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Research Article

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Thyroid Hormone Transport in the Serum of Patients with Thyrotoxic Graves' Disease before and after Treatment

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ABSTRACT Multiple indices of thyroid hormone binding have been studied in sera obtained from patients with thyrotoxic Graves' disease, before and after treatment, and in sera from a group of carefully matched normal controls. Specimens from patients with thyrotoxicosis displayed a decrease in the thyroxine (T_4)-binding capacities of T_4-binding globulin (TBG) and T_4-binding prealbumin (TBPA), an increase in serum protein-bound iodine (PBI), and an increase in both the proportion and absolute concentration of free T_4. In addition, a smaller than normal proportion of ^{131}I-labeled T_3 was associated with TBG during filter paper electrophoresis. After treatment of thyroxicosis, the only residual abnormality detected was a very slight persistent decrease in the T_4-binding capacity of TBPA, which did not appear to influence the overall thyroid hormone–plasma protein interaction significantly, regardless of whether this was assessed under basal conditions or after enrichment of specimens with stable T_4. It is concluded that the persistent abnormalities in the peripheral metabolism of T_4, previously reported to occur in some patients with treated Graves' disease, probably do not stem from residual abnormalities in the transport of T_4 in the plasma but must arise from abnormalities in T_4 accumulation or metabolism within the tissues themselves.

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INTRODUCTION

There is general agreement that the overall thyroid hormone–plasma protein complex is abnormal in patients with the diffuse toxic goiter of Graves' disease. This abnormality consists of a greater relative saturation of the plasma thyroxine (T_4)-binding proteins, which, in turn, results in a greater proportion of the hormone in the blood being present in the free or unbound state (1–3). The existence of this abnormality has been repeatedly demonstrated by in vitro techniques, such as red blood cell or resin uptake tests (4–6), or by the direct measurement of the proportion of free T_4 (1–3). There has been some question, however, concerning the factors which lead to the abnormality of thyroid hormone transport in the plasma of the thyrotoxic patient. Considerable evidence indicates that an increased concentration of hormone is at least partly responsible. In addition, the binding capacity of the T_4-binding prealbumin (TBPA) is usually diminished (1, 7, 8). Evidence as to whether a similar decrease in binding capacity occurs in the case of the T_4-binding globulin (TBG) has been more variable (1, 8–16).

According to concepts now widely held, the increased proportion of free T_4 in the serum should explain, at least in part, the accelerated fractional peripheral turnover of T_4 which occurs in thyro-

toxic patients; however, the operation of cellular factors, in addition, has not been excluded (9, 11, 17, 18). Since abnormalities in the peripheral metabolism of T_4 have been found to persist in some patients after treatment of thyrotoxicosis (17, 19, 20), it appeared of interest to determine whether abnormalities in the thyroid hormone–plasma protein interaction might also persist after treatment. Earlier studies of individual indices of thyroid hormone binding have provided no evidence that this is the case (16, 17, 21); however, a comprehensive study of this question has not been reported. In the present studies, multiple indices of thyroid hormone binding, including direct measurements of the T_4-binding capacities of TBG and TBPA and of free T_4, have been applied to the study of sera from patients with diffuse toxic goiter, before and after treatment, and from a group of carefully matched normal controls. The data reveal no residual abnormality in the overall binding of thyroid hormone in the sera of patients with Graves' disease after thyrotoxicosis has been controlled.

METHODS

Samples of serum were obtained from 14 patients (12 women and 2 men) with diffuse toxic goiter, ranging in age between 26 and 77 yr. The diagnosis of thyrotoxicosis was established by conventional clinical and laboratory criteria and by the subsequent response to treatment. Sera were obtained from the same patients from 2 months to 2 yr after treatment with either radioactive iodine or subtotal thyroidectomy. None of the patients was receiving antithyroid drugs when specimens of serum were obtained, but two (patients 7 and 11) were receiving desiccated thyroid in full replacement dosage because of posttreatment myxedema. For each patient with Graves' disease, serum was also obtained from a normal control of the same sex, carefully matched for age.[1] None of the normal donors was suffering from disease or receiving any medication.

Sera were kept frozen until used for analyses, at which time they were thawed and divided into several aliquots. For each patient with thyrotoxicosis, an aliquot of the serum obtained after treatment was enriched with a quantity of stable T_4 calculated to increase the PBI to a concentration equal to that in the corresponding pretreatment specimen; an aliquot of the serum from the corresponding normal control was similarly enriched. Thus, a set of five related samples of serum were obtained: pretreatment, posttreatment, and normal samples, as well as posttreatment and normal samples enriched with T_4.

The proportion of free T_4 was measured in duplicate by a method described in this laboratory (2), with the following modifications. The ^{131}I-labeled T_4 used in this analysis was partially purified by preliminary dialysis, as suggested by Schussler and Plager (22), and dilute (1:25) serum, rather than whole serum, was employed. In addition, ^{131}I-labeled T_3[2] was added to an aliquot of the five specimens in each set to produce a final concentration of 2 μg/100 ml; this addition provided approximately 0.8 μc of ^{131}I per ml. These specimens were subjected to reverse-flow electrophoresis in the Durrum apparatus with glycine-acetate buffer (pH 8.6), according to a technique described elsewhere (8, 23).

Samples of serum from thyrotoxic patients, before and after treatment, and from their corresponding controls, were enriched with stable T_4 to concentrations of 119 and 476 μg/100 ml for measurement of the T_4-binding capacities of TBG and TBPA, respectively. Analyses were conducted both by direct-flow electrophoresis in glycine-acetate buffer (pH 8.6), as described earlier from this laboratory (24), and by the reverse-flow technique. Radioactive analysis of electrophoretic strips was performed by direct counting of radioactive zones localized by radioautography.

For each type of test, all analyses of the samples belonging to each set were conducted concurrently.

Measurements of serum protein-bound iodine (PBI) were made by a modification of the method of Zak (25). For comparison of values for the various functions studied among the several groups, the "paired *t*" test was employed; this and other analyses were conducted according to methods described by Snedecor (26).

RESULTS

The principal results obtained are presented in Tables I and II. For each test, the statistical significance of differences among the several groups of specimens is shown in Table III.

Serum protein-bound iodine (PBI). Values for the PBI averaged 12.9 ± 2.5 μg/100 ml (mean $\pm$ SD) in specimens from thyrotoxic patients and were significantly lower after treatment, averaging 5.5 ± 0.9 μg/100 ml. The mean PBI in the normal controls was 5.3 ± 0.8 μg/100 ml, a value significantly lower than in specimens obtained during thyrotoxicosis, but not significantly different from those in the patients with treated thyrotoxicosis.

Thyroxine-binding capacity of TBG. As judged by reverse-flow electrophoresis, the T_4-binding

[1] The largest discrepancy between the age of a thyrotoxic patient and that of the corresponding normal control was 7 yr. For the entire group, the average ages in the thyrotoxic and normal groups differed by approximately 1 yr.

[2] ^{131}I-labeled hormones were purchased from Abbott Laboratories, North Chicago, Ill.

TABLE I

*Indices of Thyroid Hormone Binding in Sera from Patients with Thyrotoxic Graves' Disease before and after Treatment**

Patient No.	Sex	Age	TBG capacity‡		TBPA capacity‡		PBI		Per cent free T_4			Free T_4			T_3 bound by TBG		
			T	TT	T	TT	T	TT	T	TTE	TT	T	TTE	TT	T	TTE	TT
			μg T_4/100 ml		μg T_4/100 ml		μg/100 ml					mμg T_4/100 ml			%		
1	F	42	18.9	26.5	101	177	15.0	5.6	0.031	0.022	0.017	7.16	5.08	1.46	38.9	50.9	66.0
2	M	38	16.1	19.6	152	228	10.2	6.5	0.034	0.024	0.019	5.30	3.74	1.24	43.9	48.4	57.1
3	F	34	23.0	30.2	146	188	12.2	5.0	0.030	0.021	0.015	5.64	3.95	1.16	44.8	63.3	70.7
4	F	33	22.2	26.1	145	221	15.0	6.0	0.034	0.017	0.015	7.85	3.93	1.38	44.7	56.9	68.6
5	F	26	24.0	22.1	157	189	8.6	5.3	0.020	0.017	0.015	2.64	2.24	1.23	62.2	63.0	66.7
6	F	65	17.5	30.7	137	178	16.0	6.0	0.035	0.021	0.016	8.61	5.17	1.47	49.4	64.7	72.9
7	F	50	16.3	19.2	173	226	12.0	6.2							41.5	52.7	58.2
8	F	46	19.5	26.6	171	227	11.4	4.0	0.032	0.016	0.014	5.60	2.80	0.87	40.2	62.5	70.1
9	F	56	22.0	34.4	99	179	16.0	6.6	0.034	0.021	0.015	8.36	5.40	1.53	47.7	64.1	72.8
10	F	30	27.2	25.2	141	201	10.4	4.4	0.021	0.014	0.012	3.36	2.21	0.82	53.3	59.9	65.8
11	F	61	14.0	19.4	67	144	14.4	4.2	0.034	0.026	0.018	7.55	5.77	1.17	31.1	46.7	61.7
12	M	50	17.5	26.1	161	244	16.6	6.2	0.031	0.022	0.015	7.91	5.54	1.42	37.8	55.1	65.2
13	F	77	16.1	24.0	115	163	12.0	6.0	0.040	0.022	0.018	7.40	4.05	1.66	45.5	57.6	61.4
14	F	35	13.4	19.6	159	215	11.6	5.2	0.029	0.017	0.014	5.16	3.03	1.12	34.6	47.8	58.0
Mean		45.9	19.1	25.0	137	199	12.9	5.5	0.031	0.020	0.016	6.35	4.1	1.27	44.0	56.7	65.6
±SD		14.8	4.0	4.7	31	29	2.5	0.9	0.005	0.003	0.002	1.90	1.2	0.24	7.9	6.5	8.9

* The following abbreviations are used to designate the groups studied: T, thyrotoxic; TT, treated thyrotoxic; TTE, treated thyrotoxic enriched with stable thyroxine in concentrations calculated to yield the same PBI as was present in that patient before treatment; TBG, thyroxine (T_4)-binding globulin; TBPA, thyroxine (T_4)-binding prealbumin; PBI, serum protein-bound iodine.

‡ Based on measurements made by reverse-flow filter paper electrophoresis in glycine-acetate buffer, pH 8.6.

TABLE II

*Indices of Thyroid Hormone Binding in Sera from Normal Patients**

Patient No.	Sex	Age	TBG capacity‡	TBPA capacity‡	PBI	Per cent free T_4		Free T_4		T_3 bound by TBG	
			N	N	N	N	NE	N	NE	N	NE
			μg T_4/100 ml	μg T_4/100 ml	μg/100 ml			mμg T_4/100 ml		%	
1 N§	F	35	22.5	204	5.8	0.017	0.020	1.51	4.56	65.3	54.4
2 N	M	38	21.4	283	6.0	0.017	0.019	1.56	2.96	60.6	55.4
3 N	F	35	25.4	268	6.4	0.016	0.017	1.57	3.23	66.7	58.8
4 N	F	39	25.0	204	5.0	0.014	0.021	1.08	4.52	73.2	60.2
5 N	F	20	23.9	203	5.6	0.014	0.015	1.20	2.03	65.7	59.5
6 N	F	60	19.7	256	4.0	0.017	0.022	1.05	5.32	62.4	48.1
7 N	F	50	25.7	230	6.0					72.7	64.9
8 N	F	42	22.0	238	5.0	0.016	0.019	1.23	3.36	62.0	53.9
9 N	F	58	19.2	210	5.0	0.020	0.026	1.54	6.68	57.9	44.2
10 N	F	34	20.2	235	4.2	0.016	0.018	1.04	2.83	63.0	50.5
11 N	F	59	15.7	256	4.2	0.019	0.024	1.23	5.33	59.7	45.1
12 N	M	48	24.7	251	6.4	0.018	0.022	1.76	5.61	64.1	52.0
13 N	F	72	21.4	207	5.2	0.021	0.025	1.66	4.62	60.5	53.1
14 N	F	35	19.8	206	5.8	0.015	0.026	1.34	4.63	53.2	46.9
Mean		44.6	22.0	232	5.3	0.017	0.021	1.37	4.28	63.3	53.4
±SD		13.8	3.1	27	0.8	0.002	0.003	0.24	1.33	5.2	6.1

* The following abbreviations are used to designate the groups studied: N, normal; NE, normal enriched with stable thyroxine in concentrations calculated to yield the same values of the PBI as was present in the corresonding thyrotoxic specimens.

‡ Based on measurements made by reverse-flow filter paper electrophoresis in glycine-acetate buffer, pH 8.6.

§ N indicates normal control donor. Number indicates the number of the corresponding age and sex-matched patient with Graves' disease in Table I.

TABLE III

*Table of "P Values" for the Comparison among Clinical Groups of the Various Indices of Thyroid Hormone Binding (Paired t Test)**

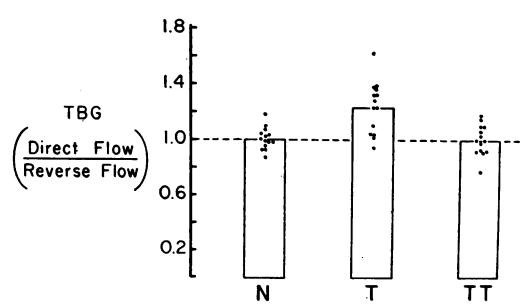
Function	P values							
	N vs. T	N vs. TT	N vs. NE	NE vs. T	T vs. TT	NE vs. TTE	T vs. TTE	TT vs. TTE
TBG capacity, *μg T_4/100 ml*	<0.05	NS			<0.001			
TBPA capacity, *μg T_4/100 ml*	<0.001	<0.01			<0.001			
PBI, *μg/100 ml*	<0.001	NS			<0.001			
Per cent free T_4	<0.001	0.05	<0.001	<0.001	<0.001	NS	<0.001	<0.001
Free T_4 concn., *mμg/100 ml*	<0.001	NS			<0.001			
T_3-TBG, *% total*	<0.001	NS	<0.001	<0.01	<0.001	NS	<0.001	<0.001

* The following abbreviations are used to designate the groups studied: N, normal; T, thyrotoxic; TT, treated thyrotoxic; NE and TTE, normal and treated thyrotoxic, respectively, enriched with stable thyroxine in concentrations calculated to yield the same PBI as was present in the corresponding thyrotoxic specimens.

capacity of TBG in normal subjects averaged 22.0 ± 3.1 μg/100 ml. In sera from patients with active thyrotoxicosis, the corresponding values averaged 19.1 ± 4.0 μg/100 ml, a difference of borderline statistical significance ($P < 0.05$). After treatment of thyrotoxicosis, an increase occurred in all but two patients, the mean T_4-binding capacity reaching a value (25.0 ± 4.7 μg/100 ml) significantly different ($P < 0.001$) from that present before treatment. No significant difference was found between values in normal patients and the slightly higher values found in the patients with treated thyrotoxicosis.

Values for the T_4-binding capacity of TBG as judged by conventional electrophoresis differed in some respects from those found by the reverse-flow technique. A comparison of the results obtained by the two methods is shown in Fig. 1. Average values for the T_4-binding capacity of TBG in normal patients were essentially the same by the two methods (22.2 vs. 22.0 μg/100 ml), and the same was true in specimens from patients with treated thyrotoxicosis (25.1 vs. 25.0 μg/100 ml). In the specimens from thyrotoxic patients, however, the T_4-binding capacity of TBG, as indicated by conventional electrophoresis, averaged 23.2 μg/100 ml, a value significantly higher ($P < 0.001$) than that found by the reverse-flow technique. Hence, values found by conventional electrophoresis did not differ significantly among the three groups.

Thyroxine-binding capacity of TBPA. From measurements with the reverse-flow technique, the T_4-binding capacity of TBPA in normal sera averaged 232 ± 27 μg/100 ml. In sera from patients with active thyrotoxicosis, the corresponding val-



TBG Capacity (μg T_4/100ml)	Direct Flow	22.2 ± 4.1	23.2 ± 3.9	25.1 ± 5.3
	Reverse Flow	22.0 ± 3.1	19.1 ± 4.0	25.0 ± 4.7

FIGURE 1 The effect of electrophoretic technique on measured values of the thyroxine (T_4)-binding capacity of T_4-binding globulin (*TBG*). Each point represents, for an individual sample of serum, the ratio between the maximal T_4-binding capacity of TBG measured by direct-flow electrophoresis and that measured by the reverse-flow technique. Abbreviations used: *N*, normal sera; *T*, sera from patients with active thyrotoxicosis; *TT*, sera from the same patients after treatment. Absolute values for T_4-binding capacity shown below are the means ± SD.

ues were uniformly decreased, averaging 137 ± 31 μg/100 ml ($P < 0.001$). After treatment, values increased in all patients, reaching a mean of 199 ± 29 μg/100 ml. The latter value was, nevertheless, significantly lower than that found in normal specimens ($P < 0.01$).

Per cent of free T_4 in serum. In normal specimens, the per cent of free T_4 averaged 0.017 ± 0.002.[3] Values in patients with active thyrotoxicosis were much higher, averaging 0.031 ± 0.005. After treatment of thyrotoxicosis, the per cent of free T_4 in serum decreased in all patients. The difference between the mean value in specimens from patients with treated thyrotoxicosis (0.016 ± 0.002) and that in normal specimens was of borderline statistical significance ($P = 0.05$).

Enrichment of normal sera with stable T_4 increased the value for the per cent of free T_4 in all specimens, the mean reaching 0.021 ± 0.003. A similar increase occurred consistently upon enrichment of sera from patients with treated thyrotoxicosis. The mean value achieved (0.020 ± 0.003) was essentially the same as that found in normal serum upon enrichment with T_4. In both groups, values for the per cent of free T_4 achieved by enrichment with stable hormone were not nearly as high as those found in the patients with active thyrotoxicosis.

Concentration of free T_4 in serum. Values for the absolute concentration of free T_4 were calculated only in specimens obtained from patients with thyrotoxicosis, before and after treatment, and from normal controls. In the latter group, the concentration of free T_4 averaged 1.37 ± 0.24 mμg/100 ml. Very similar values were obtained in specimens from patients with treated thyrotoxicosis (1.27 ± 0.24 mμg/100 ml). Values in both groups were far lower than in patients with active thyrotoxicosis, in whom values averaged 6.35 ± 1.90 mμg/100 ml.

Binding of T_3 by TBG. The per cent of added labeled T_3 associated with TBG averaged 63.3 ± 5.2 in normal specimens. In speciments from patients with treated thyrotoxicosis, values were not significantly different, averaging 65.6 ± 8.9. In contrast, a pronounced decrease in the per cent of added T_3 associated with TBG was seen in specimens from patients with active thyrotoxicosis, values averaging 44.0 ± 7.9.

Enrichment with stable T_4 of both normal specimens and those from patients with treated thyrotoxicosis decreased the per cent of T_3 bound by TBG in all subjects in each group. Although a slightly higher percentage (56.7 ± 6.5) was found in sera from patients with treated thyrotoxicosis than in sera from normal controls (53.4 ± 6.1), this difference was not significant. In neither group did specimens enriched with stable T_4 display values for the per cent of T_3 bound by TBG as low as those seen in sera from patients with active thyrotoxicosis.

DISCUSSION

The proportion of T_4 in serum that is unbound or free is a direct measure of the overall intensity of T_4 binding by serum proteins, to which it is inversely related. In the present as in earlier reports (1–3), the per cent of free T_4 was found to be increased, often greatly so, in the serum of patients with active thyrotoxicosis. Two general factors seem to cause this increase. That the first factor is an increase in the concentration of T_4 in the serum is indicated by the increased proportion of free T_4 that occurred when, in this and earlier studies (2), normal sera were enriched with stable T_4 to concentrations equal to those present in thyrotoxicosis. Nevertheless, an increase in the concentration of T_4 cannot be the sole factor, since values for the per cent of free T_4 as large as those found in thyrotoxicosis did not result. Therefore, a second factor must be invoked, and this would appear to be a decrease in the level or capacity of the T_4-binding proteins, TBG and TBPA. Several studies have revealed a decrease in the T_4-binding capacity of TBPA in the serum of thyrotoxic patients, similar to that found in the present studies (1, 7, 8). Available data concerning the T_4-binding capacity of TBG in thyrotoxicosis have been more variable, however (1, 8–16). Possible reasons for these discrepancies will be discussed below. However, the slight decrease in the T_4-binding capacity of TBG noted in the present studies is similar to that reported by Inada and Sterling, who used similar electrophoretic tech-

[3] Values for the per cent of free T_4 were calculated as previously described for dilute specimens (2). The values herein reported are lower than those previously reported for undiluted specimens (2). This difference may be ascribed to an effect of dilution itself (2) and to the predialysis of the labeled T_4 (22).

niques (8). Furthermore, we have demonstrated that when thyrotoxicosis is treated, the T_4-binding capacity of TBG in the serum of individual patients almost invariably increases. This observation strongly suggests that the T_4-binding capacity had previously been depressed.

It is not certain how much the increased per cent of free T_4 in the sera of thyrotoxic patients results from a decrease in the T_4-binding activity of TBG and how much from the decrease in TBPA. In previous studies from this laboratory, it was shown that small or moderate increments in the concentration of stable T_4 increased the per cent of free T_4 only slightly (a finding confirmed in the present studies), unless T_4 binding by TBPA was diminished (2). Viewed in another light, the same findings indicate that a decrease in T_4 binding by TBPA has only a small effect on the per cent of free T_4, unless the concentration of stable T_4 is increased. Additional studies to be published separately also indicate that only a small increase in the proportion of free T_4 is produced when TBPA is removed from normal serum by specific immunoadsorption (27). When, however, the relative saturation of TBG is increased by either enrichment of specimens with T_4 or by a primary reduction in TBG binding sites, removal of TBPA results in a large increase in the per cent of free T_4. In the serum of thyrotoxic patients, the relative saturation of TBG is indeed increased, owing to both a decrease in TBG binding sites and an increase in the total concentration of T_4. Hence, in sera of thyrotoxic patients, for any prevailing concentration of T_4, the decrease in the T_4-binding capacity of TBG increases the importance of a decrease in the binding capacity of TBPA.

Several factors may explain the discrepant data in the literature concerning the changes which occur in the T_4-binding capacity of TBG in the serum of patients with thyrotoxicosis. First, it seems possible that the extent of decrease in TBG depends on the severity or duration of the disease; this factor is difficult to evaluate from the present or previous data. Failure to detect a lowering of TBG in thyrotoxicosis also may have resulted from differences in the preponderant sex or age of the patients in the thyrotoxic and control groups, since the binding capacity of TBG has been shown to vary significantly with these factors (24, 28). Finally, the present studies strongly indicate that small decreases in the T_4-binding capacity of TBG may be obscured when the binding capacity of TBPA is also reduced if conventional, rather than reverse-flow, electrophoresis is employed. In normal sera and in those obtained from patients after treatment of thyrotoxicosis, in both of which TBPA was essentially normal, no significant difference between values for the T_4-binding capacity of TBG assessed by the two electrophoretic techniques was found. In sera from thyrotoxic patients, however, in which the binding capacity of TBPA was subnormal, higher values were found by conventional than by reverse-flow electrophoresis, and by the former technique no difference between normal specimens and those from thyrotoxic patients was detected. It seems likely that this phenomenon can be ascribed to trailing of albumin-bound T_4 across the TBG zone during conventional electrophoresis. This possibility, originally suggested by Robbins, first prompted development of the reverse-flow technique (29). In sera containing little TBPA, a greater proportion of labeled T_4 is associated with albumin than is the case in sera in which TBPA is normal, particularly at the high concentrations of stable T_4 used to measure the binding capacity of TBG. Hence, during conventional electrophoresis of sera in which the binding of T_4 by TBPA is decreased, trailing of albumin becomes a significant factor in producing a spuriously high value for the binding capacity of TBG. In some previous studies which indicated that no decrease in the binding capacity of TBG occurs during thyrotoxicosis, conventional electrophoresis was employed (1, 11).

The major objective of the present study was to ascertain whether any abnormalities in the thyroid hormone–plasma protein interaction which were present during active thyrotoxicosis would persist after restoration of a euthyroid state. Previous studies have provided suggestive evidence bearing upon this point. In a group of patients showing persistent abnormalities in T_4 turnover, no abnormality in TBG could be detected (17). Schussler, who estimates the T_4-binding capacity of TBG indirectly, has reported an increase to follow treatment of thyrotoxic patients with propylthiouracil (16). Mitchell, Bradford, and Collins found that the per cent of ^{131}I-labeled T_3

associated with TBG during electrophoresis, which is decreased during active thyrotoxicosis, returns toward normal after treatment (21). In none of these studies were values for the T_4-binding capacity of TBPA or the per cent of free T_4 after treatment reported. In the present studies, the only abnormality similar to that present during thyrotoxicosis which could be detected after treatment was a persistent significant decrease in the T_4-binding capacity of TBPA. This decrease was slight, however, compared to that present before treatment. The T_4-binding capacity of TBPA averaged 232 ± 27 μg/100 ml in normal specimens and 199 ± 29 μg/100 ml in sera from patients with treated thyrotoxicosis. As indicated earlier, this slight lowering of TBPA would not be expected to influence the overall binding of T_4 appreciably, especially in the presence of a normal PBI and TBG (2, 27). The lowering of TBG found during active thyrotoxicosis was completely reversed by treatment; indeed, T_4-binding capacities of TBG were slightly, though not significantly, higher than normal in the serum of patients with treated thyrotoxicosis. This finding may account for the very slight lowering of the per cent of free T_4 found in this group as compared to the normal controls. In all other respects, including PBI, absolute concentration of free T_4, per cent of labeled T_3 bound by TBG, and the response to T_4 loading of both the per cent of free T_4 and the binding of T_3 by TBG, no difference between normal sera and those obtained after treatment of thyrotoxicosis was observed.

Despite some data which suggest the contrary (30), several studies indicate that abnormalities in the peripheral turnover of T_4 persist at least in some patients with diffuse toxic goiter after relief of thyrotoxicosis. Lennon, Engbring, and Engstrom, who studied the early phase disappearance of T_4 from plasma, i.e. that between 20 and 50 min after administration of the labeled hormone, found that the half-time of disappearance in hypothyroid patients after treatment was significantly faster than in patients with spontaneous myxedema. Furthermore, in patients rendered euthyroid by treatment, the half-time of early disappearance was significantly faster than in normal controls, although only marginally so ($P < 0.05$) (19). Nicoloff and Dowling have reported in abstract form that both the early rate of disappearance of T_4 and its early hepatic uptake are increased in patients with treated diffuse toxic goiter (20). Examination of their data reveals the early (3 hr) hepatic uptake of T_4 to be 40% higher than normal, and 3 hr deiodination rate 33% greater than normal, in patients euthyroid after treatment.[4] As regards the late phase metabolism of T_4, i.e. that which occurs after distribution equilibrium, Ingbar and Freinkel have noted (17) a persistent increase in the fractional rate of T_4 turnover in some patients with diffuse toxic goiter long after restoration of a euthyroid state. These data are in accord with those of Nicolof and Dowling,[4] which demonstrate an increase of approximately 45% in mean daily T_4 disposal and an increase in mean hepatic T_4 content of more than 100% in patients with Graves' disease rendered euthyroid. Although the studies of Sterling were taken to indicate that no abnormality of T_4 turnover persists after treatment of thyrotoxicosis (30), several aspects of the data in that publication suggest that many of the patients were mildly hypothyroid. Since hypothyroidism may itself retard the turnover of T_4 (30, 31), a persistent acceleration of turnover associated with Graves' disease may have been obscured.

Since none of the foregoing studies included measurements of T_4 binding by techniques currently employed, it is pertinent to note that in a group of four patients studied in the author's laboratory before and approximately 6 months after treatment of thyrotoxicosis, normal values for the T_4-binding capacity of TBG and TBPA were found after treatment, whereas the average half-time of T_4 remained less than 4 days.[5]

From all of the foregoing evidence, it may be concluded that abnormalities of T_4 metabolism frequently, if not invariably, persist after relief of thyrotoxicosis in Graves' disease. It has generally been agreed that the proportion of free T_4 in plasma is a major determinant of its fractional rate of turnover, and that the concentration of free T_4 is a major determinant of total T_4 disposal. Our inability to demonstrate any persistent in-

[4] The authors are indebted to Dr. J. T. Nicoloff and Dr. J. T. Dowling for permission to examine their data and describe a portion of their findings before publication.

[5] Sobel, R. J., K. A. Woeber, and S. H. Ingbar. Unpublished observations.

crease in the proportion or concentration of free T_4 in the serum of patients with treated thyrotoxicosis suggests that persistent abnormalities in T_4 turnover do not result from disturbances in extracellular hormonal binding. Rather, they appear to result from factors within one or more cellular sites, such as the liver, where T_4 is bound and metabolized (20, 32, 33). The concept that cellular factors, in addition to extracellular binding, act as important determinants of the overall metabolism of T_4 has been recognized for a considerable period (9, 11, 17, 18), but has only recently been verified by direct evidence (34). Other instances of altered T_4 turnover without evidence of abnormal extracellular binding are to be found in the progressive slowing of fractional T_4 turnover that occurs with advancing age (28, 35), and in the accelerated fractional turnover of T_4 produced by thyroid-suppressive doses of T_3 (36). Whether altered cellular metabolism of T_4 in these and possibly other conditions arises from changes in the cellular binding of the hormone or in the enzymatic mechanisms for hormonal degradation or excretion is as yet unknown.

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REFERENCES

1. Oppenheimer, J. H., R. Squef, M. I. Surks, and H. Hauer. 1963. Binding of thyroxine by serum proteins evaluated by equilibrium dialysis and electrophoretic techniques. Alterations in nonthyroidal illness. *J. Clin. Invest.* **42**: 1769.
2. Ingbar, S. H., L. E. Braverman, N. A. Dawber, and G. Y. Lee. 1965. A new method for measuring the free thyroid hormone in human serum and an analysis of the factors that influence its concentration. *J. Clin. Invest.* **44**: 1679.
3. Sterling, K., and M. A. Brenner. 1966. Free thyroxine in human serum: simplified measurement with the aid of magnesium precipitation. *J. Clin. Invest.* **45**: 153.
4. Hamolsky, M. W., M. Stein, and A. S. Freedberg. 1957. The thyroid hormone-plasma protein complex in man. II. A new *in Vitro* method for study of "uptake" of labelled hormonal components by human erythrocytes. *J. Clin. Endocrinol. Metab.* **17**: 33.
5. Mitchell, M. L., A. B. Harden, and M. E. O'Rourke. 1960. The *in Vitro* resin sponge uptake of triiodothyronine-I^{131} from serum in thyroid disease and in pregnancy. *J. Clin. Endocrinol. Metab.* **20**: 1474.
6. Sterling, K., and M. Tabachnick. 1961. Resin uptake of I^{131}-triiodothyronine as a test of thyroid function. *J. Clin. Endocrinol. Metab.* **21**: 456.
7. Richards, J. B., J. T. Dowling, and S. H. Ingbar. 1959. Alterations in the plasma transport of thyroxine in sick patients and their relation to the abnormality in Graves' disease. *J. Clin. Invest.* **38**: 1035. (Abstr.)
8. Inada, M., and K. Sterling. 1967. Thyroxine transport in thyrotoxicosis and hypothyroidism. *J. Clin. Invest.* **46**: 1442.
9. Robbins, J., and J. E. Rall. 1960. Proteins associated with the thyroid hormones. *Physiol. Rev.* **40** (Suppl. 4): 415.
10. Tanaka, S., and P. Starr. 1959. Clinical observations on serum globulin thyroxine-binding capacity, using a simplified technique. *J. Clin. Endocrinol. Metab.* **19**: 84.
11. Ingbar, S. H., and N. Freinkel. 1960. Regulation of the peripheral metabolism of the thyroid hormones. *Recent Progr. Hormone Res.* **16**: 353.
12. Cavalieri, R. R. 1961. Hyperthyroidism and decreased thyroxine binding by serum proteins. *J. Clin. Endocrinol. Metab.* **21**: 1455.
13. Bakker, A., M. G. Woldring, and H. Doorenbos. 1961. Thyroxine-binding capacity of the carrier protein fraction in serum of patients with thyroid dysfunction. *Clin. Sci.* **21**: 241.
14. Silverstein, J. N., H. L. Schwartz, E. B. Feldman, D. M. Kydd, and A. C. Carter. 1962. Correlation of the red blood cell uptake of I^{131}-L-triiodothyronine and thyroxine binding globulin capacity in man. *J. Clin. Endocrinol. Metab.* **22**: 1002.
15. Cuaron, A. 1966. Relationship between the *in Vitro* uptake of ^{131}I-triiodothyronine by erythrocytes and its binding by serum proteins in thyroid disease. *J. Clin. Endocrinol. Metab.* **26**: 53.
16. Schussler, G. C. 1966. Thyroxine binding globulin (TBG) in thyrotoxicosis and nonthyroidal illness. Abstracts of the 48th meeting of The Endocrine Society, Chicago, Ill. 113.
17. Ingbar, S. H., and N. Freinkel. 1958. Studies of thyroid function and the peripheral metabolism of I^{131}-labeled thyroxine in patients with treated Graves' disease. *J. Clin. Invest.* **37**: 1603.
18. Braverman, L. E., and S. H. Ingbar. 1961. The metabolism of thyroid hormones as related to protein binding. *J. Chronic Diseases.* **14**: 484.
19. Lennon, E. J., N. H. Engbring, and W. W. Engstrom. 1961. Studies of the rate of disappearance of labeled thyroxine from the intravascular compartment. *J. Clin. Invest.* **40**: 996.
20. Nicoloff, J. T., and J. T. Dowling. 1964. Compartmental distribution of thyroxine in man. Abstracts of the 46th meeting of The Endocrine Society, San Francisco, Calif. 29.

21. Mitchell, M. L., A. H. Bradford, and S. Collins. 1964. Differences in the interaction of triiodothyronine-^{131}I with serum proteins *in vitro*. *J. Clin. Endocrinol. Metab.* **24**: 867.
22. Schussler, G. C., and J. E. Plager. 1967. Effect of preliminary purification of ^{131}I-thyroxine on the determination of free thyroxine in serum. *J. Clin. Endocrinol. Metab.* **27**: 242.
23. Elzinga, K. E., E. A. Carr, Jr., and W. H. Beierwaltes. 1961. Adaptation of the standard Durrum-type cell for reverse-flow paper electrophoresis. *Am. J. Clin. Pathol.* **36**: 125.
24. Braverman, L. E., A. E. Foster, and S. H. Ingbar. 1967. Sex-related differences in the binding in serum of thyroid hormones. *J. Clin. Endocrinol. Metab.* **27**: 227.
25. Benotti, J., and N. Benotti. 1963. Protein-bound iodine, total iodine, and butanol-extractable iodine by partial automation. *Clin. Chem.* **9**: 408.
26. Snedecor, G. W. 1956. Statistical Methods Applied to Experiments in Agriculture and Biology. Iowa State University Press, Ames, Iowa. 5th edition.
27. Woeber, K. A., and S. H. Ingbar. 1967. Contribution of prealbumin to thyroxine-binding in normal and abnormal sera. *Clin. Res.* **15**: 267. (Abstr.)
28. Braverman, L. E., N. A. Dawber, and S. H. Ingbar. 1966. Observations concerning the binding of thyroid hormones in sera of normal subjects of varying ages. *J. Clin. Invest.* **45**: 1273.
29. Robbins, J. 1956. Reverse-flow zone electrophoresis. A method for determining the thyroxine-binding capacity of serum protein. *Arch. Biochem. Biophys.* **63**: 461.
30. Sterling, K. 1958. Radiothyroxine turnover studies in thyroid disease after therapy. *J. Clin. Invest.* **37**: 1348.
31. Ingbar, S. H., and N. Freinkel. 1955. Simultaneous estimation of rates of thyroxine degradation and thyroid hormone synthesis. *J. Clin. Invest.* **34**: 808.
32. Cavalieri, R. R., and G. L. Searle. 1966. The kinetics of distribution between plasma and liver of ^{131}I-labeled L-thyroxine in man: observations of subjects with normal and decreased serum thyroxine-binding globulin. *J. Clin. Invest.* **45**: 939.
33. Oppenheimer, J. H., G. Bernstein, and J. Hasen. 1967. Estimation of rapidly exchangeable cellular thyroxine from the plasma disappearance curves of simultaneously administered thyroxine-^{131}I and albumin-^{125}I. *J. Clin. Invest.* **46**: 762.
34. Bernstein, G., N. I. Surks, J. Hasen, S. Artz, and J. H. Oppenheimer. 1967. Increased thyroidal secretion and thyroxine turnover following stimulation of hepatocellular binding by phenobarbital and chlordane. Program of the American Thyroid Association. 51. (Abstr.)
35. Gregerman, R. I., G. W. Gaffney, and N. W. Shock. 1962. Thyroxine turnover in euthyroid man with special reference to changes with age. *J. Clin. Invest.* **41**: 2065.
36. Schussler, G. C., and V. K. Vance. 1967. Effect of triiodothyronine (T_3) on thyroxine binding and turnover. *Clin. Res.* **15**: 266. (Abstr.)