [125I]T3 and T4 added. Results were expressed as a fraction of the total 131I in the extract.

131I 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 μg 131I per mg wet weight of thyroid.

Serum and thyroidal T3 and T4 content. T3 and T4 content in the thyroid hydrolysates and serum were determined by radioimmunooassays 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/NH4OH (3:1, vol:vol). 5 or 10 μl of this dilution was then added to tubes containing either T4-free or T3-free human serum in 1 ml total volume. This concentration of MeOH/NH4OH does not affect the assays. In measuring T3 and T4 in thyroid hydrolysates from iodine sufficient rats, the MeOH/NH4OH 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 1/4 and 1/1 were sometimes necessary for accurate quantitation, indicating that there was no artifactual contribution to either the calculated T3 or T4 value from non-iodine-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 T3, MIT, or DIT. Since there is essentially no cross-reaction of the T3 antibody with T4, prior separation of T3 and T4 is unnecessary.

An asterisk will be used to denote 131I-labeled compounds present in thyroid glands or in serum.

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not required for this method. Total thyroid T₄ and T₃ were corrected for losses in extraction and expressed as ng T₃ or T₄ per mg wet weight of thyroid.

Analysis of serum T₃ was performed by using a modification of the T₃-free human serum system that employs sodium salicylate to block T₃-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 T₃-free human serum added to make the serum concentration 20% in all tubes. Duplicates of the two dilutions were in excellent agreement. T₃-free rat serum prepared as we have previously described for human serum contained no T₃ (10). Recovery of T₃ from pooled rat sera was 96±8% (mean±SD) for 100 ng T₃/100 ml and 99±8% (mean±SD) for 200 ng T₃/100 ml. Similar recoveries were obtained using serum from iodine-deficient rats in a smaller series of studies. Likewise, the T₃ determinations could be performed in the system containing T₃-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 ¹³¹I 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. T₃⁺ and T₄⁺ were determined by the method of Sterling, Dellabarba, Newman, and Brenner, as modified in our laboratory (9, 12). [¹³¹I]T₃ and T₄ were added before chromatography to allow correction for losses. T₄⁺ was corrected for the artificial increases due to deiodination of T₃⁺ as described above. Results were expressed as a fraction of the total serum radioactivity.

RESULTS

Effect of iodine deprivation on thyroidal weight, ¹³¹I content, ¹³¹I uptake and the distribution of ¹³¹I 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⁺, 0.5–0.75); 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 1 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 ¹³¹I 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 ¹³¹I at 24 h increased progressively with iodine deficiency. However, since the maximal uptake of ¹³¹I 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 ¹³¹I uptake.
Table I

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>I*</th>
<th>Thyroid weight</th>
<th>RAUI*</th>
<th>MIT*</th>
<th>DIT*</th>
<th>T3*</th>
<th>T4*</th>
<th>MIT*/DIT*</th>
<th>T3*/T4*</th>
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<tr>
<td></td>
<td></td>
<td>ng/mg thyroid</td>
<td>mg/100 g body wt</td>
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<td></td>
<td></td>
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<tr>
<td>A</td>
<td>12</td>
<td>880±180</td>
<td>4.1±0.7</td>
<td>15±10</td>
<td>4.8±1.0</td>
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<td>23</td>
<td>18</td>
<td>14</td>
<td>1.7</td>
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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 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 B animals with regard to the relative distribution of isotope in T3 and T4 while in group C there is a significant increase in the percent T4* 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 T4* 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 T3*/T4* is 0.12 in group A, and not significantly different (0.11) in group B. However in group C the ratio is reversed, T4* being 1.8 times T3*. The distribution of the isotope in these normal and iodine-deficient glands is substantially the same as that observed by previous investigators (1).

Serum T3 and T4 levels in control and iodine-deficient rats. Mean levels of serum T3 and T4 are presented in Table II. Where thyroid glands from two animals were pooled, the mean of the values for serum T3 and T4 were used. Serum T3 levels in the control animals ranged from 29 to 54 ng/100 ml with a mean of 44. T3 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 T3 concentration was unchanged from the group A animals, being 43 ng/100 ml. Thus there was no change in the serum T3 concentration in the iodine-deficient rat.

In the second column are given the concentrations of immunoassayable T4. 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, T4 was undetectable. With current methods, we were able to quantitate T4 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 T4 estimates obtained by immunoassay.
animals shows that in animals 9 and 10, which had been on the diet for a period of only 4 wk, T₃ had already disappeared. These animals were examined at 10 days after institution of the low-iodine diet and still had normal T₄ levels at that time.

**Thyroid glands.** The T₃ and T₄ concentrations of the rat thyroid hydrolysates are also shown in Table II. In the thyroid glands of the control rats, the T₃ concentration varied from 27 to 58 ng/mg wet wt with a mean of 39 ng/mg. In mildly deficient animals, T₃ 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 T₄ content in this group was 1.7 ng/mg. While the T₄ concentration was markedly reduced in the group C animals, the larger size of the thyroid gland resulted in an overall decrease of the gland T₄ content of approximately 85%, from a mean of 0.64 µg to about 0.10 µg/total gland.

T₄ content of the normal rat thyroid gland was 400 ng/mg wet wt. Like T₃, 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 T₄ content in the C animals was 3.2 ng/mg wet wt, less than 1% of the control T₄ concentration. This is similar in magnitude to the reduction in the ¹³¹I content of the gland. The total T₄ content of the goitrous glands was only 3% of the control value.

Of considerable interest is the fact that the molar ratio of T₄ to T₃ in these thyroid glands is similar to the ratio of T₄* / T₃* 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 T₄ / T₃ 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 T₄* / T₃* ratio even as soon as 24 h after labeling is an accurate reflection of the absolute ratio of T₄ to T₃ 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 ¹³¹I 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 T₄*, T₃*, and T₄* were possible only in this group, due to the high ratio of T₄* / T₃* present in normal animals. Since there is artificial deiodination of a variable fraction of T₄* during chromatographic separation, it is difficult to determine accurately how much T₄* present in normal serum arises in vivo.

Direct chromatography of serum in collidine/NH₄OH indicated that the only labeled components in serum of iodine-deficient rats were I*, T₃*, and T₄*, 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

<table>
<thead>
<tr>
<th>Group</th>
<th>T₃ Concentrations</th>
<th>T₄ Concentrations</th>
<th>Molar ratio T₄ / T₃</th>
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<tr>
<td></td>
<td>Serum</td>
<td>Thyroid</td>
<td></td>
</tr>
<tr>
<td></td>
<td>µg/100 ml</td>
<td>µg/100 ml</td>
<td>µg/gland</td>
</tr>
<tr>
<td>A</td>
<td>12</td>
<td>44±9</td>
<td>39±10</td>
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<tr>
<td>B</td>
<td>9</td>
<td>48±6</td>
<td>26±6</td>
</tr>
<tr>
<td>C$</td>
<td>1</td>
<td>41 &lt;0.50 (0.4)‡</td>
<td>0.79</td>
</tr>
<tr>
<td>2</td>
<td>45 &quot; (0.6)</td>
<td>0.55</td>
<td>0.039</td>
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<td>3</td>
<td>40 &quot;</td>
<td>0.88</td>
<td>0.065</td>
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<tr>
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<td>53 &quot; (0.6)</td>
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‡ PBI, µg 1/100 ml.
§ In Group C, data are provided for individual animals.
|| Animals on iodine restriction for 4 wk.

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label as T₃ varied from 5 up to 17% at this time, whereas T₄* ranged from 70 to 102% of the total. Since T₃* and T₄* content of the original serum was determined independently by ¹³¹I-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 T₄*/T₃* 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 T₄*/T₃* is presented based on the gland T₄*/T₃*. The derivation of these ratios is discussed subsequently.

Estimation of the respective contributions of the thyroid and peripheral T₄-to-T₃ conversion to the peripheral T₃ pool in the rat. It has been previously shown that peripheral T₄-to-T₃ conversion contributes substantially to the circulating T₃ pool in man (15). A similar pathway for T₄ metabolism has been shown in the rat by Schwartz, Surks, and Oppenheimer (16). If the clearances of T₄ and T₃ do not change in the iodine-deficient rat, it should be possible to calculate the relative contributions of the thyroid and peripheral T₄-to-T₃ conversion to the serum T₃ pool in this situation. If plasma T₄ or T₃ 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 T₄ and T₃ in rats deprived of iodine for 3 mo (4) Using the kinetic data for T₄ and T₃ clearance in the rat, previously published by Oppenheimer et al. (17), and Silva’s conclusions (4), it is possible to calculate total T₃ and T₄ utilization in the control and iodine-deficient rats by using our values for serum T₄ and T₃. It is then possible to estimate the relative proportion of T₃ coming from T₄ and that coming from the thyroid gland with the T₄-to-T₃ conversion ratio of 0.17 determined by Schwartz et al. in the rat (16). The estimates of the quantities of T₄ and T₃ metabolized by these rats are presented in Table IV (see Table III: Labeled Compounds in the Serum of Severely Iodine-Deficient Rats 24 h After ¹³¹I Administration

<table>
<thead>
<tr>
<th>% Total</th>
<th>T₁*</th>
<th>T₄*</th>
<th>T₄*/T₃*</th>
<th>Predicted‡</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>4.5</td>
<td>10</td>
<td>74</td>
<td>7.4</td>
</tr>
<tr>
<td>2</td>
<td>6.3</td>
<td>17</td>
<td>70</td>
<td>4.1</td>
</tr>
<tr>
<td>3</td>
<td>4.4</td>
<td>10</td>
<td>74</td>
<td>7.7</td>
</tr>
<tr>
<td>4</td>
<td>2.6</td>
<td>5.6</td>
<td>84</td>
<td>15</td>
</tr>
<tr>
<td>5</td>
<td>2.4</td>
<td>5.0</td>
<td>92</td>
<td>18</td>
</tr>
<tr>
<td>6</td>
<td>4.1</td>
<td>5.6</td>
<td>89</td>
<td>16</td>
</tr>
<tr>
<td>7</td>
<td>3.2</td>
<td>7.6</td>
<td>83</td>
<td>11</td>
</tr>
<tr>
<td>8</td>
<td>4.0</td>
<td>7.5</td>
<td>90</td>
<td>12</td>
</tr>
<tr>
<td>9</td>
<td>4.4</td>
<td>8.6</td>
<td>83</td>
<td>9.7</td>
</tr>
<tr>
<td>10</td>
<td>4.6</td>
<td>7.4</td>
<td>102</td>
<td>14</td>
</tr>
<tr>
<td>Mean</td>
<td>4.1</td>
<td>8.4</td>
<td>84</td>
<td>11</td>
</tr>
<tr>
<td>SEM</td>
<td>0.4</td>
<td>1.1</td>
<td>3</td>
<td>1</td>
</tr>
</tbody>
</table>

‡ Predicted on the basis of the gland T₄*/T₃* (see Discussion).

Appendix for formulae). Total T₃ degradation is 710 and 760 ng/100 g body wt/day in groups A and B. Total T₄ metabolism is 150 and 160 ng/100 g/day with approximately 67% of this T₄ arising from T₃ to T₄ deiodination. In group C, the total T₄ metabolism is reduced to approximately 11% of that present in the A and B groups even if the maximum T₄ value of 0.5 μg/100 ml is used as the estimate of the T₄ level. Nevertheless, T₄ utilization is essentially unchanged. Since there is little T₄ arising from T₃, over 90% of this T₄ 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 T₄ secretion rate must occur in the iodine-deficient rat. Even if the T₄ to T₃ conversion rate increased to 100% in these animals, a maximum of 71 ng of T₃/100 g body wt/day could be generated. This is still less than one-half of the T₄ utilized per day.

Table IV: Analysis of the Quantity and Origin of T₃ and T₄ Utilized by the Rat‡

<table>
<thead>
<tr>
<th>Group</th>
<th>T₃</th>
<th>T₄</th>
<th>T₄/Thyroid</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ng 100 g day⁻¹</td>
<td>ng 100 g day⁻¹</td>
<td>ng</td>
</tr>
<tr>
<td>A</td>
<td>710</td>
<td>150</td>
<td>101</td>
</tr>
<tr>
<td>B</td>
<td>760</td>
<td>160</td>
<td>108</td>
</tr>
<tr>
<td>C</td>
<td>856</td>
<td>150</td>
<td>12</td>
</tr>
</tbody>
</table>

‡ Values based on mean serum iodothyronine concentrations in the three groups.
§ Calculated if maximum serum T₃ (0.5 μg/100 ml) is assumed.

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Inspection of Table IV also reveals that the calculated molar ratio of secreted $T_s$ to secreted $T_i$ in the group C animals is about 2:1, similar to the ratio of $T_s^*/T_i^*$ (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 $T_s^*/T_i^*$ ratios were determined in the iodine-deficient animals, it was possible to test this hypothesis rigorously by attempting to predict the serum $T_s^*/T_i^*$ ratio based on the gland $T_s^*/T_i^*$. This requires the assumption that there is relative equilibrium of the $T_s^*/T_i^*$ ratios in the gland and serum at 24 h. This has been verified experimentally for the gland $T_s^*/T_i^*$ by previous investigators (1). Since the half-lives of $T_s$ and $T_i$ 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 $T_s$ and $T_i$ 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 $T_i$ and $T_s$ 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 $T_s^*/T_i^*$ and serum $T_s^*/T_i^*$ in the group C animals where accurate estimates of serum $T_s^*$ are possible. There is an excellent agreement of these points with the predicted curve over a fourfold range of gland $T_s^*/T_i^*$ ratios. Furthermore, in the last column of Table III, the individual predicted ratios of serum $T_s^*$ to $T_i^*$ 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 $T_s^*/T_i^*$ 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 $T_s$ 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 $T_s$, no changes were detected in the levels of serum immunoassayable $T_i$ 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 T3 and T4 values in control and iodine-deficient rats using equilibrium labeling and iodine specific activity (18). In normal rats, the serum T3 was estimated to be 43 ng/100 ml, similar to our control values. In animals receiving iodine-deficient diet for 2 mo, a serum T3 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 T3 values in rats fed a low iodine diet for an unspecified period of time while T4 levels decreased to less than 25% of control (19). However, the estimates of normal T3 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 T3 might have to undergo deiodination to T4 in order to be metabolically active and that, therefore, T4 might act primarily as a precursor of the active thyroid hormone, T3 (20). Oppenheimer, Schwartz, and Surks have demonstrated that T3-to-T4 conversion occurs in the rat and that this process can be inhibited by propylthiouracil (16, 21). In order to maintain hepatic mitochondrial alpha glycero phosphatase dehydrogenase at normal levels in the propylthiouracil-treated animals, 2.5 times the usual replacement dose of T3 had to be given. This also restored the net T3 production from T4 to normal levels. If, as these studies suggest, T4 must be converted to T3 to exert its metabolic effect, feedback regulation of the thyroid-pituitary-hypothalamic axis would most likely be dependent on tissue (pituitary) T4 levels. The presence of specific binding sites for T4 but not for T3 in the nuclei of pituitary cells provides further support for this concept (22). The T3 present in the pituitary could be derived either from T4 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 T3 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 T4, as the primary source of T3, 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 T3/T4 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 T3 to T4 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 these animals are euthyroid and T3 levels remain normal with markedly decreased serum T4, 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 T3, in the normal rat, is primarily a precursor of T4. Other investigators, as well as ourselves, have observed that normal serum T3 concentrations may be associated with substantial TSH elevations and low serum T4 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 T3 and T4 by equilibrium labeling techniques is tedious. Inspection of Tables I and II shows that in groups A and B, multiplication of the fraction of T3* by the radioactivity content results in calculated T3 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 T3 content in the control group agrees well with the values of about 350 ng T3/mg wet wt in rat thyroid gland.

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that can be calculated from recent \([^{131}I]T_4\) 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 thyrotrypine is essentially the same. Since the immunoassay does not require separation of \(T_4\) and \(T_3\) 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:

\[
\text{Total } T_x = [T_x] \times V_{T_x} \times K_{T_x}
\]

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

Under steady-state conditions, the \(T_4\) cleared daily equals the \(T_3\) secreted by the thyroid. It has been shown in the rat that approximately 17% of the \(T_3\) is metabolized via conversion to \(T_4\) (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:

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

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

\[
\begin{align*}
\text{Secreted } T_3/100 \text{ g/day} & = [T_3] \times 16.4 \times 1.032 \\
\text{Secreted } T_4/100 \text{ g/day} & = ([T_4] \times 165 \times 2.07) - 0.17 ([T_4] \times 16.4 \times 1.032)
\end{align*}
\]

If \(T_3^*\) and \(T_4^*\) 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 ratio of \(T_4^*\) to \(T_3^*\) in the gland and in the serum are in relative equilibrium:

\[
\begin{align*}
\text{Secreted } T_4^* & = \frac{\text{Gland } T_4^*}{\text{Gland } T_3^*} \\
\text{Secreted } T_3^* & = \frac{\text{Gland } T_3^*}{\text{Gland } T_4^*} \\
& = \frac{[T_3^* \times 16.4 \times 1.032]}{[T_4^* \times 165 \times 2.07 - 0.17]}
\end{align*}
\]

\[
= T_3^* \times 165 \times 2.07 - 0.17
\]

\[
\times (T_4^* \times 16.4 \times 1.032) \quad (1)
\]

Eq. (1) reduces to

\[
\begin{align*}
\text{Gland } T_3^* & = \frac{16.9 \text{ T}_3^*}{340.96 \text{ T}_3^* - 2.88 \text{ T}_4^*} \\
\text{Gland } T_4^* & = \frac{16.9 \text{ T}_4^*}{340.96 \text{ T}_4^* - 2.88 \text{ T}_3^*}
\end{align*}
\]

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

\[
\frac{\text{T}_4^*}{\text{T}_3^*} = \frac{20.2 \text{ Gland } T_4^*}{\text{Gland } T_3^* + 0.17 \text{ Gland } T_4^*} \quad (3)
\]

**ACKNOWLEDGMENTS**

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