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### Research Article

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(c) In hypothyroidism, the TBPA capacities showed no consistent deviation from the normal range. 11 of the 16 sera had elevated TBG capacities as well as markedly diminished free thyroxine fractions. In contrast, five hypothyroid sera had normal TBG capacities and normal or nearly normal free thyroxine fractions.

(d) In thyrotoxicosis and hypothyroidism, the inverse correlation between free thyroxine fraction and TBG was much closer than that with TBPA. When diagnostic categories were considered separately, only TBG bore a significant inverse relation to the free thyroxine fraction. It is therefore suggested that in thyroid diseases TBG may sometimes play a more important role than TBPA in determining the free thyroxine fraction.

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## Thyroxine Transport in Thyrotoxicosis and Hypothyroidism \*

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**Summary.** (a) The thyroxine-binding proteins were investigated in 23 cases of untreated thyrotoxicosis and 16 cases of untreated hypothyroidism, employing reverse flow paper electrophoresis with the glycine acetate system at pH 8.6 in the Durrum type cell.

(b) In active thyrotoxicosis, all sera exhibited diminished thyroxine-binding prealbumin (TBPA) capacities. However, 17 of the 23 sera also had diminished thyroxine-binding alpha globulin (TBG) capacities, as well as markedly elevated free thyroxine fractions. In contrast, six thyrotoxic sera had normal TBG capacities and normal or slightly elevated free thyroxine fractions.

(c) In hypothyroidism, the TBPA capacities showed no consistent deviation from the normal range. 11 of the 16 sera had elevated TBG capacities as well as markedly diminished free thyroxine fractions. In contrast, five hypothyroid sera had normal TBG capacities and normal or nearly normal free thyroxine fractions.

(d) In thyrotoxicosis and hypothyroidism, the inverse correlation between free thyroxine fraction and TBG was much closer than that with TBPA. When diagnostic categories were considered separately, only TBG bore a significant inverse relation to the free thyroxine fraction. It is therefore suggested that in thyroid diseases TBG may sometimes play a more important role than TBPA in determining the free thyroxine fraction.

(e) The demonstrated variations in the binding proteins were considered sufficient to explain the abnormalities of the free thyroxine fractions in thyroid disease.

### Introduction

Previous studies (1-4) have suggested diminished thyroxine-binding prealbumin (TBPA) in sera from patients with thyrotoxicosis without significant alteration in thyroxine-binding alpha globulin (TBG); slight but significant elevation of TBG was reported in hypothyroidism (2-4).

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In more recent works (4-7) the free thyroxine fraction in serum has been measured; it appears to be determined in large part by the thyroxine-binding proteins. Thus, the elevated free thyroxine fractions in sera from patients with thyrotoxicosis have been ascribed to their diminished TBPA capacities, as well as to the elevated thyroxine concentrations (4, 5). However, published findings do not appear to have excluded definitely the possibility that free thyroxine fractions might also be related to TBG capacities.

In the present report, we have shown that approximately three-fourths of 23 sera from thyrotoxic patients had diminished TBG capacities and, moreover, the free thyroxine fractions were correlated inversely with TBG capacities in thyrotoxi-

cosis and in hypothyroidism. These findings suggested that in thyroid disease, TBG may play a more important role than TBPA in determining the free thyroxine fraction.

## Methods

### Clinical material

**Thyrotoxicosis.** Sera from 23 untreated hyperthyroid patients were obtained from the Veterans Administration Hospital and its Radioisotope Service, the Thyroid Clinic, and the Radiotherapy Department of the Presbyterian Hospital. The diagnoses were verified by thyroidal uptake of  $^{131}\text{I}$ , as well as thyroidal clearance (8), in the patients from the Veterans Administration Hospital Radioisotope Service. Protein-bound iodine (PBI)<sup>1</sup> or thyroxine iodine by column<sup>2</sup> was also available, as well as  $\text{T}_3$  resin uptake and other diagnostic tests. All were considered cases of toxic diffuse goiter (Graves' disease).

**Hypothyroidism.** Sera from 16 untreated hypothyroid subjects were obtained from the same sources as the patients with thyrotoxicosis. The diagnoses were verified by thyroidal uptake, PBI,<sup>1</sup> or thyroxine iodine by column,<sup>2</sup> and usually by other tests as well.

Cases C.K., R.C., I.H., J. E., and E.C. were hypothyroid after  $^{131}\text{I}$  therapy, and W.P. after total thyroidectomy for thyroid carcinoma. Case E.W. became hypothyroid

after subacute thyroiditis. M.C. had unexplained goitrous hypothyroidism. A.T., M.S., J.M., E.G., M.O., J.V., F.R., and O.L. had spontaneous idiopathic myxedema or hypothyroidism.

All patients had moderate or severe clinical pictures, and patients with mild thyrotoxicosis or hypothyroidism were not included in this study.

**Normal group.** Sera were obtained from 19 healthy volunteers (12 men, 7 women) with ages ranging from 22–45 yr.

### Determination of free thyroxine in serum

The free thyroxine fraction in serum was determined in duplicate or triplicate by the magnesium precipitation method of Sterling and Brenner (6). The free thyroxine iodine concentration in  $\mu\text{g}/100\text{ ml}$  represents the product of free thyroxine percentage and PBI value or thyroxine iodine by column.

$^{131}\text{I}$ -labeled L-thyroxine<sup>3</sup> in 50% propylene glycol solution was tested for purity by descending paper chromatography with the *n*-butanol-dioxane-ammonia system. The radiothyroxine was free of appreciable radioactive contaminants other than 1–5% iodide. The precise per cent of radioactivity due to iodide- $^{131}\text{I}$  was determined by paper electrophoresis as previously described (6).

### Paper electrophoretic study

Reverse flow electrophoresis (9) with a glycine acetate system, pH 8.6 (10), was performed to determine the

<sup>1</sup> J. R. Leonards Medical Laboratory, Cleveland, Ohio, or Bio-Science Laboratories, Van Nuys, Calif.

<sup>2</sup> Bio-Science Laboratories, Van Nuys, Calif.

<sup>3</sup> Abbott Laboratories, North Chicago, Ill. Cysteine (0.2%) was added by the supplier for stabilization.

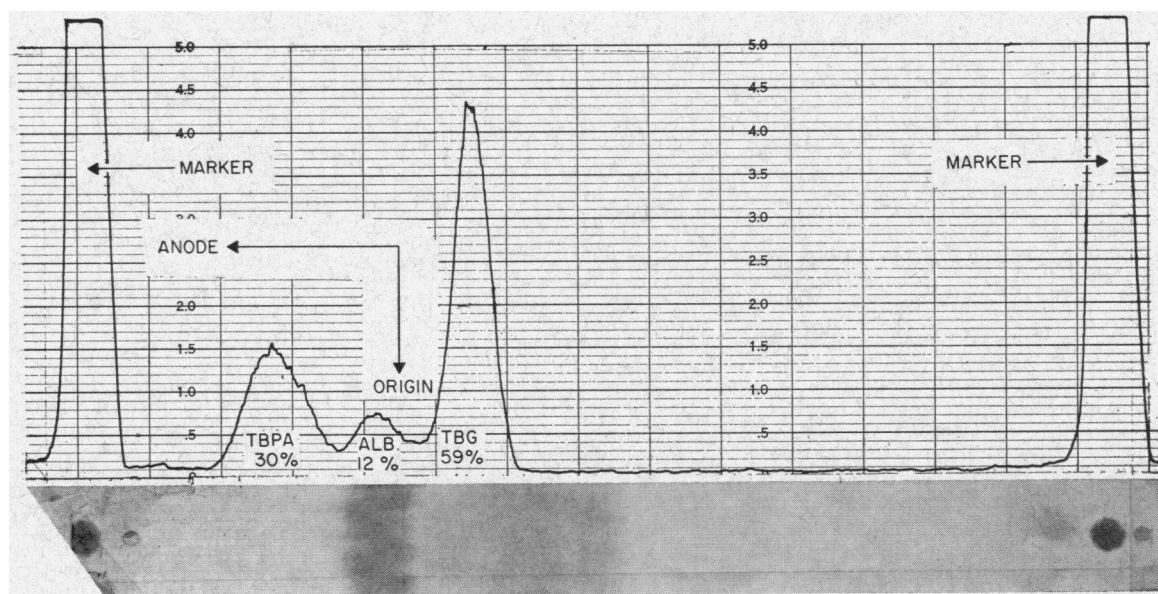


FIG. 1. REVERSE FLOW PAPER ELECTROPHORESIS, GLYCINE ACETATE SYSTEM, pH 8.6. Radioactive scan aligned with stained paper strip below. The above normal serum had been enriched with a "tracer" amount of thyroxine- $^{131}\text{I}$ , 3  $\mu\text{g}/100\text{ ml}$ . The scan is typical of sera with "tracer" as opposed to "loading" or saturating concentrations of the hormone.

maximal binding capacities of thyroxine-binding alpha globulin (TBG) and thyroxine-binding prealbumin (TBPA), using the Spinco-Durrum apparatus. 580 ml glycine acetate solution was poured into the anodal cell and 500 ml solution into the cathodal cell. Fresh solution was always employed. Serum samples of 8  $\mu$ l were applied 3.5 inches from the anodal end of each Whatman filter paper strip (grade 1, SP Pattern). Samples were applied on the paper strips in the cold room at 4°C to minimize the evaporation of the solution from the paper during application. Electrophoresis was performed for 19 hr at a constant current reading of 12 mA (0.5 mA/cm of paper strip) at room temperature. After completion

of electrophoresis, the paper strips were dried at 120°C for 30 min and then scanned with the Nuclear-Chicago Actigraph II (model C-100B) equipped with an automatic recorder, before they were stained with bromophenol blue. From both the tracings and the stained paper strips, the position of each thyroxine-binding protein was determined, and the maximal binding capacities of TBG and TBPA were calculated by planimetry of the tracing. In subsequent work planimetry was obviated by automatic integration (Disc Integrator in Nuclear-Chicago Actigraph III System, model 1004) which yielded the same values.

TABLE I  
*Findings in sera from 23 cases of untreated thyrotoxicosis*

Subjects	Age and sex	PBI	Free thyroxine fraction	Free thyroxine iodine	TBG	TBPA
		$\mu\text{g}/100\text{ ml}$	%	$m\mu\text{g}/100\text{ ml}$	$\mu\text{g}/100\text{ ml}$	$\mu\text{g}/100\text{ ml}$
Group I (diminished TBG)						
H.P.	50F	10.9	0.091	9.92	12.7	172
E.H.	63F	13.1*	0.111	14.54	17.5	135
S.B.	45F	10.3	0.146	15.04	8.9	132
W.H.	46F	9.1	0.088	8.01	14.8	157
B.P.	25F	8.1	0.076	6.16	15.0	113
B.S.	42F	9.5	0.125	11.88	9.4	156
A.R.	36M	12.6*	0.073	9.20	11.5	165
H.F.	38F	15.8*	0.094	14.85	16.0	145
J.P.	55M	13.9	0.065	9.04	15.6	199
I.A.	62F	11.7	0.112	13.10	17.1	204
B.L.	40F	14.6	0.113	16.50	17.1	169
H.A.	45M	12.2	0.072	8.78	15.5	147
L.T.	45F	12.0	0.092	11.04	15.2	167
R.R.	50M	8.9	0.104	9.26	15.3	148
M.L.	30F	10.0	0.066	6.60	13.6	194
G.B.	35F	11.6	0.184	21.34	8.8	193
L.P.	35M	8.1	0.091	7.37	14.3	168
Mean $\pm$ SD		11.3 $\pm$ 2.3	0.100 $\pm$ 0.031	11.33 $\pm$ 4.0	14.0 $\pm$ 2.8	163 $\pm$ 25
Group II (normal TBG)						
P.W.	12F	18.5	0.067	12.40	22.3	120
E.S.	45F	12.6	0.065	8.19	20.8	160
P.R.	45M	17.3	0.071	12.28	20.9	141
A.B.	55F	16.2	0.057	9.23	23.5	176
B.L.	22F	10.3	0.048	4.94	21.0	168
A.N.	45F	13.6	0.053	7.21	21.8	159
Mean $\pm$ SD		14.8 $\pm$ 3.1	0.060 $\pm$ 0.008	9.04 $\pm$ 2.9	21.7 $\pm$ 1.1	154 $\pm$ 21
$P\ddagger$		<0.01	<0.01	NS	<0.001	NS
Mean $\pm$ SD in all cases of thyrotoxicosis		12.2 $\pm$ 2.9	0.090 $\pm$ 0.032	10.73 $\pm$ 3.88	16.0 $\pm$ 4.2	160 $\pm$ 24
$P\S$		<0.001	<0.001	<0.001	<0.001	<0.001
Normal mean $\pm$ SD (12 volunteers)		6.2 $\pm$ 1.0	0.042 $\pm$ 0.006	2.56 $\pm$ 0.49	21.0 $\pm$ 2.1	259 $\pm$ 21

PBI, protein-bound iodine; TBG, maximal binding capacity of thyroxine-binding alpha globulin; TBPA, maximal binding capacity of thyroxine-binding prealbumin; NS, not significant ( $P > 0.05$ ).

\* Thyroxine iodine by column.

$\ddagger$  Probability that the value in group I is identical to the corresponding value in group II.

$\S$  Probability that the value in the whole diagnostic category is identical to the corresponding value in the normal group.

|| Mean  $\pm$  SD of 19 normal volunteers, rather than 12.

As shown in Fig. 1, quite satisfactory separation of thyroxine-binding proteins was obtained with this method.

It was found by studies with various thyroxine enrichments from 50–1000  $\mu\text{g}/100\text{ ml}$  that TBG and TBPA were saturated by the addition of 75 and 600  $\mu\text{g}/100\text{ ml}$ , nonradioactive L-thyroxine to serum, respectively. Therefore, samples of sera, enriched with the above amounts of nonradioactive L-thyroxine were used to determine the maximal binding capacities of TBG and TBPA, respectively. The appropriate carrier was prepared from a stock solution of 2 mg/ml nonradioactive L-thyroxine<sup>4</sup> dissolved in 0.067 N NaOH. This was diluted 1:3 with 0.125 g/100 ml human serum albumin in isotonic saline solution and stored in a brown plastic bottle at 4°C. Although this stock solution was stable for at least 2 wk, it was ordinarily depleted within 5 days. Appropriate dilutions of the stock solution were made immediately before addition to serum.

All measurements of maximal binding capacities were made at least in duplicate or triplicate and were run with concomitant analysis of a serum pool and, occasionally, a normal serum as standards. Replicates were in good agreement, and mean values were determined for each serum sample. The mean values and standard deviation of maximal binding capacities of TBG and TBPA in the serum pool were  $21.5 \pm 0.3$  and  $186 \pm 3\text{ }\mu\text{g}/100\text{ ml}$ , respectively. The mean value for TBPA in the serum pool (which included sick patients) was appreciably lower than values in healthy volunteers ( $259 \pm 21\text{ }\mu\text{g}/100\text{ ml}$ ).

<sup>4</sup> Mann Research Laboratories, Inc., New York, N. Y.

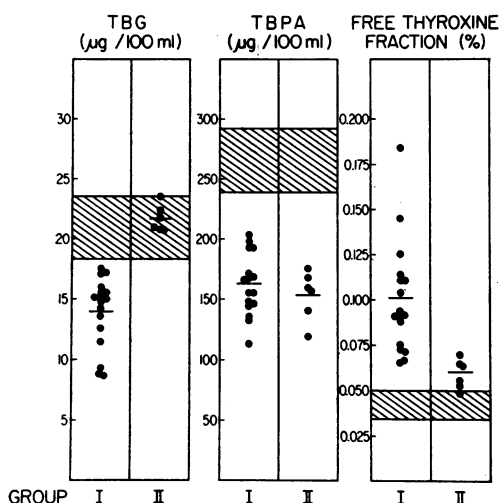


FIG. 2. TBG, TBPA, AND FREE THYROXINE FRACTION IN THYROTOXICOSIS. The cross-hatched areas indicate the normal ranges. The horizontal lines indicate the mean values in each group of thyrotoxicosis. Group I has diminished TBG capacity; group II has normal TBG.

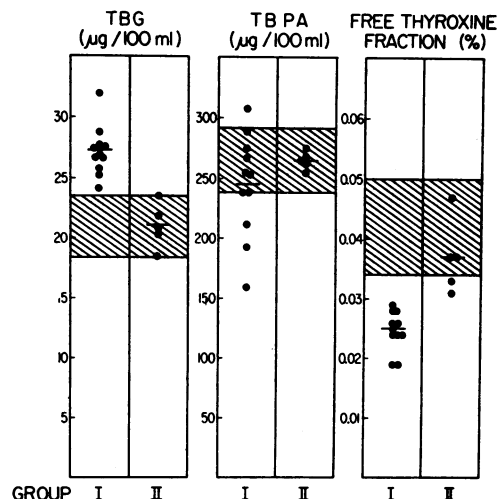


FIG. 3. TBG, TBPA, AND FREE THYROXINE FRACTION IN HYPOTHYROIDISM. The cross-hatched areas indicate the normal ranges. The horizontal lines indicate the mean values in each group of hypothyroidism. Group I has elevated TBG capacity; group II has normal TBG.

## Results

In 23 cases of thyrotoxicosis, the TBG capacities ranged from 8.8–23.5  $\mu\text{g}/100\text{ ml}$  (Table I and Fig. 2). Despite the wide scatter, the mean value for TBG capacities in thyrotoxicosis was significantly reduced (mean  $\pm$  SD =  $16.0 \pm 4.2\text{ }\mu\text{g}/100\text{ ml}$  in thyrotoxicosis vs. normal of  $21.0 \pm 2.1$ ,  $P < 0.001$ ). The TBPA capacities were diminished in all instances of thyrotoxicosis (Table I and Fig. 2). The mean TBPA capacity in thyrotoxicosis (mean  $\pm$  SD =  $160 \pm 24\text{ }\mu\text{g}/100\text{ ml}$ ) was significantly below the normal TBPA capacity (mean  $\pm$  SD =  $259 \pm 21\text{ }\mu\text{g}/100\text{ ml}$ ,  $P < 0.001$ ).

In 16 cases of hypothyroidism, the TBG capacities were normal or slightly elevated (Table II and Fig. 3). The mean value (mean  $\pm$  SD =  $25.4 \pm 3.5\text{ }\mu\text{g}/100\text{ ml}$ ) for TBG capacities in hypothyroidism was significantly elevated ( $P < 0.001$ ). The TBPA capacities in hypothyroidism showed some scatter (mean  $\pm$  SD =  $251 \pm 36\text{ }\mu\text{g}/100\text{ ml}$ ), but there was no significant difference from normal (Table II).

The scatter diagram of the relation between free thyroxine fraction and PBI value in thyroid disease revealed the expected direct relation (Fig. 4). The correlation coefficient of +0.63 was highly significant statistically ( $P < 0.001$ ). The results resembled the previous findings from this

TABLE II  
Findings in sera from 16 cases of untreated hypothyroidism

Subjects	Age and sex	PBI	Free thyroxine fraction	Free thyroxine iodine	TBG	TBPA
		$\mu\text{g}/100\text{ ml}$	%	$\text{m}\mu\text{g}/100\text{ ml}$	$\mu\text{g}/100\text{ ml}$	$\mu\text{g}/100\text{ ml}$
Group I (elevated TBG)						
C.K.	23F	1.4	0.019	0.27	25.8	256
M.C.	36F	2.8*	0.028	0.78	27.8	194
A.T.	40F	1.5	0.019	0.29	27.7	267
R.C.	42M	2.2	0.026	0.57	27.6	238
W.P.	45M	3.3	0.024	0.79	32.2	287
M.S.	50F	1.1	0.025	0.28	28.9	213
E.W.	52F	2.2	0.029	0.64	27.0	238
J.M.	25M	2.0	0.024	0.48	26.8	160
E.G.	50F	1.0	0.026	0.26	24.4	255
I.H.	72M	2.0	0.028	0.56	25.3	308
M.O.	35F	2.6	0.024	0.62	26.9	275
Mean $\pm$ SD		$2.0 \pm 0.7$	$0.025 \pm 0.003$	$0.50 \pm 0.20$	$27.3 \pm 2.1$	$245 \pm 43$
Group II (normal TBG)						
J.V.	45F	0.8	0.031	0.25	21.9	266
J.E.	46F	1.3	0.037	0.48	20.6	264
F.R.	72M	1.1	0.047	0.52	21.0	255
E.C.	38M	3.1	0.033	1.02	18.5	269
O.L.	60F	1.1	0.037	0.41	23.5	275
Mean $\pm$ SD		$1.5 \pm 0.9$	$0.037 \pm 0.006$	$0.54 \pm 0.3$	$21.1 \pm 1.8$	$266 \pm 7$
$P\ddagger$		NS	$<0.001$	NS	$<0.001$	NS
Mean $\pm$ SD in all cases of hypothyroidism		$1.8 \pm 0.8$	$0.029 \pm 0.007$	$0.51 \pm 0.22$	$25.4 \pm 3.5$	$251 \pm 36$
$P\S$		$<0.001$	$<0.001$	$<0.001$	$<0.001$	NS
Normal mean $\pm$ SD (12 volunteers)		$6.2 \pm 1.0$	$0.042 \pm 0.006$	$2.56 \pm 0.49$	$21.0 \pm 2.1\parallel$	$259 \pm 21\parallel$

PBI, protein-bound iodine; TBG, maximal binding capacity of thyroxine-binding alpha globulin; TBPA, maximal binding capacity of thyroxine-binding prealbumin; NS, not significant ( $P > 0.05$ ).

\* Thyroxine iodine by column.

$\ddagger$  Probability that the value in group I is identical to the corresponding value in group II.

$\S$  Probability that the value in the whole diagnostic category is identical to the corresponding value in the normal group.

$\parallel$  Mean  $\pm$  SD of 19 normal volunteers, rather than 12.

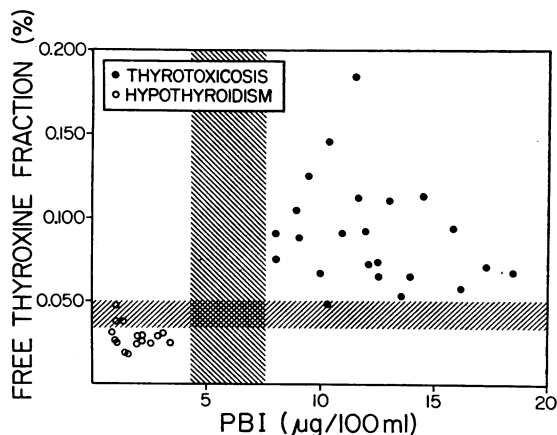


FIG. 4. RELATION BETWEEN FREE THYROXINE FRACTION AND PBI IN THYROTOXICOSIS AND HYPOTHYROIDISM. The cross-hatched areas indicate the normal ranges.

laboratory (6), which showed elevation of percentage free thyroxine in thyrotoxicosis and diminution in hypothyroidism. However, the PBI value is not considered to be the main factor regulating the fraction of unbound hormone. Since loading normal sera with nonradioactive thyroxine in the clinical range produces much less elevation of the free thyroxine percentage than is usually observed in sera from patients with thyrotoxicosis (5, 6), the relation illustrated does *not* imply that the thyroxine concentration of serum per se is a major determinant. The effects of the binding proteins are discussed below.

The scatter diagram of free thyroxine fraction against TBPA capacity (Fig. 5) revealed the expected highly significant inverse correlation

( $-0.59$ ,  $P < 0.001$ ). However, an even more striking inverse correlation was evident in the plot of free thyroxine fraction against TBG (Fig. 6), which had a correlation coefficient of  $-0.84$  ( $P < 0.001$ ). The data were subjected to additional statistical treatment, including computation of partial and multiple correlation coefficients, as well as simple correlation coefficients within the diagnostic categories.

As may be anticipated by inspection of Fig. 4, no significant correlation existed between free thyroxine fraction and PBI value if either diagnostic category was considered separately ( $P > 0.1$ ). Similarly, within either the thyrotoxic or hypothyroid group, the free thyroxine fraction bore no significant relation to the TBPA capacity (Fig. 5). On the other hand, TBG capacity bore a statistically significant inverse relation to the free thyroxine fraction both in thyrotoxicosis ( $P < 0.001$ ) and in hypothyroidism ( $0.025 < P < 0.05$ ).

The partial and multiple correlation coefficients of free thyroxine fraction against PBI and/or TBPA and/or TBG revealed highly significant correlations only when TBG was included, regardless of inclusion or exclusion of PBI and TBPA. For thyrotoxicosis,  $P < 0.001$ ; and for hypothyroidism,  $0.01 < P < 0.025$ . All the statistical data were compatible with a more signifi-

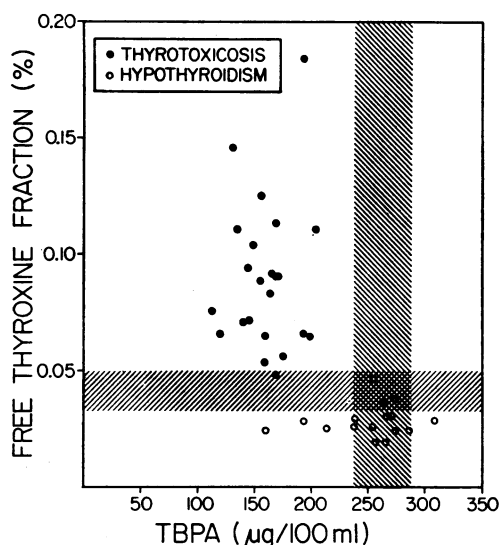


FIG. 5. RELATION BETWEEN FREE THYROXINE FRACTION AND TBPA IN THYROTOXICOSIS AND HYPOTHYROIDISM. The cross-hatched areas indicate the normal ranges.

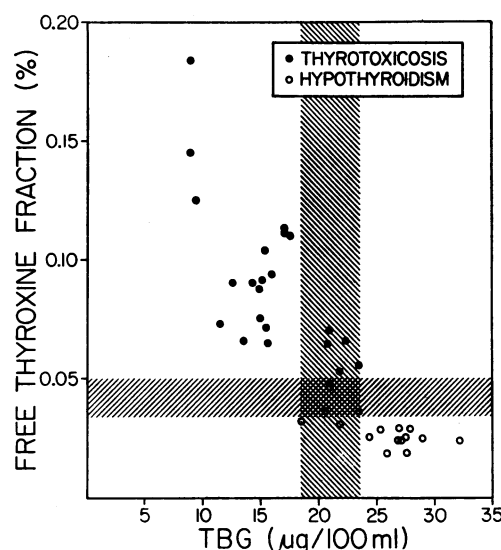


FIG. 6. RELATION BETWEEN FREE THYROXINE FRACTION AND TBG IN THYROTOXICOSIS AND HYPOTHYROIDISM. The cross-hatched areas indicate the normal ranges.

cant role of TBG than TBPA as a determinant of the free thyroxine fraction in thyroid disease (see discussion).

For purposes of illustration, it appeared reasonable to divide the patients with thyrotoxicosis into two groups based upon the TBG capacities: group I with diminished TBG and group II with normal TBG. The difference between TBG capacity in group I ( $14.0 \pm 2.8 \mu\text{g}/100 \text{ ml}$ ) and that in group II ( $21.7 \pm 1.1 \mu\text{g}/100 \text{ ml}$ ) was appreciable, whereas the TBPA capacity in group I was not different from that in group II (Table I and Fig. 2). Despite the diminution of TBPA capacities in all thyrotoxic sera, the free thyroxine fractions in group I with diminished TBG capacities were much higher than those in group II with normal TBG capacities (Table I and Fig. 2).

The patients with hypothyroidism could also be divided into two groups based upon TBG capacities: group I with elevated TBG capacities and group II with normal TBG capacities. As shown in Table II and Fig. 3, group I with elevated TBG capacities had markedly diminished free thyroxine fractions, while group II with normal TBG capacities had normal or nearly normal free thyroxine fractions. The difference between free thyroxine fractions in groups I and II was highly significant statistically ( $P < 0.001$ ).

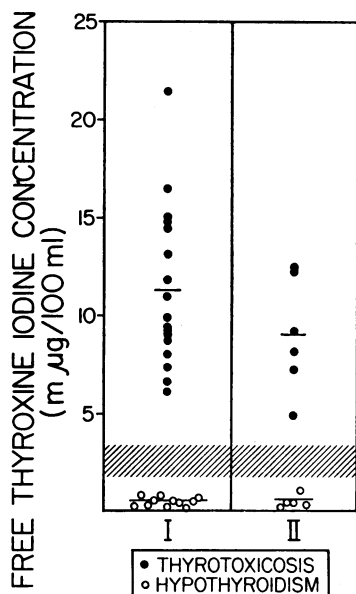


FIG. 7. FREE THYROXINE IODINE CONCENTRATION IN THYROTOXICOSIS AND HYPOTHYROIDISM. The cross-hatched area indicates the normal range. Group I has abnormal TBG capacity; group II has normal TBG.

The calculated free thyroxine iodine concentrations, representing the products of PBI values and free thyroxine fractions, revealed marked deviations from the normal range in both thyrotoxicosis and hypothyroidism, regardless of subdivision into groups I and II. The magnitudes of these abnormalities (Fig. 7) are similar to differences previously observed between the normal range and thyroid diseases, when estimations of the absolute free hormone concentration have been compared (5, 6). There was no statistically significant difference in each diagnostic category between groups I and II in this parameter (Tables I and II), as anticipated from inspection of Fig. 7. The PBI values were significantly higher in thyrotoxicosis group II with normal TBG than in group I with diminished TBG; evidently the higher PBI concentrations offset the lower free thyroxine fractions in yielding similarly elevated unbound hormone concentrations. In hypothyroidism a similar offsetting difference in PBI concentrations was present, although in this instance the difference was not statistically significant (Table II).

### Discussion

Reverse flow paper electrophoresis (9) with barbital buffer or ammonium bicarbonate buffer

at pH 8.6 has been used as a standard method to determine the maximal binding capacity of TBG. However, since it became evident that the barbital (Veronal) buffer inhibited thyroxine binding to TBPA, Tris-maleate buffer, pH 8.6, was introduced by Ingbar to determine TBG and TBPA capacities (11, 12). Recently, thyroxine-binding prealbumin has been purified by Purdy, Woeber, Holloway, and Ingbar (13) and Oppenheimer, Surks, Smith, and Squef (14). Studies with the addition of purified TBPA to serum revealed correlation of added protein with maximal binding capacity and also with stainable protein in prealbumin-1 on starch gel (14, 15). Therefore, it was concluded that the normal serum maximal binding capacity was approximately 270  $\mu\text{g}/100\text{ ml}$ , as determined by glycine acetate electrophoresis—a value considerably higher than that obtained with Tris-maleate buffer, under the conditions employed (11, 12).

Sterling and Tabachnick (10) first reported the use of a glycine acetate system at pH 8.6 to separate thyroxine-binding proteins on paper electrophoresis. Oppenheimer et al. (4) reported that the patients with thyrotoxicosis had normal TBG capacities; although they employed the glycine acetate system, they used conventional paper electrophoresis. Consequently, it is entirely possible that trailing albumin and prealbumin were associated with TBG and obscured alterations which might have been evident with the reverse flow technique. Silverstein et al. (16) and Elzinga, Carr, and Beierwaltes (17) adapted the Durrum type cell for reverse flow paper electrophoresis, a procedure which has distinct technical advantages. An important advantage of the Durrum type cell is that the buffer reservoir contains four baffles to prevent electrode products from reaching the paper strips. Therefore, it was possible to minimize the effect of pH change, which is pronounced during electrophoresis with the glycine acetate system. In immediate proximity to the anode, pH may fall to 5.0, whereas at the cathode it may rise to 9.8. However, in the outer side of each reservoir, into which the paper strips were dipped with the paper wick, pH was almost constant ( $8.6 \pm 0.1$ ). In three successive runs without change of the solution the same samples gave values of TBG and TBPA capacities within the error of simultaneous



replicates. Nevertheless, we always employed fresh buffer in the present study.

Thus, we reinvestigated the thyroxine-binding proteins in thyrotoxic and hypothyroid sera, using reverse flow paper electrophoresis with the glycine acetate system, pH 8.6, in the Durrum type cell.

The diminished binding capacity of TBPA in sera of patients with thyrotoxicosis was first noted by Richards, Dowling, and Ingbar (1). The markedly elevated free thyroxine fractions in sera of patients with thyrotoxicosis have been ascribed to their diminished TBPA capacities, as well as to the increased total hormone concentration (4, 5). This tentative explanation did not appear sufficient to account for the wide variation in free thyroxine fractions in thyrotoxic sera, some of which showed strikingly high values. Moreover, the TBPA was not found to be diminished in all of the thyrotoxic sera in the preliminary report by Richards, Dowling, and Ingbar (1).

Diminution of TBG in thyrotoxicosis has been reported by Silverstein and coworkers who used Veronal buffer with reverse flow technique (16) and by Cuarón, who used conventional Tris-maleate electrophoresis (18). Moreover, simultaneous with the present work, Schussler, employing quite different methodology, reported in an abstract (19) the finding of diminution of TBG in thyrotoxicosis.

In the present paper, the reduction of TBG capacities present in 17 of 23 thyrotoxic sera and the elevation of TBG capacities in 11 of 16 hypothyroid sera appeared sufficient to explain the pronounced abnormalities of free thyroxine fractions. Indeed, in thyrotoxicosis and hypothyroidism, the inverse correlation between free thyroxine fractions and TBG capacities was much closer than that with TBPA capacities. When diagnostic categories were considered separately, only TBG bore a significant inverse relation to the free thyroxine fraction. These results suggested that in thyroid diseases, TBG may sometimes play a more important role than TBPA in determining the free thyroxine fraction. The regulation of the free thyroxine fraction by TBG was illustrated most clearly by the division of the sera into two groups, based upon their TBG capacities.

In thyrotoxicosis, both the sera of group I with diminished TBG capacities and those of group II with normal TBG capacities had simi-

larly diminished TBPA capacities. Nevertheless, the elevation of free thyroxine fractions was significantly greater where diminution of TBG occurred, that is, in group I. It appeared reasonable, therefore, to attribute the more marked elevation of free thyroxine fractions to the reduction not only of TBPA but also of TBG capacities in the group I sera.

Furthermore, TBPA capacities showed no consistent deviations from normal in hypothyroidism. The sera of group I with slightly but significantly elevated TBG capacities had markedly diminished free thyroxine fractions, whereas those of group II with normal TBG capacities had normal or nearly normal free thyroxine fractions.

Thus, these results suggested a significant clinical role of a variation in TBG.

Recent studies (20, 21) suggested that the diminished TBPA capacity in chronic illness and acute injury was due to reduction of actual concentration of prealbumin due to diminished synthesis. Presumably, the diminished TBPA capacities in thyrotoxicosis might also be due to diminished prealbumin synthesis; however, they could be due to accelerated turnover of serum proteins (22, 23). The diminished TBG capacities are not explained by the present findings, and it is not proven that diminished TBG capacity reflects reduction of the actual concentration of TBG itself, although this is a plausible hypothesis.

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