Estimation of the Maximal T₄-Binding Capacity of TBG Using the Triosorb Test in Serum Treated with Dextran-Coated Charcoal

MANABU YOSHIMURA, YUKIO OCHI, TAKASHI HACHIYA AND TADAYOSHI MIYAZAKI

Second Department of Internal Medicine, Kyoto Prefectural University of Medicine, Kyoto, 602

Synopsis
A new method for the estimation of the maximal T₄-binding capacity of TBG (max. TBG) in serum (dextran-coated charcoal-triosorb method) was devised. Thyroxine (T₄) concentration in test sera were determined by the Tetrasorb method and then T₃ resin sponge uptake (T₃ RSU) was determined after extraction of 70% of the endogenous T₄ with dextran-coated charcoal. The binding capacity of unsaturated TBG in treated sera was estimated from the reciprocal of T₃ RSU. Max. TBG was calculated from the arithmetic sum of the unsaturated binding capacity of TBG and the serum T₄ concentration multiplied by 0.3. The normal range for max. TBG was 20.1±2.6 (mean±SD) µg T₄/100 ml (14~24) and it was 19.2±3.5 µg T₄/100 ml in hyperthyroidism which was lower compared with hypothyroidism (21.1±2.6 µg T₄/100 ml). Max. TBG in 8 hyperthyroid patients did not change significantly after treatment in half of the case, although in the other half it was slightly increased. Increased values of max. TBG were observed in pregnancy (32.9±4.4) and in a hyperthyroid state associated with pregnancy (31.4±4.7). Decreases were observed in TBG deficiency (<5.6 µg T₄/100 ml). These procedures can be applied for the determination of both the unsaturated binding capacity of TBG and that of max. TBG.

It is well known that thyroxine (T₄) is bound to thyroxine binding globulin (TBG), thyroxine-binding prealbumin (TBPA) and albumin in normal human serum. Literatures indicate that TBG may play a predominant role in thyroxine transport due to its high affinity for thyroxine, although TBG concentration in serum is the lowest in comparison with the other T₄-binding proteins (Ingbar & Freinkel, 1960; Osorio, 1962; Hamada et al., 1970).

Evidence has been accumulated that changes in TBG content occur in various disorders of thyroid function and in non-thyroidal illnesses and physiological states. For example, TBG content is increased in pregnancy (Robbins & Nelson, 1958; Nicoloff et al., 1970; Refetoff et al., 1972), during estrogen therapy (Levy et al., 1971; Refetoff et al., 1972) and in an inherited trait (Levy et al., 1971). On the contrary TBG content is decreased in the patient with liver cirrhosis (Levy et al., 1971), nephrosis (Musa et al., 1967), acute and chronic illness (Refetoff et al., 1972) and in an inherited TBG deficient trait (Levy et al., 1971). Several methods for the determination of the maximal binding capacity of TBG (max. TBG), electrophoretic separation of serum protein (Albright et al., 1955; Sterling & Tabachnick, 1961; Musa et al., 1967), dextran-coated charcoal procedure (Roberts & Nikolai, 1969) or ion-exchange resin method (Keane et al., 1969; Refetoff et al., 1972), have been reported.

Received for publication November 12, 1975
June 1975

131I-T3 resin sponge uptake (T3 RSU or Triosorb; Dainabot RI Laboratory), a popular test of thyroid function is proportional to the degree of the unsaturated binding capacity of TBG. It has been previously indicated that the reciprocal value of T3 RSU showed a positive correlation with the unsaturated binding capacity of TBG (Hamada et al., 1970; Nicoloff et al., 1970). Using this relationship, it is possible to estimate the max. TBG by the addition of endogenous thyroxine levels to the unsaturated binding capacity of TBG.

Nicoloff et al., (1970) demonstrated a method to measure the unsaturated TBG capacity using a modification of Triosorb test in which Triosorb test was measured under the enrichment of T4 to neglect the influence of endogenous T4 level on Triosorb test. However, in the present study, Triosorb test was carried out under low T4 levels with undersaturated TBG in serum treated with dextran-coated charcoal (DCC) to neglect the influence of endogenous T4 level and also the other T4 binding proteins such as TBPA and albumin on Triosorb test. Max. TBG was calculated from the determination of both T3 RSU and endogenous T4.

Materials and Methods

Principle of the method

The principle of the method is based on the observation that T3 RSU test is dependent upon the number of unsaturated binding sites of TBG available to T4. Unsaturated TBG capacity can be calculated from the equation using the reciprocal values of T3 RSU:

\[ T_3 \text{ RSU} = \frac{K_1 R}{K_1 R + K_2 X} \]  
(Equation 1)

where \( R \): the binding capacity of resin sponge  
\( X \): the unsaturated binding capacity of TBG  
\( K_1 \) and \( K_2 \): the affinity constants for binding of T4 to resin sponge and TBG respectively

\[ Y = \frac{1}{U} = 1 + \frac{K_2 X}{K_1 R} \]  
(Equation 2)

where \( K = \frac{K_2}{K_1} \)

\[ Y = \frac{K}{R} X + 1 \]  
(Equation 3)

rearranging equation 3

\[ X = R \left( \frac{Y - 1}{K} \right) \]  
(Equation 4)

Accordingly the relation between the reciprocal value of T3 RSU (\( Y \)) and the unsaturated binding capacity of TBG (\( X \)) can be expressed as simple linear correlation. The slope of the straight line (\( K/R \)) in equation 3 was obtained experimentally by the standard electrophoretic TBG determination using sera from myxedematous patients. The unsaturated binding capacity of TBG was calculated by the subtraction of the endogenous T4 value, determined by Tetrasorb procedure, from max. TBG. A regression line was obtained by the least squares method plotting the reciprocal values of the Triosorb test in the ordinate (\( Y \)) and the unsaturated binding capacity of TBG in the abscissa (\( X \))(Fig. 1).

The intercept at the ordinate proved to be 1, as theoretically suggested and the slope 0.171. Therefore the relation between the reciprocal value of T3 RSU (\( Y \)) and the unsaturated binding capacity of TBG (\( X \)) could be obtained by substitution of these values in equation 3 or equation 4.

\[ Y = \frac{1}{U} = 1 + 0.171 X \]  
(Equation 5)

\[ X = \frac{1}{0.171} = \left( \frac{1}{U} - 1 \right) \]  
(Equation 6)

Fig. 1. Relationship between the reciprocal values of T3 RSU and the unsaturated binding capacity of TBG measured by saturation method, in patients with myxedema. Triosorb results were expressed as a fraction rather than percent, therefore the ordinate (1/U) is greater than 1. T3 RSU is used as T3 RSU(%).

\[ Y = 1 + 0.171 X \]
The unsaturated binding capacity of TBG in various thyroidal diseases was calculated using the equations 5 and 6.

In patients with high endogenous $T_4$, usually a larger proportion of the total endogenous $T_4$ is bound to albumin and TBPA, and the endogenous TBG is oversaturated limiting the sensitivity of $T_3$ RSU test. Accordingly the endogenous $T_4$ should be removed. Using DCC solution, 70% of endogenous $T_4$ was removed from $T_4$-binding protein in serum, which is described in method section. The Triosorb test was performed with sera that had been treated with DCC. The unsaturated binding capacity of TBG of sera was determined from the Triosorb value using equation 6. Accordingly, max. TBG was calculated as follows:

$$\text{Max. TBG} = \text{unsaturated binding capacity of TBG} + \text{$T_4$ concentration of unextracted serum} \times 0.3$$

(Equation 7)

Of course when the myxedematous sera are used for TBG determination, the procedure of DCC treatment is unnecessary and max. TBG is calculated by the addition of the endogenous $T_4$ value (determined by Tetrasorb procedure) to the unsaturated binding capacity of TBG.

**Materials**

All test sera were obtained from patients and normal volunteers. Diagnosis was made from clinical signs and laboratory data. Triosorb ($T_3$ RSU) and Tetrasorb ($T_4$ determination by competitive binding assay) measurements were made using commercial kits obtained from Dainabot Laboratory (Tokyo Japan). Normal values for $T_3$ RSU and $T_4$ in our laboratory were 25-35% and 5.4-13.7 pg $T_4$/100 ml respectively. All radioactive materials were purchased from Abbott Laboratory (Chicago, U.S.A.).

**Electrophoretic determination of TBG**

Max. TBG by the saturation method was estimated by the addition of 100 or 200 pg $^{131}$I-$T_4$ per 100 ml of serum prior to conventional electrophoresis for 1 hr (Albright et al., 1955). These concentrations of $T_4$ ensure complete saturation of TBG. The excess $T_4$ not associated with TBG is bound to albumin and TBPA. Cellulose acetate electrophoresis was carried out at 150 volts, 0.1 mA/cm for 1 hr using veronal buffer, pH 8.0, 0.06 $\mu$ Sanyo Junyaku Comp., Japan). In the present electrophoresis, albumin stayed at applied spot and globulin, especially $\gamma$-globin moved to cathode side like reverse-flow electrophoresis. After electrophoresis the strip was cut for measurement of radioactivity in each fraction and stained with 0.4% Ponceau 3R trichlor acetic acid solution. Max. TBG was calculated from the percentage of total radioactivity associated with the TBG fraction.

**Removal of $T_4$ by DCC treatment**

For the determination of the unsaturated binding capacity of TBG, the Triosorb test should be performed in undersaturated state of TBG with $T_4$. Treatment with DCC solution was used for this purpose. 1.5 ml of undiluted test serum was added to 1.5 ml of DCC solution containing 1% dextran (MW: 80,000) and 10% charcoal in saline. For the removal of $T_4$ from TBG the mixed solution was shaken vigorously for 1 min, and allowed to stand at room temperature for 30 min. The supernatant fraction was separated by centrifugation for 20 min at 8,000 rpm. The $T_4$ RSU was determined after the addition of 0.01 ml of a $^{131}$I-$T_4$ solution to 1 ml supernatant fraction. To calculate the percent of extractable $T_4$ from the test serum, 0.1 $\mu$g of $^{131}$I-$T_4$/100 ml was added to test serum one hour before the addition of DCC solution. DCC solution was added stirringly to test serum equilibrated with $^{131}$I-$T_4$ at room temperature. The suspension was allowed to stand at room temperature for 30 min after the vigorous shake for one min. From remaining radioactivity in the supernatant fraction after centrifugation for 20 min at 8,000 rpm, it was determined that the percentage of $T_4$ extracted was constant at 70.2±2.8% (mean±SD) by the addition of DCC solution (Fig. 2).

**Effect of DOC treatment on Triosorb test, Tetrasorb test and max. TBG**

To estimate the effect of DCC treatment on the Triosorb and Tetrasorb test, max. TBG in sera from myxedematous patients and from pregnant women was determined by two methods. One method was calculated by the addition of the residual $T_4$ to the unsaturated binding capacity of TBG in sera that had been treated with DCC solution. The other method was determined by the addition of the endogenous $T_4$ value to the unsaturated binding capacity of TBG without DCC treatment. Both values were then compared.

![Fig. 2. The percent of extractable $^{131}$I-$T_4$ from test serum with $^{131}$I-$T_4$ by DCC solution. The abscissa expressed the ratio of DCC solution in amount (ml) divided by serum volume (ml).](image-url)
Effect of DCC treatment on serum protein

Estimating the change of TBG in quality by DCC treatment, 1.0 ml of DCC solution was given to pooled human serum of 1.0 ml with tracer amounts of an $^{131}$I-T$_4$ solution. Protein concentration was determined by ultra-violet absorption at 280 m$\mu$. A three percent decrease in serum protein was observed after treatment with DCC solution. In another experiment 1.0 ml of DCC solution was added to pooled human serum of 0.1 ml with tracer amounts of a $^{125}$I-RISA (Radioiodinated serum albumin) and the fraction of RISA removed from serum was determined. In 3 experiments, less than 5% of RISA was removed.

In general, albumin of which molecular weight is low compared with globulins in serum is easily absorbed to DCC (Ochi et al., 1973). The molecular weight of TBG is close to that of albumin, so the fraction of TBG removed by DCC treatment is thought to be under 5%.

Results

To evaluate the DCC treatment, max. TBG in sera from hypothyroid patients and from pregnant women was determined with and without DCC treatment (Table 1). The difference in the max. TBG determined by these two hypothyroid series of patients was less than 8%. In two pregnant subjects having less than 25% in the T$_3$ RSU, max. TBG was determined with or without DCC treatment. There was less than 5% difference in the values by either method.

To assess the applicability of the equations 5 and 6 to the non-myxedematous thyroid state, max. TBG in serum from 3 patients or

Table 1. Maximal binding capacity of TBG in hypothyroid patients and in pregnant women that was determined with or without DCC treatment

<table>
<thead>
<tr>
<th>Test serum</th>
<th>D.C.C. treatment</th>
<th>T$_3$ RSU</th>
<th>1</th>
<th>Unsaturated binding capacity of TBG (μg%)</th>
<th>Serum T$_4$ (μg%)</th>
<th>serum T$_4$ x 0.3 (μg%)</th>
<th>Max. TBG (μg%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hypothyroid</td>
<td></td>
<td>23.1</td>
<td>4.33</td>
<td>19.6</td>
<td>4.1</td>
<td></td>
<td>23.7</td>
</tr>
<tr>
<td>patient</td>
<td>+</td>
<td>22.2</td>
<td>4.51</td>
<td>20.8</td>
<td></td>
<td>1.2</td>
<td>22.0</td>
</tr>
<tr>
<td>Hypothyroid</td>
<td></td>
<td>24.4</td>
<td>4.10</td>
<td>18.4</td>
<td>3.8</td>
<td></td>
<td>22.2</td>
</tr>
<tr>
<td>Patient</td>
<td>+</td>
<td>21.9</td>
<td>4.57</td>
<td>21.0</td>
<td></td>
<td>1.1</td>
<td>22.1</td>
</tr>
<tr>
<td>Pregnancy</td>
<td></td>
<td>23.7</td>
<td>4.22</td>
<td>19.0</td>
<td>14.0</td>
<td></td>
<td>33.0</td>
</tr>
<tr>
<td></td>
<td>+</td>
<td>16.7</td>
<td>5.99</td>
<td>29.5</td>
<td></td>
<td>4.2</td>
<td>33.7</td>
</tr>
<tr>
<td>Pregnancy</td>
<td></td>
<td>20.0</td>
<td>5.00</td>
<td>23.5</td>
<td>13.0</td>
<td></td>
<td>36.5</td>
</tr>
<tr>
<td></td>
<td>+</td>
<td>16.0</td>
<td>6.25</td>
<td>31.0</td>
<td></td>
<td>3.9</td>
<td>34.7</td>
</tr>
</tbody>
</table>

T$_3$ RSU is used as T$_3$ RSU(%)/100
DCC: Dextran-coated charcoal

Table 2. Maximal binding capacity of TBG in hyperthyroid patients, euthyroid subjects and hyperthyroid state associated with pregnancy

<table>
<thead>
<tr>
<th>Test serum</th>
<th>T$_3$ RSU</th>
<th>T$_3$ RSU after DCC treatment</th>
<th>Unsaturated binding capacity of TBG (μg%)</th>
<th>Serum T$_4$ x 0.3 (μg%)</th>
<th>Max. TBG (Triosob method) (μg%)</th>
<th>Max. TBG (Saturation method) (μg%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hyperthyroid</td>
<td>1 61.3</td>
<td>41.3</td>
<td>8.5</td>
<td>9.3</td>
<td>17.8</td>
<td>17.1</td>
</tr>
<tr>
<td>patient</td>
<td>2 44.1</td>
<td>34.0</td>
<td>11.5</td>
<td>7.4</td>
<td>18.9</td>
<td>18.7</td>
</tr>
<tr>
<td></td>
<td>3 43.5</td>
<td>28.9</td>
<td>14.5</td>
<td>5.8</td>
<td>20.3</td>
<td>19.3</td>
</tr>
<tr>
<td>Euthyroid</td>
<td>1 28.0</td>
<td>23.2</td>
<td>19.5</td>
<td>3.2</td>
<td>22.7</td>
<td>21.6</td>
</tr>
<tr>
<td>subject</td>
<td>2 29.5</td>
<td>25.5</td>
<td>17.2</td>
<td>3.3</td>
<td>20.5</td>
<td>20.2</td>
</tr>
<tr>
<td></td>
<td>3 31.2</td>
<td>24.5</td>
<td>19.0</td>
<td>2.6</td>
<td>21.6</td>
<td>21.0</td>
</tr>
<tr>
<td>Hyperthyroid</td>
<td>1 41.3</td>
<td>21.1</td>
<td>22.0</td>
<td>9.2</td>
<td>31.2</td>
<td>32.0</td>
</tr>
<tr>
<td>state associated</td>
<td>2 45.0</td>
<td>18.7</td>
<td>25.5</td>
<td>11.0</td>
<td>36.5</td>
<td>36.8</td>
</tr>
<tr>
<td>with pregnancy</td>
<td>3 40.8</td>
<td>20.2</td>
<td>23.3</td>
<td>8.7</td>
<td>32.0</td>
<td>32.4</td>
</tr>
</tbody>
</table>
Table 3. Reproducibility of TBG determination

<table>
<thead>
<tr>
<th>Test serum</th>
<th>N</th>
<th>Mean±SD</th>
<th>Coefficient of variation %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hyperthyroid</td>
<td>5</td>
<td>19.6±1.1</td>
<td>5.6</td>
</tr>
<tr>
<td>Euthyroid</td>
<td>5</td>
<td>20.7±1.2</td>
<td>5.8</td>
</tr>
<tr>
<td>Hypothyroid</td>
<td>5</td>
<td>21.2±1.0</td>
<td>4.7</td>
</tr>
</tbody>
</table>

Fig. 3. Max. TBG using the Triosorb test in patients with various thyroid diseases and in normal subjects.

subjects each of hyperthyroidism, euthyroid, or pregnancy with hyperthyroidism was determined by the Triosorb method and also by the electrophoretic saturation method. All sera were treated with DCC solution before the determination of max. TBG, to eliminate the effect of DCC treatment. Table 2 shows the example for the determination of max. TBG by Triosorb method. Both values of max. TBG determined by Triosorb method and electrophoretic saturation method differed by less than 5%.

The reproducibility of TBG determinations using the sera from hyperthyroidism, euthyroid and hypothyroid patient is shown in Table 3. The coefficient of variation was between 4.7 and 5.8%. Max. TBG in various thyroid diseases are shown in Fig. 3. Using the above procedure max. TBG in sera from euthyroid subjects (normal subject) was 20.1±2.6 μg T₄/100 ml (mean±standard deviation). Max. TBG was increased in euthyroid pregnancy (32.9±4.4 μg T₄/100 ml) and in hyperthyroid pregnancy (31.4±4.7 μg T₄/100 ml). It was decreased in TBG "deficiency" (<5.6 μg T₄/100 ml). In hyperthyroidism max. TBG was 19.2±3.5 μg T₄/100 ml. In hypothyroidism it was 21.1±2.6 which was slightly higher than in hyperthyroidism. In other thyroid diseases (simple goiter, thyroid cancer and Hashimoto's thyroiditis) it was also within the normal range (Fig. 3).

The 8 hyperthyroid patients max. TBG was examined after symptoms had returned to a euthyroid level. As shown in Fig. 4, max. TBG decreased in half the patients but not in the rest.

A longitudinal study showing changes in max. TBG during pregnancy in a single thyrotoxic patient is shown in Fig. 5. Follow-
ing the first stage of pregnancy, it increased gradually and returned to pre-pregnancy levels one month after delivery.

Discussion

In the electrophoretic saturation methods for the measurement of T₄ binding capacity of TBG, excess T₄ is added to the test serum usually in concentrations of 100 or 200 μg/100 ml (Albright et al., 1955; Nikolae & Seal, 1966). Under these conditions TBG is fully saturated. The amount of T₄ bound to TBG is derived by examining the percentage of the radioactive T₄ in the TBG region on electrophoresis. From our personal experience we know that it is difficult to obtain accurate values by the saturation method with conventional electrophoretic separation, because of nonspecific binding of T₄ to proteins and poor reproducibility. Recently methods for the estimation of the T₄ binding capacity of TBG by non-electrophoretic procedures using either DCC (Roberts & Nikolai, 1969) or ion-exchange resin (Keane et al., 1969; Refetoff et al., 1972), competitive ligand-binding assay (Chopra et al., 1972) and radioimmunoassay of TBG (Levy et al., 1971) have been reported and these procedures were limited in some ways.

Attempts to determine the normal distribution of endogenous T₄ among serum binding protein utilizing the saturation method have yielded inconsistent results. For instance, a distribution of 83% for TBG, 2% for TBPA, 10% for albumin and 5% for others has been reported (Osorio, 1962), contrasted to 76%, 15% and 9% (Hamada et al., 1970) or 45%, 40% and 15% (Ingbar & Freinkel, 1960) for TBG, TBPA and albumin respectively. Therefore it is not clear whether the percentage of T₄ distribution among carrier proteins determined by various electrophoretic methods actually reflects the situation in vivo. It has been reported that the binding of T₄ to TBG or TBPA or albumin in human serum was changed by experimental pH conditions (Lutz & Gregerman, 1967; Coutsofides & Gordon, 1970). In the present state of knowledge the physiological role of TBPA in the transport of T₄ remains unclear.

In the present procedure, 70% of the endogenous T₄ in sera was removed by DCC, creating an analogous situation to that present in the serum from hypothyroid patients. The loss of TBG quantity by DCC treatment was within 5%, which could be neglected in practical use. Under these conditions it is therefore legitimate to calculate the unsaturated binding capacity of TBG utilizing the reciprocal value of T₃ RSU and the equation derived. Max. TBG can be obtained from the arithmetic sum of the unsaturated binding capacity of TBG and the concentration of residual T₄ in the serum treated by DCC. The residual T₄ after the extraction procedure by DCC accounts for 30% of original serum T₄ level determined by Tetrasorb method. When T₃ RSU was less than 25% in hypothyroid patients, max. TBG showed no significant difference by the method, with or without DCC treatment.

The values obtained by the present method in euthyroid subjects and patients with various thyroidal diseases are compatible with those previously reported (Albright et al., 1955; Robbins & Rall, 1957; Roberts & Nikolai, 1969; Refetoff et al., 1972). Using the saturation method with electrophoretic separation, a decreased max. TBG was observed in some cases of hyperthyroidism, especially when endogenous T₄ levels exceeded max. TBG. An elevated max. TBG was observed in hypothyroidism (Albright et al., 1955; Robbins & Rall, 1957; Ingbar & Freinkel, 1960). In contrast, TBG levels were normal in hypothyroidism and slightly lower in hyperthyroidism using the radioimmunoassay (Levy et al., 1971). In the present procedure,
max. TBG in hypothyroidism showed a slightly higher value compared with hyperthyroidism within the normal range. Braverman et al., (1968), Levy et al. (1971) (using radioimmunoassay) and Chopra et al. (1972) (using a radioligand method) have reported that the low binding capacity in hyperthyroid patients gradually increase after treatment.

From the present study, the half of the 8 patients with hyperthyroidism showed an increase of binding capacity of TBG, and in the rest it was almost the same. The present method requires the T3 RSU test and DCC technique. Therefore, it has some technical limitations like other non-electrophoretic techniques, but it can be used for measuring binding capacity of TBG with accuracy.

Acknowledgements

We wish to thank Dr. Hamao Iijichi, Professor of Internal Medicine, for his valuable advice and unflagging encouragement. We also wish to thank Dr. Samuel Refetoff for his kind help in the preparation of this manuscript.

References


