TECHNICAL NOTE

Tables to Estimate Total Binding Capacity of Thyroxine-binding Globulin from the In Vitro Thyroid Function Tests

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Equations for an estimate of the total binding capacity of serum thyroxine-binding globulin (TBG-TC) were developed relating this parameter to serum thyroxine concentration (T_4) and in vitro uptake (T_3U). This estimate demonstrated a highly significant, positive correlation with TBG-TC as measured by electrophoresis in ten normal subjects, 20 patients with thyroid dysfunction (ten hypo- and ten hyperthyroid) and in 40 individuals with altered TBG-TC but without thyroid dysfunction (ten normal pregnant women, ten healthy women receiving anovulatories, ten nephrotics, and ten patients with severe malnutrition). True-positive (sensitivity) and true-negative (specificity) ratios were calculated for total and free T_4 in serum, T_3U, Free T_4 Index, and both measured and calculated TBG-TC. False-positive results for free T_4 index (12%) were due to altered TBG-TC. In such cases, 93% were recognized by the calculated TBG-TC from the values of the in vitro tests. It is concluded that this estimate should be added to the in vitro thyroid tests for their proper interpretation in cases where altered TBG-TC could be misleading. This estimate applies only to the particular in vitro testing system used herein.

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Alterations in the binding capacity of the main thyroxine-binding globulin (TBG) result in abnormal values of serum thyroxine (T_4) concentration and of the in vitro uptake (T_3U) of radiactive triiodothyronine by a secondary substrate. In such cases, knowledge of total TBG binding capacity is of great value for the proper interpretation of these in vitro thyroid function tests (*1,2*). Unfortunately, both direct assessment of serum TBG concentration by radioimmunoassay (*3*) and the indirect estimation of total TBG binding capacity by electrophoretic techniques (*2,4*) are time-consuming and impractical for a daily routine.

In 1975, Nusynowitz and Benedetto (*5*) developed an equation to estimate total TBG binding capacity from two ordinary tests: a) for T_3U by a surface-adsorbant, and b) from the total T_4 concentration in serum.

The purpose of this study was to evaluate such an indirect estimate in selected patients with thyroid dysfunction and in other subjects with altered T_3U and Total T_4 serum concentrations due to changes in total TBG binding capacity.

MATERIALS AND METHODS

The study was performed with sera from 70 subjects: ten normal, ten hypothyroid, ten thyrotoxic,

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ten nephrotic, ten with severe malnutrition, ten women in the third trimester of normal pregnancy, and ten otherwise normal women under chronic treatment with various anovulatories.

Serum samples were assayed by triplicate for the following parameters:

In vitro uptake of radioactive triiodothyronine by a surface adsorbant*, and total thyroxine concentration in serum†. A "Free T_4 Index" was calculated as the product of T_3U and total T_4 concentration in serum (*1*). Serum free T_4 concentration was assayed by equilibrium dialysis (*2*), and total TBG binding capacity was directly estimated by paper electrophoresis (*4*).

Total TBG binding capacity was computed for every sample from the values of T_3U and total T_4 concentration in serum by the original equation developed by Nusynowitz and Benedetto (5):

$$TBG_c = 15.35 \left[\frac{40}{T_3U}\right]^{1.5} + 14.96 \left[\frac{T_4}{8.0}\right]^{0.5} - 9.7$$

Variable I — Variable II — Constant

To facilitate routine calculations, tables can be established for all values of T_3U and T_4. Total TBG binding capacity can then be calculated for each serum sample by adding the values for the two variables and subtracting the constant 9.7. The resulting estimate of total TBG binding capacity will be in micrograms of T_4 bound to TBG, per 100 ml of serum.

True-positive (TP) and true-negative (TN) ratios were calculated for each parameter, both for the patients with thyroid dysfunction (TD) and for the subjects with altered serum proteins (ASP) but without thyroid dysfunction:

TP in TD = abnormal results in cases with TD;
TP in ASP = abnormal results in cases with ASP;
TN in TD = normal results in subjects with no TD;
TN in ASP = normal results in subjects without ASP.

The TP ratio is referred to as the "sensitivity" of the test, whereas the TN ratio is called its "specificity" (*6*).

RESULTS

The values determined (mean ± s.d.) for each parameter in the different clinical groups are summarized in Table 1.

The frequency distribution of cases according to the selected normal ranges for each parameter are shown in Table 2 for the diverse clinical groups studied, while Table 3 includes the true-positive and true-negative ratios calculated for each parameter, both for the patients with thyroid dysfunction and for those with altered serum proteins but without thyroid dysfunction.

Figure 1 shows a highly significant, positive correlation between measured total TBG binding ca-

TABLE 1. VALUES (means ± s.d.) OBTAINED FOR EACH PARAMETER IN THE VARIOUS CLINICAL GROUPS

	N	Serum thyroxine (mg/100 ml)	T_3U (%)	Free T_4 Index	Serum-free Thyroxine (mg/100 ml)	Total TBG (measured) (mg T_4/100 ml)	Total TBG (calculated) (mg T_4/100 ml)
Normal	10	7.9 ± 1.9	40.0 ± 3.1	3.17 ± 0.89	2.96 ± 0.61	19.8 ± 1.7	20.5 ± 1.3
Hyperthyroidism	10	14.3 ± 3.1 p 0.005	53.8 ± 6.5 p 0.005	7.85 ± 2.59 p 0.005	14.07 ± 6.77 p 0.005	21.0 ± 2.3 NS	20.3 ± 1.1
Hypothyroidism	10	2.6 ± 1.3 p 0.005	31.9 ± 2.6 p 0.005	0.85 ± 0.45 p 0.005	1.01 ± 0.45 p 0.005	20.8 ± 1.4 NS	20.4 ± 1.0 NS
Normal pregnancy	10	13.1 ± 1.7 p 0.001	30.2 ± 4.2 p 0.001	3.93 ± 0.49 NS	3.09 ± 0.58 NS	33.4 ± 5.3 p 0.001	31.5 ± 4.9 p 0.001
Anovulatories	10	12.1 ± 2.5 p 0.001	32.9 ± 3.6 p 0.001	3.91 ± 0.54 NS	3.23 ± 0.43 NS	29.5 ± 5.4 p 0.001	28.1 ± 8.1 p 0.001
Malnutrition	10	4.2 ± 1.0 p 0.001	43.9 ± 3.6 NS	1.81 ± 0.39 p 0.005	2.71 ± 0.40 NS	14.2 ± 2.1 p 0.001	15.6 ± 2.1 p 0.001
Nephrosis	10	4.6 ± 1.0 p 0.001	42.0 ± 2.3 NS	1.93 ± 0.44 p 0.005	3.08 ± 0.49 NS	15.4 ± 1.5 p 0.001	16.7 ± 1.6 p 0.001

pacity and its estimated value from the in vitro thyroid function tests. The regression line closely approximates the identity line.

Correlation between Free T_4 Index and the measured concentration of the free hormone in serum showed two different regression lines (Fig. 2) demonstrating poor linearity. The first regression line follows the identity line and includes the values obtained in sera from normal subjects, from patients with hypothyroidism, and from individuals with altered TBG capacity. The values obtained from thyrotoxic patients follow a less steep regression line.

DISCUSSION

Our present results are in accordance with the accepted mechanisms by which the interaction between serum T_4 and TBG is affected by both thy-

TABLE 2(A). FREQUENCY DISTRIBUTION OF CASES ACCORDING TO SELECTED NORMAL RANGES FOR EACH PARAMETER

		Serum T_4 concentration (μg/100 ml)			In vitro T_3 Uptake (%)			"Free T_4 Index"		
Clinical groups	N	<4.5	4.5–11.5	>11.5	<35	35–45	>45	<1.6	1.6–5.2	>5.2
Normal	10	0	10	0	1	8	1	0	10	0
Thyroid dysfunction:										
Hyperthyroidism	10	0	1	9	0	1	9	0	1	9
Hypothyroidism	10	9	1	0	7	3	0	9	1	0
Altered serum proteins:										
Normal pregnancy	10	0	5	5	8	2	0	0	10	0
Anovulatories	10	0	5	5	8	2	0	0	10	0
Malnutrition	10	4	6	0	0	2	8	4	6	0
Nephrosis	10	2	8	0	0	1	9	2	8	0

TABLE 2(B). FREQUENCY DISTRIBUTION OF CASES ACCORDING TO SELECTED NORMAL RANGES FOR EACH PARAMETER

		Serum free T_4 concentration (μg/100 ml)			Measured total TBG capacity (μg T_4/100 ml)			Estimated total TBG capacity (μg T_4/100 ml)		
Clinical groups	N	<1.8	1.8–5.2	>5.2	<18	18–23	>23	<18	18–23	>23
Normal	10	0	10	0	0	10	0	0	9	1
Thyroid Dysfunction:										
Hyperthyroidism	10	0	0	10	0	10	0	0	10	0
Hypothyroidism	10	10	0	0	0	10	0	0	10	0
Altered serum proteins:										
Normal pregnancy	10	0	10	0	0	0	10	0	0	10
Anovulatories	10	0	10	0	0	0	10	0	1	9
Malnutrition	10	0	10	0	10	0	0	10	0	0
Nephrosis	10	0	10	0	10	0	0	8	2	0

TABLE 3. TRUE-POSITIVE (SENSITIVITY) AND TRUE-NEGATIVE (SPECIFICITY) RATIOS FOR THE INDICATED PARAMETERS

	Thyroid dysfunction		Altered serum proteins	
Procedure	True-Positive *ratio* (sensitivity)	True-Negative *ratio* (specificity)	True-Positive *ratio* (sensitivity)	True-Negative *ratio* (specificity)
Serum T_4 concentration	0.90	0.68	0.40	0.40
In vitro T_3 uptake	0.80	0.30	0.83	0.40
Free T_4 Index	0.90	0.88	0.15	0.40
Serum Free T_4 concentration	1.00	1.00	0.00	0.33
Total TBG capacity (measured)	0.00	0.20	1.00	1.00
Total TBG capacity (calculated)	0.00	0.19	0.93	0.97

roidal and nonthyroidal illness, and with the influence that those mechanisms exert on the in vitro thyroid function tests (*1,2,7*).

The total T_4 concentration in serum, the T_3U, and the corresponding Free T_4 Index, were adequate to separate the normal subjects from the hypothyroid and thyrotoxic patients (Tables 1 and 2). Measured and estimated values for total TBG capacity were alike and showed no significant differences among these three clinical groups (Figure 1, Tables 1 and 2).

In thyrotoxicosis, total TBG binding capacity, whether measured or calculated, was normal. The increased concentration of serum T_4 increased the saturation of TBG and decreased the free TBG binding capacity, with a resulting rise of T_3U, Free T_4 Index, and concentration of free T_4 in serum. In hypothyroidism the mechanism was inverted: the combination of a normal total TBG capacity with a low concentration of T_4 in serum decreased the saturation of TBG, enhancing free TBG capacity. As a result, T_3U, Free T_4 Index, and free serum T_4 were all reduced.

When the trigger mechanisms for the alteration of these interactions were changes of TBG serum concentration, the findings were different. Both normal pregnancy and treatment with anovulatories significantly increased the measured and calculated values for total TBG binding capacity (Tables 1 and 2, Fig. 1). As a consequence, free TBG binding capacity was also raised, enhancing the fraction of T_4 that is bound to TBG, and decreasing T_3U. Both free T_4 concentration in serum and Free T_4 Index remained within their normal limits.

On the other hand, severe malnutrition and nephrosis significantly reduced both measured and calculated total TBG binding capacity. Hence, the free TBG binding capacity was also decreased, diminishing the fraction of T_4 that is bound to this protein and elevating T_3U. In these cases, however, the Free T_4 Index was found to be significantly reduced, notwithstanding the normal values found for the actual concentration of the free hormone in serum (Tables 1 and 2).

The disparity between Free T_4 Index and the actual concentration of free T_4 in serum is more obvious in Fig. 2, which depicts two different regression lines between these two parameters. The first, approaching the line of identity ($y = 1.02 \times - 0.14$), includes the values obtained in sera from normal subjects and those observed in sera from patients with hypothyroidism and from individuals with altered TBG capacity. The values obtained in sera from thyrotoxic patients followed a less steep regression line ($y = 0.37 \times + 2.71$). Similar results were previously reported by Hamada et al. (*7*), who

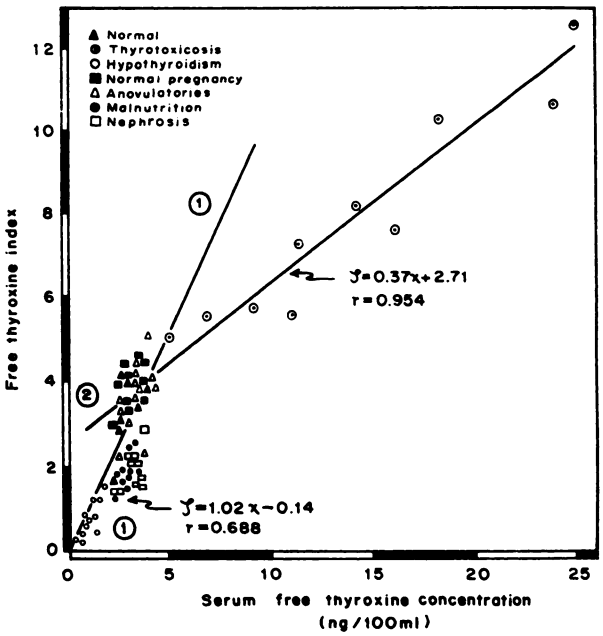

FIG. 1. Correlation between total TBG binding capacity as measured by electrophoresis and its calculated value from the in vitro thyroid function tests.

explained this poor proportionality by the "spilling over" of serum T_4 to thyroxine-binding prealbumin (TBPA) and albumin, owing to the limited capacity of TBG. This disparity was all the more notorious in nephrotic patients and in subjects with severe malnutrition, where total TBG binding capacity is

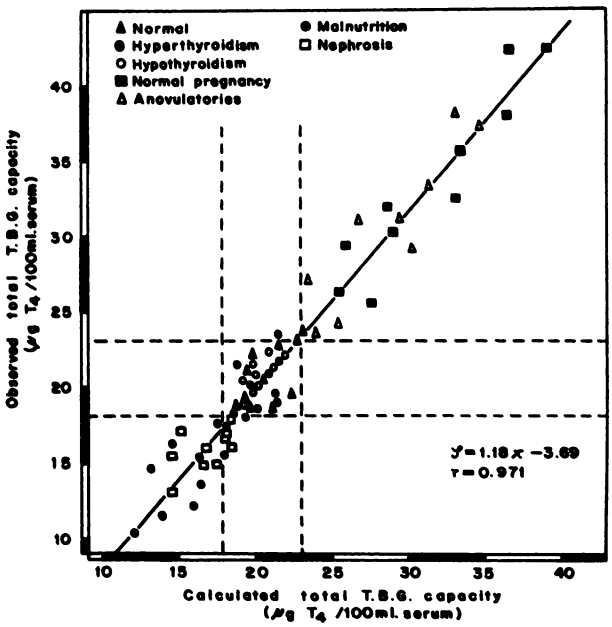

FIG. 2. Correlation between Free T_4 Index and measured concentration of free hormone in serum by dialysis, showing two different regression lines.

limited, and in thyrotoxic patients where the TBG is oversaturated by endogenous T_4. It should also be mentioned that the calculation of Free T_4 Index does not take into account the association constant between serum T_4 and TBG. For this very reason, Hamada et al. (*7*) proposed a new Free T_4 Index based on PBI and T_3U, allowing for the association constants between T_4 and its various binding proteins: TBG, TBPA, and albumin. They claim that this new index provides a more nearly linear relationship with the actual free serum T_4 concentration. Unfortunately, this new index was calculated by using a commercial kit that gives values for T_3U quite different from those obtained with the kit used for the present study. This prevented us from evaluating its significance.

Our results (Table 3) showed that the most specific and sensitive test for thyroid dysfunction is the direct measurement of the concentration of free T_4 in serum (TP = 1.00; TN = 1.00), but this procedure is seldom in routine clinical use. The most sensitive and specific procedure among those commonly used was Free T_4 Index (TP = 0.90; TN = 0.88), followed by the estimate of total T_4 concentration in serum (TP = 0.90; TN = 0.68), which has the same sensitivity as the Free T_4 Index but a lower specificity. T_3U was the least sensitive and least specific procedure for thyroid dysfunction (TP = 0.80; TN = 0.30).

On the other hand, the most specific and sensitive test for alterations of serum TBG is the direct estimation of total TBG binding capacity by electrophoresis (TP = 1.00; TN = 1.00), but this procedure is rarely used in clinical practice. Fortunately, its calculation from the values for the in vitro thyroid tests (*5*) has nearly the same sensitivity and specificity (TP = 0.93; TN = 0.97). In cases where the results are in doubt, they should be confirmed or rejected by a direct radioimmunoassay for TBG (*3*).

It follows that by using the in vitro thyroid function tests and the corresponding Free T_4 Index, one may obtain the correct diagnosis of thyroid dysfunction in 90% of cases, and also that it is possible to obtain abnormal results in 12% of subjects without thyroid dysfunction but with altered concentration of TBG in serum. Among these, 93% can be sorted out by the estimate of total TBG binding capacity from the values obtained with the in vitro thyroid function tests.

Other causes of TBG alterations—such as congenital absence or deficiency of TBG, or those caused by the administration of androgens or steroidal protein anabolic agents—were not explored in this study. They will be included in a future report involving a larger group made up of unselected patients.

It is very important to stress that the original equation of Nusynowitz and Benedetto (*5*) and the equations presented here apply only to the particular in vitro testing system employed, and only if the normal ranges for T_3U and T_4 serum concentrations are the same as those reported by them and similarly found by us. Otherwise the equation must be appropriately revised.

We conclude that the results of the in vitro thyroid function tests and the Free T_4 Index should be complemented with an estimate of total TBG binding capacity in order to recognize those false-positive results due to alterations in the binding protein in serum. This precaution might be of greater importance in developing countries, where severe malnutrition is common. Its calculation has been simplified by the development of pertinent tables for easier handling. With these tables the physician can take full advantage of two routine laboratory determinations that provide information related to four important physiologic parameters: total and free T_4 concentrations in serum, and total and free TBG binding capacities—all with no increase in expense. In this way, the direct estimation of TBG concentration in serum by radioimmunoassay can be restricted to doubtful cases. We must stress, however, that the equation and the tables presented here should be corrected if different commercial kits for T_3U and T_4 are used.

FOOTNOTES

* Tri-Tab, Nuclear Medical Laboratories
† Tetra-Tab, Nuclear Medical Laboratories
‡ Triosorb, Abbott Laboratories, North Chicago, IL.

REFERENCES

1. CLARK F, HORN DB: Assessment of thyroid function by the combined use of the serum protein-bound iodine and resin uptake of 131-I-triiodothyronine. *J Clin Endocrinol Metab* 25: 39-45, 1965

2. OPPENHEIMER JH, SQUEF R, SURKS MI, et al: Binding of thyroxine by serum proteins evaluated by equilibrium dialysis and electrophoretic techniques. Alterations in nonthyroidal illness. *J Clin Invest* 42: 1769-1782, 1963

3. DRYSDALE IP, RAMSDEN DB, HOFFENBERG R: A routine method for the estimation of thyroxine-binding globulin in human serum. In *Thyroid Hormone Metabolism*, Harland WA, Ott JS, eds. New York, Academic Press, 1975, p 261-279

4. MYANT NB, OSORIO C: Paper electrophoresis of thyroxine in tris-maleate buffer. *J Physiol* 152: 601-612, 1960

5. NUSYNOWITZ ML, BENEDETTO AR: Calculation of an estimate of thyroxine-binding globulin capacity. *J Nucl Med* 16: 1076-1079, 1975

6. VECCHIO TJ: Predictive value of a single diagnostic test in unselected populations. *N Engl J Med* 274: 1171-1173, 1966

7. HAMADA S, NAKAGAWA T, MORI T: Re-evaluation of thyroxine binding and free thyroxine in human serum by paper electrophoresis and equilibrium dialysis, and a new free thyroxine index. *J Clin Endocrinol Metabol* 31: 166-179, 1970