Triiodothyronine, Thyroxine, and Iodine in Purified Thyroglobulin from Patients with Graves' Disease

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ABSTRACT Previous studies have suggested that there is an overproduction of triiodothyronine (T_3) relative to thyroxine (T_4) in patients with thyrotoxicosis associated with Graves' disease. To evaluate whether or not an increased ratio of T_3 to T_4 in thyroidal secretion could be contributing to this relative T_3 hyperproduction, T_3, T_4, and iodine were measured in thyroglobulin (Tg) from controls and patients with Graves' disease who had been treated either with propranolol only or with antithyroid drugs plus iodide before surgery. To avoid possible artifacts associated with pulse labeling and chromatography, T_3 and T_4 were determined by radioimmunoassay of Pronase hydrolysates of purified Tg. Results of analyses of Tg from six control patients and seven with Graves' disease, not receiving thiourea drugs or iodide, showed that the iodine content of Graves' disease Tg was not different from normal. Both contained 3.4 residues of T_4/molecule Tg, but there was 0.39±0.08 (mean±SD) residue of T_3/molecule Tg in Graves' Tg as opposed to 0.23±0.07 residue T_3 molecule Tg in controls matched for iodine content ($P < 0.01$). This difference resulted in a significantly lower T_4/T_3 molar ratio (9±2) in Graves' Tg as opposed to control (15±2, $P < 0.001$). In Tg from patients with treated Graves' disease, iodine, T_3, and T_4 were reduced, but the reduction in the latter was more substantial, resulting in a T_4/T_3 molar ratio of 3.4 ±1. Fractionation of Tg from all groups by RbCl density gradient ultracentrifugation indicated that at physiological levels of Tg iodination, the molar ratio of T_3/Tg was consistently higher in Graves' disease. The specific mechanism for this difference is not known, but it is not due to iodine deficiency. If T_3 and T_4 are secreted in this altered ratio in patients with Graves' disease, the magnitude of the difference could explain the relative T_3 hyperproduction which is characteristic of this state.

INTRODUCTION

There is considerable evidence that the hyperthyroidism associated with Graves' disease is accompanied by hyperproduction of triiodothyronine (T_3)[1] relative to thyroxine (T_4) (1). The most extreme example of this phenomenon is found in the patient with the clinical stigmata of hyperthyroidism, a normal serum T_4 and elevated serum T_3, a condition which has been called "T_3 thyrotoxicosis" (2). Previous studies from our laboratory have suggested that the contribution of direct thyroidal secretion to the peripheral T_3 pool in thyrotoxicosis is greater than in the euthyroid subject (3, 4). Studies of digests of thyroid homogenates from normal subjects and patients with treated Graves' disease showed that the molar ratio of T_3 to T_4 in the thyroid glands of patients with treated Graves' disease was higher than in normal subjects (4). However, because of the pretreatment of these subjects with antithyroid agents, iodine content was reduced. Since reduction in iodine content per se gives rise to thyroglobulin (Tg) with an increased T_3/T_4 molar ratio (5), it was not possible to determine if the high T_3/T_4 ratio was due to Graves' disease or iodine deficiency itself. Preliminary data obtained in homogenates of thyroid tissue from two patients who were treated only with propranolol before therapy showed only a modest reduction in iodine content, but a marked increase in the T_3/T_4 ratio (4). Because this suggested that there might be an additional factor(s) besides iodine regulating the ratio of

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[1] *Abbreviations used in this paper*: T_3, 3,5,3′-triiodo-L-thyronine; T_4, 3,5,3′,5′-tetraiodo-L-thyronine; Tg, thyroglobulin.

TABLE I

Results of Analyses of Serum T_4 and T_3 at the Time of Surgery in Patients with Graves' Disease

Subject	Presurgical therapy	T_4	T_3 Resin uptake	T_3 Radioimmunoassay
		μg/100 ml	*%*	*ng/100 ml*
Normal range		5.4–13.4	22–34	80–210
1	Propranolol	>25	52	>500
2	Propranolol	>25	54	—
3	Propranolol	18	45	—
4	Propranolol	20	44	—
5	Propranolol	22	51	—
6	Propranolol	20	50	>300
7	Propranolol	23	51	—

		T_4 Radioimmunoassay	T_4-Binding globulin index*	T_3 Radioimmunoassay
		μg/100 ml		*ng/100 ml*
Normal range		5–10.2	0.85–1.10	65–160
8	6-*n*-Propylthiouracil, SSKI, propranolol	5.8	0.66	100
9	6-*n*-Propylthiouracil, SSKI, propranolol	7.9	—	—
10	1-methyl,2-mercaptoimdazole	3.3	0.96	48

* T_4-binding globulin index-normalized T_3 charcoal uptake.

T_3/T_4 synthesized in the stimulated thyroid of Graves' disease, the following investigation was performed.

METHODS

Sources of thyroid tissue. Specimens of human thyroid tissue were obtained from two sources. Patients whose only preoperative treatment was propranolol (referred to as "untreated" since they did not receive thiourea drugs or iodide) underwent subtotal thyroidectomy at Georgetown University in Washington, D. C. Specimens of thyroid tissue from these patients were kindly provided by Dr. J. J. Canary of that institution and were shipped frozen in dry ice, having been stored at −20°C for various periods of up to several months. Specimens of thyroid tissue from patients with treated Graves' disease (thiourea drugs plus iodide) were obtained with the cooperation of members of the Departments of Surgery and Pathology at the Peter Bent Brigham Hospital. Control samples of tissue were obtained from portions of microscopically normal thyroid tissue in surgical specimens which were resected for thyroid nodules. All patients were clinically euthyroid at the time of surgery. Specimens were frozen immediately after their removal from the operating room and kept at −20°C. Control studies indicated that the results obtained using frozen and unfrozen tissue from the same patient with treated Graves' disease were identical.

Thyroglobulin preparation. Approximately 2–3 g of thyroid was cut into small pieces and put into an equal volume of 0.1 M phosphate buffer (pH 7.19). This was homogenized with a Teflon pestle and centrifuged at 40,000 g for 20 min at 4°C in a Sorvall RC 2B centrifuge (Ivan Sorvall, Inc., Norwalk, Conn.). The supernate was saved and pooled with the supernatant fraction of a single wash with the same buffer. Ammnoium sulfate precipitation was performed with a collection of insoluble material between the concentrations of 1.4 and 1.8 M (6). Gel filtration on Bio-Gel A5M, 200–400 mesh, (Bio-Rad Laboratories, Richmond, Calif.) was performed using a 90 × 2.6-cm column in phosphate buffer at 4°C according to the method of Bilstad et al. (7). The concentration of Tg in the eluate of the 5-ml fractions was determined spectrophotometrically (extinction coefficient, 280 nm, 1% solution = 10). The 19S Tg was identified by its position in the elution pattern and the single peak fraction used for further studies. This protein gave a single band on disc gel electrophoresis.

Equilibrium density gradient centrifugation on RbCl. Isopycnic centrifugation in 34.5% RbCl was performed according to the method of Schneider and Edelhoch (8). A model L Beckman preparative centrifuge with SW 50.1 rotor was used (Beckman Instruments, Inc., Spinco Div., Palo Alto, Calif.) and centrifugation was performed for 5–7 days at 20°C at 33,000 rpm. Fractionation was obtained using the Buchler Auto Densi-Flow II C apparatus (Buchler Instruments Div., Searle Analytic Inc., Fort Lee, N. J.). 35 0.15–0.2-ml fractions were obtained from each tube. After protein quantitation, appropriate fractions were pooled and dialyzed against 500 ml of 0.04 M Tris-0.11 M NaCl, pH 8.5 for three 2-h periods to remove RbCl. Measurements of iodine content and Pronase digestion were then performed.

Digestion of Tg. 20–40 μg of purified Tg (either after gel filtration or RbCl equilibrium centrifugation) in Tris-NaCl was placed in a small plastic tube and digested according to the method of Inoue and Taurog (9). In addition to 100 μl of the Tg solution, each tube contained 30 μl of 0.15 M 1-methyl-2-mercaptoimidazole, (Aldrich Chemical Co. Milwaukee, Wis.) and 20 μl of Pronase (B grade Calbiochem San Diego, Calif.) 3 mg/ml in Tris NaCl with 10 μl toluene. Incubation was carried out for 24 h at 37°C under N_2. Completeness of digestion was determined by quantitation of iodine remaining at the origin of a butanol-acetic acid chromatogram of unextracted digests of Tg from three control and three propranolol-treated patients. The appearance of I^- during digestion (also in six samples) was determined by quantitation of iodine in the appropriate area of the chromatographic strip (butanol-acetic acid) as indicated by the location of $^{125}I^-$ which had been added in tracer amounts. The digests were extracted with 0.8 ml methanol/concentrated ammonium hydroxide (99/1, vol/vol). Appropriate dilutions of this extract were made and radioimmunoassay of T_3 and T_4 performed in duplicate at two dilutions using minor modifications of methods previously described (10). Specimens from all controls and all Graves' thyroids were assayed simultaneously to avoid interassay variation. Iodine determinations were performed by Boston Medical Laboratory. Statistical comparisons were carried out using methods described by Snedecor and Cochran or, where indicated, by Fisher's Exact Treatment (11, 12).

RESULTS

Clinical data for subjects with Graves' disease. In Table I are presented the presurgical therapy and biochemical results relative to thyroid status of the patients with Graves' disease. The results of the first seven patients who received propranolol alone are typical of patients with untreated hyperthyroidism due to Graves' disease. T_3 determinations were available in only two patients and both were elevated.

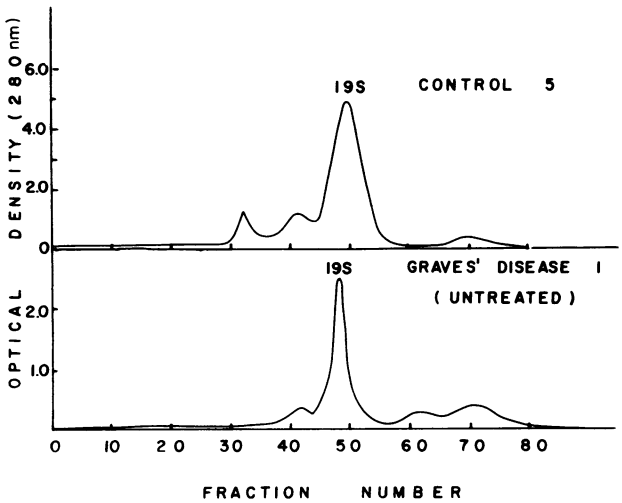

FIGURE 1 Typical elution patterns obtained by column chromatography of ammonium sulfate fractions of extracts from human thyroid tissue. Chromatography was performed with Bio-Gel A5M (Bio-Rad Laboratories), at 4°C. Each fraction contains 5 ml. Representative patterns from a control thyroid (upper portion) and from thyroid tissue from a patient with Graves' disease are depicted. The term "untreated" indicates that the patient received propranolol but not thiourea drugs or iodide.

Patients 8–10 received various antithyroid drugs, and T_4 results were in the normal range for two subjects and subnormal in subject 10. None of these patients was clinically hypothyroid.

Results of iodine, T_3, and T_4 analyses. Fig. 1 depicts typical elution patterns of Tg from euthyroid patients and those with propranolol-treated Graves' disease. Aside from differences in the width of the peak associated with the degree of column loading, there was no apparent difference in elution patterns between these two groups of samples. In all subjects the 19S peak was clearly delineated and constituted the greatest percentage of the protein present.

Measurements of T_3, T_4, and iodine, in Tg from control subjects are presented in Table II. The mean iodine content of the group was 0.42% corresponding to 22 atoms of iodine/molecule Tg. The content of T_3 and T_4 per molecule Tg was 0.18 and 2.5 with a calculated molar ratio of 13. When expressed as a percentage of the total Tg iodine, this corresponds to 2.4 and 41% for T_3 and T_4, respectively. The iodine content of two of the control Tg preparations was modestly reduced for reasons which were not apparent from the clinical history. In subject 1 this was associated with a T_4 content which appeared to be considerably below that of the remainder of the group. Because of the reduced iodine content, and because of the fact that, as will be seen in Table III, the Graves' disease Tg was relatively rich in iodine, mean values were also calculated excluding subjects 1 and 2 to allow valid comparisons.

The results of analyses of T_3, T_4, and iodine content in the Tg from patients with Graves' disease is presented in Table III. *P* values are calculated for comparison of results in the propranolol-treated subjects with the four control subjects with the higher iodine content using Students *t* test (11). The mean iodine content of the Tg of the untreated Graves' disease patients was 0.56% corresponding to 29 atoms/molecule. This was identical to the group of four patients used as controls. Despite this, the T_4/Tg ratio was 3.4 which was not different from the control group. This resulted in a significantly lower T_4/T_3 molar ratio of 9.0 for the Tg from patients with Graves' disease.

The lower portion of Table III presents the results of Tg analyses from patients with treated Graves' disease. Because of antithyroid drug treatment, the iodine content was markedly reduced in these subjects, being 0.16% or 6.5 atoms/molecule. While the ratio of T_3/Tg was reduced in these subjects, it was not reduced to as great a degree as was the T_4/Tg ratio. Therefore, the T_4/T_3 ratio of 3.4 is lower than in Tg from propranolol-treated Graves' disease patients. The percent T_3 iodine was increased in these subjects and the percent T_4 iodine was reduced when compared with normals.

During Pronase digestion, 8.2±1.0% (mean±SE) of the Tg iodine was found as I^- in incubations involving either control or Graves' disease Tg. If this iodine were derived, at least partly, from T_4 with T_3 as a by-product, then it is theoretically possible that greater deiodination of T_4 to T_3 could occur with

TABLE II
T_3, T_4, and I Content of Purified Human Tg from Euthyroid Subjects

Subjects	I Content	I/Tg	T_3/Tg	T_4/Tg	T_4/T_3	T_3I/I	T_4I/I
	%	atoms/molecule	molar ratio			%	%
Control							
1	0.13	6.6	0.046	0.33	7.2	2.1	20
2	0.20	10	0.087	1.0	12	2.6	40
3	0.44	23	0.13	2.2	18	1.6	39
4	0.56	29	0.28	3.9	14	2.9	54
5	0.54	28	0.24	3.6	15	2.6	52
6	0.65	34	0.28	3.7	13	2.5	43
Mean	0.42	22	0.18	2.5	13	2.4	41
SD	0.21	11	0.10	1.5	4	0.5	12
Mean*	0.55	29	0.23	3.4	15	2.4	47
SD	0.09	5	0.07	0.8	2	0.6	7

* Excluding subjects 1 and 2.

TABLE III

T_3, T_4, and I Content of Purified Tg from Patients with Graves' Disease

Subjects	I Content	I/Tg	T_3/Tg	T_4/Tg	T_4/T_3	T_3I/I	T_4I/I
	%	*atoms/ molecule*		*molar ratio*		%	%
Presurgical treatment with propranolol alone							
1	0.33	17	0.32	2.9	9.0	5.6	67
2	0.48	25	0.26	3.1	12	3.2	50
3	0.55	28	0.45	2.6	5.7	4.8	36
4	0.56	29	0.37	3.4	9.3	3.8	47
5	0.63	33	0.45	4.4	9.8	4.1	54
6	0.65	34	0.41	3.5	8.4	3.6	41
7	0.69	36	0.47	3.9	8.9	3.9	44
Mean	0.56	29	0.39	3.4	9.0	4.1	48
SD	0.12	6	0.08	0.6	2.0	0.8	10
*P**	NS	NS	<0.01	NS	<0.001	<0.005	NS
Presurgical treatment with thiourea drugs and iodide							
8	0.13	6.6	0.18	0.46	2.6	8.1	28
9	0.17	8.7	0.15	0.43	3.0	6.9	20
10	0.19	4.2	0.13	0.58	4.5	4.2	24
Mean	0.16	6.5	0.15	0.49	3.4	6.4	24
SD	0.03	2.3	0.02	0.08	1.0	2.0	4

* For difference from mean of control subjects three to six.

Graves' disease Tg as opposed to control Tg. This could result in an artifactual reduction of the T_4/T_3 ratio in Graves' Tg. To eliminate this possibility, labeled T_4 was incubated with unlabeled control or Graves' disease Tg during Pronase digestion. Labeled T_3 was then isolated by specific immunoadsorption to Sepharose–anti-T_3 antibody conjugates prepared by methods to be published subsequently.[2] A maximum of 0.74±0.15% (mean±SD) of labeled T_4 appeared as labeled T_3 in incubations using Graves' disease Tg and 0.60±0.07% in the presence of control Tg. These results were not different and even the maximum percent conversion of T_4 to T_3 is not significant under these conditions. Both control and Graves' disease Tg were digested equally well by Pronase. The origin iodine was less than 5% of the total for three different samples from both control and propranolol-treated Graves' Tg.

Heterogeneity of human Tg. Human goiter Tg is known to be heterogeneous in its iodine content (8). To determine whether or not the lower T_4/T_3 ratio was characteristic of all of the Tg present in the thyroids of patients with Grave's disease, it was fractionated into proteins of varying iodine content, using RbCl density gradient centrifugation. Typical patterns of Tg obtained using this technique are presented in Fig. 2. As with the gel-filtration elution profile, there appeared to be no significant difference between the pattern in Graves' disease and that of controls. After determination of protein concentration, the fractions were pooled into four groups, A–D, as indicated by the lines in Fig. 2. Iodine determinations and Pronase digestion were performed on the pooled subfractions. The iodine content of the four fractions followed a consistent pattern. It was lowest in the A fraction, and reached a peak in fraction C, while that of the B fraction was intermediate. The iodine content of the D fraction was the same or slightly lower than that of the C fraction. The data presented in Table IV shows the efficiency of this separation technique analyzed by comparing the Tg iodine content of the A and C fractions. In addition, the T_4/T_3 ratios calculated for the Tg in the same fractions are also presented. In the control specimens, the mean number of iodine atoms per molecule Tg was 14 in the A fractions whereas in the C fractions it was 19. These values are significantly different and demonstrate the successful partition of the Tg molecules according to iodine content. A larger gradient is seen in the Graves' disease Tg where the iodine/Tg ratio was 13 in the A fractions and 24 in the C fractions. Despite the significant differences in iodine content of the Tg from the A and C fractions, the molar ratio of T_4/T_3 was not different, either in the normals, or in the patients with Graves' disease. Thus, it appeared that the T_4/T_3 molar ratio was in-

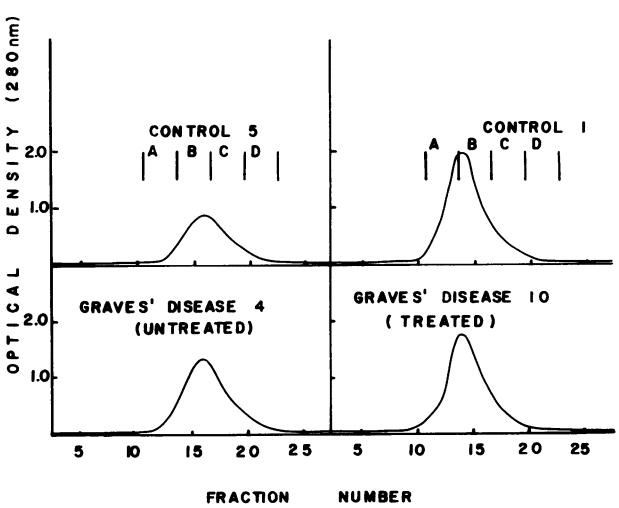

FIGURE 2 Representative profiles of Tg after isopycnic equilibrium density gradient ultracentrifugation in 34.5% RbCl. Each fraction is 0.2 ml. The upper portion of the tube is to the left. The divisions, A–D show which fractions were pooled to evaluate the relationship between T_3, T_4, and iodine content of the Tg. Results of four different preparations are depicted. The terms "treated" and "untreated" refer to thiourea drugs and iodide.

[2] Zimmerman, C. J., M. Izumi, and P. R. Larsen. Manuscript submitted for publication.

dependent of the iodination of the Tg within a single gland as it was partitioned in these studies.

T_3Tg and T_4/Tg in RbCl subfractions. The separation of Tg into four subfractions allowed an analysis of the relationship between the T_3 and T_4 and iodination of the Tg over a broad range of iodine content. There were 23 subfractions of normal Tg and 40 subfractions of Tg from treated and untreated patients with Graves' disease. Figs. 3–5 show the relationship between the T_3/Tg, T_4/Tg, and T_4/T_3 molar ratios and iodine content for all subfractions. In Fig. 3, there appears to be a higher T_3/Tg ratio in Graves' disease Tg at any level of iodine content. The T_3/Tg ratio was significantly higher in the propranolol-treated Graves' patients as opposed to control subjects

TABLE IV

Comparison of Iodine Content and T_4/T_3 Ratio in RbCl Density Gradient Fractions of Human Tg

Control				Graves' disease			
Sample	RbCl fraction	I/Tg	T_4/T_3	Sample	RbCl fraction	I/Tg	T_4/T_3
		atoms/ molecule	*molar ratio*			*atoms/ molecule*	*molar ratio*
1	A	6.4	7.2	1	A	10	14
	C	5.2	5.6		C	16	13
2	A	5.9	15	2	A	20	14
	C	11	11		C	30	15
3	A	16	19	3	A	13	9.3
	C	18	17		C	25	8.3
4	A	15	7	4	A	16	5.9
	C	22	10		C	31	12
5	A	18	17	5	A	19	12
	C	29	14		C	32	12
6	A	22	15	6	A	20	7.3
	C	31	16		C	39	8.4
Mean	A	14	13	7	A	22	7.2
SE		2.6	2.1		C	37	8.2
Mean	C	19*	12	8	A	3.6	4.4
		4.1	1.7		C	6.8	2.8
				9	A	4.4	2.4
					C	8.2	3.6
				10	A	6.0	8.4
					C	11	4.9
				Mean	A	13	8.5
				SE		2.2	3.9
				Mean	C	24‡	8.8
				SE		3.8	1.3

* Different from A, $P < 0.05$.
‡ Different from A, $P < 0.005$.

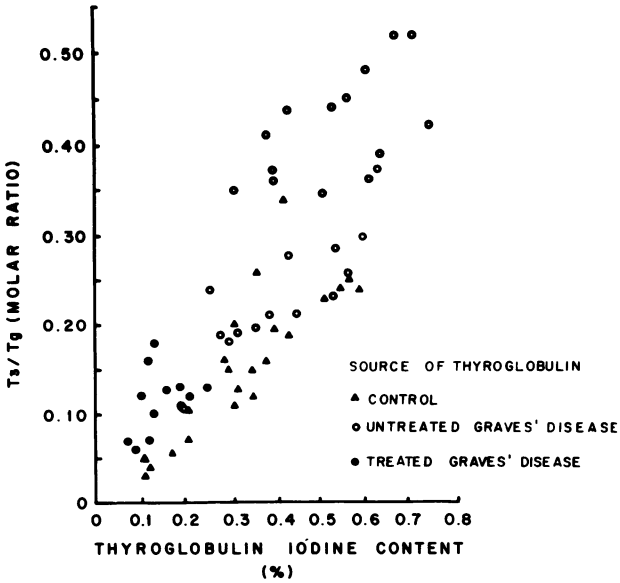

FIGURE 3 Relationship between the T_3/Tg molar ratio and iodine content of the subfractions of humn Tg obtained by RbCl density gradient centrifugation. T_3 content was determined by immunoassay after Pronase digestion of the purified Tg.

with $P = 0.003$ by the Fisher's Exact Treatment (12). On the other hand, there was a great overlap between the controls and Graves disease groups in the T_4/Tg molar ratios (Fig. 4). As was the case with the analyses of the whole Tg preparations (Table III), the T_4/T_3 molar ratios in the RbCl subfractions were significantly higher in normal Tg than in Tg from patients with propranolol-treated Graves' disease ($P = 0.013$, Fisher's Extract Treatment). These results are presented in Fig. 5.

DISCUSSION

Much of the previous data in the literature relative to the iodothyronine content of human thyroid tissue or Tg is derived from studies obtained using tissue from patients treated preoperatively with labeled iodine. In this method, the thyroid tissue was then digested and labeled compounds were separated by chromatography with iodine content being measured in each fraction. There are two technical problems with this analytical approach for T_3 and T_4 determinations. The first is that chromatography generally results in deiodination of T_4 with some of this appearing as T_3 artifactually increasing the T_3/T_4 ratio (13, 14). Secondly, tracer iodine given on a single occasion within a few days of surgery is not homogeneously distributed within the thyroid gland. Heterogeneity, both with respect to the iodination of Tg within a follicle as well as between follicles in various portions of the thyroid gland has been clearly demonstrated (15, 16). The radioimmunoassay tech-

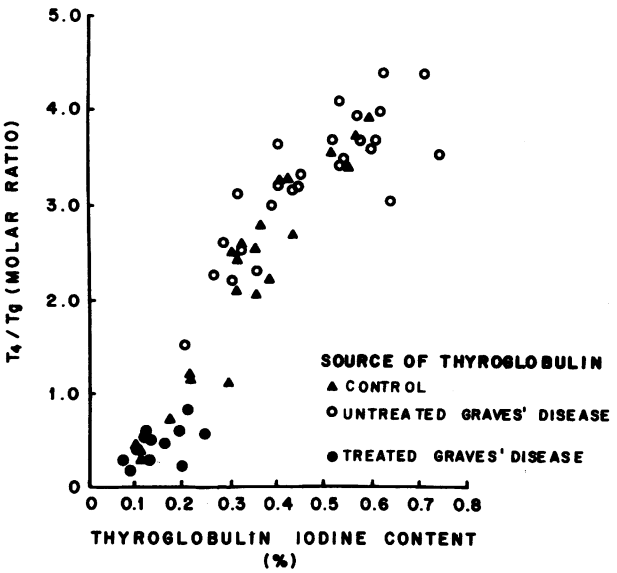

FIGURE 4 Relationship between the T_4/Tg molar ratio and the iodine content of the subfractions of human Tg obtained by RbCl density gradient centrifugation. T_4 content was determined by radioimmunoassay after Pronase digestion of the purified Tg.

niques used in this study circumvent both of these problems. The methodological studies presented above indicate that there is no significant conversion of T_4 to T_3 during the Pronase digestion process and that digestion of the two types of Tg is equally complete with respect to organic iodine.

The range of iodine content observed in normal Tg was from 0.13 to 0.65% similar to that observed by many other investigators (17). With the exception of the first Tg preparation, the T_4 iodine constituted approximately 46% of the total Tg iodine. This is not statistically different from that observed in a group of 12 normal specimens of purified Tg examined by Rolland et al. (18). Because of the difficulty in quantitating the small quantities of T_3 accurately without immunoassay due to the artifacts mentioned previously, there are no reliable estimates of its concentration in normal human Tg. The present results suggest that only about one in five molecules of well-iodinated human Tg contains a T_3 residue as opposed to an average of 3.4 residues of T_4/molecule Tg, a T_4/T_3 molar ratio of 15/1. This results can be compared to several recent studies of human thyroid homogenates, which have used either careful chromatographic separation or immunoassay techniques. Nagataki et al. reported a T_4/T_3 molar ratio of 9.2 (19) and Chopra et al. a ratio of 19 (20). Our previous studies gave results of 13 and 11 for T_4/T_3 ratio in homogenates of normal human thyroids (4, 21).

The iodine content of the Tg from seven propranolol-treated patients with Graves' disease was normal. Because the usual approach to presurgical therapy of patients with this disease includes antithyroid drugs and iodine, there have been no recent systematic studies of tissue from subjects who have not received agents known to alter thyroid function. In one series, a mean value of 0.17% was found in six apparently untreated patients living in France (18). Whether this lower value results from differences in methodology or in the iodine content of the patients' diet is unknown. The percent T_4 iodine in that report varied between 7 and 49% depending on the iodine content of the Tg. This was similar to normal Tg in that study, but the data was insufficient to allow valid comparisons with the T_3 content of normal Tg. The present studies indicate that the percent Tg iodine as T_4 in these subjects is 49%, not different from that in normal Tg. The percent T_3 iodine was significantly increased resulting in a reduction in the molar ratio of T_4/T_3 to 9/1. This percent T_3 iodine is about 1.7 times that which we found in normal Tg.

The results presented in Fig. 3 indicate that at all levels of iodination which are present in these human Tg samples, the T_3/Tg ratio is higher in the Graves' disease Tg than in the control material. Because there is little difference in the T_4/Tg molar ratio (Fig. 4), the calculated T_4/T_3 ratio is significantly lower in Graves' disease. The uniformity of these observations in the RbCl subfractions of differing iodine content (Fig. 5) suggests that the higher T_3/Tg molar ratio is characteristic of all components of the Tg pool. The possibility that two pools of Tg might exist in Graves' thyroid, one with a reduced iodine content and low T_4/T_3 ratio and a second with normal

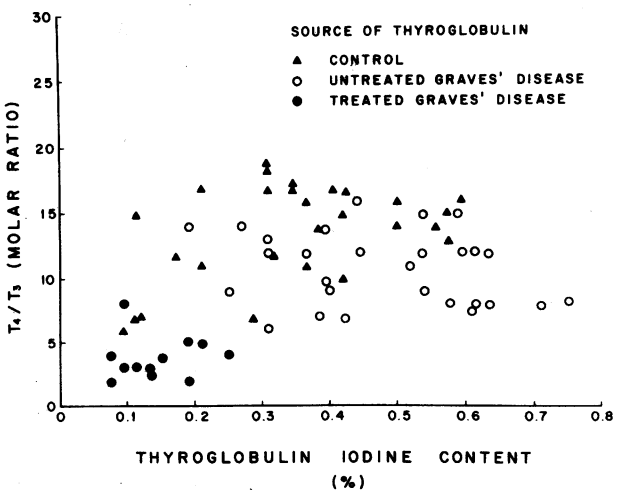

FIGURE 5 Relationship between the T_4/T_3 ratio and iodine content of the subfractions of human Tg obtained by RbCl density gradient centrifugation.

I/Tg and T_3/Tg ratios, seems therefore eliminated. The exclusion of this possibility was the major reason for performing the RbCl fractionation studies. We were somewhat surprised not to find a lower T_4/T_3 ratio in the A, as opposed to the C, fractions from both control and Graves' disease Tg as one might expect more recently synthesized Tg to have a lower I/Tg ratio and therefore a lower T_4/T_3 ratio. We have demonstrated the latter phenomenon in normal and iodine-deficient rats (22). A possible explanation is that this pool of recently synthesized Tg comprises such a small portion of the total pool that its characteristics are lost even in the A subfraction.

The reason for the higher T_3 content of Graves' Tg as opposed to normal is not known. Since the Tg did not appear to be deficient in iodine, this explanation could not be used for these particular findings. Furthermore, in iodine-deficient rats, both the T_3 and T_4 content of Tg are reduced, the reduction in the latter being greater (10). This change gives rise to the high molar ratio of T_3/T_4 characteristic of this condition. Such a situation is seen in the three patients with treated Graves' disease, where the reduction in T_4 from what is observed in either normal or propranolol-treated Graves' Tg exceeds the reduction in T_3 on a percentage basis. The molar ratio, T_3/T_4, is increased even further in these glands than in propranolol-treated patients.

Thyroid peroxidase has been demonstrated to have a stimulatory catalytic action on the rate of coupling of diiodotyrosine to T_4 in vitro (23). The activity of this enzyme has been recently reported to be three to four times normal in thyroid tissues from patients with Graves' disease (24). It is conceivable that in Graves' disease, coupling of critical iodotyrosyl residues might occur before their attaining the level of iodination which would be anticipated from the available iodine supply. Thus instead of 2-diiodotyrosyl residues combining to form T_4, earlier coupling might involve a mono- and a diiodotyrosine giving rise to T_3. An iodine-independent stimulatory effect of thyroid-stimulating hormone on coupling has been demonstrated in hypophysectomized rats by Greer et al. (25) and in thyroid-stimulating hormone-treated guinea pigs by Dunn and Ray (26). Whether or not thyroidal peroxidase is involved in this stimulation is unclear, but the Graves' disease thyroid is clearly stimulated by some factor. It would not appear that propranolol, which appears to have no effect either on thyroidal iodine metabolism or the serum concentrations of thyroid hormones in patients with hyperthyroidism could be implicated in this effect (27, 28).

Our previous measurements of serum T_3 and T_4 in patients with hyperthyroidism due to Graves' disease have indicated that the mean total serum T_3 concentration is 4.3 times normal and that of T_4, 2.7 times normal (3). The molar ratio of T_4/T_3 in the serum of normal subjects was 60±3 (mean±SE) and was 40±2 in the hyperthyroid group. If this apparent relative hyperproduction of T_3 is due solely to an increase in the ratio of T_3/T_4 in the secreted hormones, fractional peripheral T_4/T_3 conversion remaining unchanged, then one would predict an approximate 1.5-fold higher T_3/T_4 ratio in the secreted hormones. This is slightly less than the 1.7-fold difference that we observed. If the ratio of secreted hormones is similar to that present in the Tg, then this relatively small increase in the T_3/Tg ratio could explain the hyperproduction of T_3 in patients with Grave's disease. It would also suggest that the condition known as "T_3 thyrotoxicosis" is a more exaggerated form of this abnormality, perhaps influenced by the level of intrathyroidal iodine. We would conclude, therefore, that while the total quantity of the iodothyronines in the human thyroid is directly related to the iodine content of the Tg, there is another factor (or factors), one of which is present in patients with Graves' disease, which can alter the ratio of T_3 to T_4 present in the Tg and presumably in the secreted hormones.

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