Rapid Radioimmunoassay of Triiodothyronine

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The method used in this report is based on the immunological production of a specific antisera to triiodothyronine (T3) in rabbits. Conducted at 4 °C, the radioimmunoassay requires three to five days to complete. Hoping to speed this assay, we investigated the influence of time, temperature, and pH. Incubation at 37 °C and pH 8.0 for 30 min did not deleteriously affect either precision, specificity, reproducibility, or accuracy. Bound and unbound T3 were separated in 10 min by adding polyethylene glycol ("Carbowax 6000") at 0-4 °C. T3 was prevented from binding to thyroxine-binding globulin by the addition of 8-anilino-1-naphthalene sulfonic acid. To minimize the differences in protein concentration between standards and unknowns, we added T3-free serum to all standards. Thus, a simplified and convenient method is described for measurement of T3 that is suitable for clinical use because it is simpler and faster than previously reported methods.

Additional Keyphrases: assessment of thyroid function • thyrotoxicosis • hyper- and hypothyroidism

Since 1952, when Pitt-Rivers and Gross discovered 3,5,3'-l-triiodothyronine (1), this naturally occurring iodinated amino acid has been the subject of investigation and speculation by thyroid physiologists. The interest was heightened when it was observed that the potency of T3 2 is severalfold greater than that of T4 (2-4). The recent discovery that thyrotoxicosis may be caused by an inappropriate concentration of T3 rather than T4 has reinforced the importance of T3 measurement (5-9). T3 values must thus be considered in the diagnosis of thyroid disorders.

Many methods described for assay of T3 before the advent of RIA required large volumes of blood, and at the same time were difficult and time-consuming (5, 10-12). Since the discovery that T3 conjugates with protein are antigenic (13), many laboratories have successfully produced specific antibodies against synthetic T3 conjugates (14, 15, 17-19). Thyroglobulin also produces antibodies that bind both T4 and T3; the T4-antibodies are neutralized before the T3 assay by the addition of T4 (16), and recently T3-thyroglobulin conjugates have been used (20).

All of the RIA methods for T3 depend on the addition of a standard of unlabeled T3 or of an unknown solution to a fixed amount of T3 antibody followed by the addition of a fixed amount of [125I]-T3. This mixture is conventionally incubated at 4 °C for 16-72 h. Characteristically, bound and unbound ligand have been separated by incubating with a second antibody for 24 h at 4 °C (14, 16, 17). Others have used dextran-coated charcoal (15, 18). In our assay, incubation time was shortened to 30 min at 37 °C, and the need for a second antibody has been eliminated by using a solution of polyethylene glycol.

Materials and Methods

Materials

Sources of materials are as follows: l-triiodothyronine (sodium salt and free acid), l-thyroxine (sodium salt) and "Trizma Base," Reagent Grade No. I 1503 (trade name for Tris buffer) from Sigma Chemical Co., St. Louis, Mo. 63178; [125I]-l-triiodothyronine from Abbott Laboratories Chemical Marketing Division, North Chicago, Ill. 60064; human serum albumin (HSA) from Nutritional Biochemicals Corp., Cleveland, Ohio 44128; thimerosal (ethylmercurithiosalicylic acid sodium salt) from Aldrich Chemical Co., Inc., Milwaukee, Wis. 53233; polyethylene glycol ("Carbowax 6000"), a gift from Union Carbide Corp., New York, N. Y. 10017; resin ["Rexyn 202" (Cl-SO4)] from Fisher Scientific Co., Chemical Manufacturing Division, Fair Lawn, N. J.; and 8-anilino-1-naphthalene sulfonic acid from K & K Laboratories, Plainview, N. Y. 11803.

We used the following equipment: metabolic incubator P/S Dubnoff and Packard Gamma Scintillation Spectrometer.

White New Zealand male rabbits weighing 4 kg were purchased from Kennedy Small Animal Farms, Tonawanda, N. Y. and were fed with "Rabbit Ration" from Rockland Laboratories, Derby, N. Y.

Methods

Production of antisera: T3 was conjugated to HSA as reported by Gharib et al. (14), except that the precipitate that formed during dialysis was discarded because it was protein-free as judged by the microscale biuret method (21). The molar ratio of T3 to HSA in the conjugate was calculated to be 6:1 by spectrophotometry. Two milligrams of conjugated T3, suspended in 0.5 ml of 0.155 mol/liter sodium chloride solution plus 0.5 ml of complete Freund's adjuvant, was injected into each of two rabbits sub-
cutaneously at 10 sites on their backs. Immunization was repeated weekly for the first five weeks. At the end of five weeks, titers of 1:3000 and 1:4000 were obtained. The latter antiserum was used in subsequent studies.

Preparation of T₃-free serum: Wash 10 g of resin five times with 20-ml aliquots of Tris buffer (0.1 mol/liter, pH 7.4). Soak it overnight (or for 12 h) in the buffer, dry it by vacuum filtration, and store it at room temperature.

Add 1 g of resin to 3 ml of pooled serum from euthyroid subjects. Incubate at 37 °C for 2 h, shaking vigorously at speed 7 on the metabolic incubator (99% of the added [¹²⁵I]-T₃ is removed in these 2 h). Upon removal from the shaker-incubator, the resin quickly settles by gravity, and the supernatant fluid is stored frozen in 1-ml aliquots. Prepare weekly.

Preparation of quality control: Dissolve 1.0 mg of T₃ (sodium salt) in 95% ethanol to give a final concentration of 1.0 ng/ml. Pipet 500 µl of the solution into 50 12 × 75 mm disposable tubes and evaporate the solvent under nitrogen gas. To each tube add 50 µl of the T₃-free serum. Mix well and keep at 0 °C until used.

Preparation of standard solutions and buffers: Dissolve 12.1 g of Trizma base in 500 ml of distilled, deionized water. Add about 56 ml of HCl (0.1 mol/liter) to adjust to pH 8.0; dilute to 1 liter. Dissolve 25 mg of ANS in 100 ml of the above Tris buffer. Prepare daily.

The standard solutions are made in the Tris buffer so that 10, 50, 150, 200, 250, 300, 400, 500, and 1000 pg of T₃ (sodium salt) (X in calculations below) are contained in 50 µl of buffer.

Assay procedure: Even though incubation is at 37 °C, the tubes are kept in an ice-bath during pipetting, for best reproducibility. Pipet 250 µl of buffer (containing ANS) into each tube. Pipet in duplicate 50 µl of each standard solution and unknowns, leaving out the first four tubes (tubes No. 1 and 2 for N values and 3 and 4 for B₀ values). Add 50 µl of T₃-free serum to all tubes containing standard solutions, including the first four tubes. Include quality-control tubes in duplicate for each assay. Add 100 µl of buffer (without ANS) to the first two tubes and 50 µl to the next two tubes and to all tubes containing unknowns (50 µl) and quality controls. Add 50 µl of antibody in appropriate dilution (B₀/T ~ 60%) to each tube except the first two tubes. Finally, add 100 µl of [¹²⁵I]-T₃ (~10,000 cpm) solution to all the tubes. The final volume in each incubation mixture should be 500 µl. Mix well, and incubate at 37 °C for 30 min with constant agitation at speed 5 (70 cycles/min) on a Dubnoff metabolic incubator. (We are not certain that shaking is an important variable.) Cool the tubes in an ice-water bath for 10 min, and add 500 µl of a solution of Carbowax 6000 (300 g/liter of Tris buffer) followed by 50 µl of T₃-free serum. Mix vigorously and pre-count all tubes for 1 min (T). Centrifuge at 2000 rpm for 20 min, aspirate off the supernatant fluid and count the precipitate (B).

The order of pipetting is the same as above if the incubation is done at 4 °C, except that before the addition of [¹²⁵I]-T₃, the solutions are pre-incubated at 4 °C for 24 h. Then [¹²⁵I]-T₃ is added and incubation resumed for 72 additional hours.

Calculations: Calculate either B/T or B/B₀ as below:

\[
\frac{B}{T} = \frac{B - N}{T - N} \% \quad \text{or} \quad \frac{B}{B_0} = \frac{B - N}{B_0 - N} \%
\]

B, number of counts per minute that are precipitated or bound; N, number of counts per minute that are nonspecifically precipitated or "trapped"; T, total number of counts per minute added to each incubation mixture; X, amount of standard; and B₀, total number of counts per minute precipitated in the tubes where X = 0.

The values for y-intercept, slope, and coefficient of correlation were obtained by calculating the least-square line in each case in the range of 0 to 500 pg of X. B/B₀ (defined as y) is a linear function of X up to 500 pg. The equation of the least-squares line is: \( y = mx + b \) where \( (y, X) \) are coordinates of any point on the line \( (B/B₀ \text{ vs. } X) \). The coefficient of correlation is the degree of association or correlation between the two variables.

Results

Table 1 shows that the steepest slope was obtained at an incubation temperature of 37 °C. The slope decreased below and above 37 °C. The lowest slope and coefficient of correlation were obtained at 10 °C, showing that equilibrium had not been established at 10 °C in 30 min. At 60 °C, the slope was higher than that at 10 °C, but the coefficient of correlation \( (r) \) was somewhat lower than that obtained at 37 °C. The y-intercepts increased with increasing temperature. The N values were relatively constant over the temperature range.

Optimum time was established as 30 min at an incubation temperature of 37 °C (Table 2). The slope was steepest for the 30-min incubation period. The lowest slope was obtained at 10 min. indicating that

<table>
<thead>
<tr>
<th>Temp. °C</th>
<th>Slope</th>
<th>y-intercept B/B₀ curve</th>
<th>r</th>
<th>B₀/B₇</th>
<th>Blank (N) % (±SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>44.6</td>
<td>81.2</td>
<td>0.558</td>
<td>23.4</td>
<td>10.3 ± 0.05</td>
</tr>
<tr>
<td>23</td>
<td>91.5</td>
<td>89.7</td>
<td>0.941</td>
<td>32.0</td>
<td>8.9 ± 0.07</td>
</tr>
<tr>
<td>37</td>
<td>107.1</td>
<td>88.4</td>
<td>0.947</td>
<td>50.8</td>
<td>8.8 ± 1.20</td>
</tr>
<tr>
<td>47</td>
<td>98.3</td>
<td>93.3</td>
<td>0.972</td>
<td>51.3</td>
<td>10.3 ± 0.21</td>
</tr>
<tr>
<td>60</td>
<td>62.9</td>
<td>99.9</td>
<td>0.920</td>
<td>52.0</td>
<td>8.3 ± 0.39</td>
</tr>
<tr>
<td>109.8</td>
<td>92.3</td>
<td>99.9</td>
<td>0.964</td>
<td>52.1</td>
<td>8.8 ± 0.03</td>
</tr>
</tbody>
</table>

| a | Values were obtained by linear regression. |

Table 1. The Slopes, y-Intercepts, Coefficient of Correlation, B₀/B₇ and N Values Obtained at Various Temperatures for 30 Min in the Range of 0.00 to 500 pg/ml, and at 4 °C for 72 h in the Same Range.
Table 2. Effect of Time on Standard Curves Obtained at 37 °C in the Range of 0.00 to 500 pg/ml of T3

<table>
<thead>
<tr>
<th>Time, min</th>
<th>Slope</th>
<th>y-intercept</th>
<th>Blank (N) ±(SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>69.2</td>
<td>88.1</td>
<td>0.859 42.8</td>
</tr>
<tr>
<td>20</td>
<td>80.7</td>
<td>91.4</td>
<td>0.964 46.5</td>
</tr>
<tr>
<td>30</td>
<td>92.3</td>
<td>90.9</td>
<td>0.960 49.3</td>
</tr>
<tr>
<td>40</td>
<td>85.8</td>
<td>94.6</td>
<td>0.985 48.7</td>
</tr>
<tr>
<td>50</td>
<td>70.4</td>
<td>94.0</td>
<td>0.197 48.3</td>
</tr>
<tr>
<td>60</td>
<td>73.0</td>
<td>97.9</td>
<td>0.986 50.4</td>
</tr>
</tbody>
</table>

a Values were obtained by linear regression.

Table 3. Standard Curves Were Compared at pH Values of 6.0, 7.0, 8.0, 9.0, and 10.0 in the Range of 0.00 to 500 pg/ml of T3 at 37 °C for 30 Min

<table>
<thead>
<tr>
<th>pH</th>
<th>Slope</th>
<th>y-intercept</th>
<th>Blank (N) ±(SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>6.0</td>
<td>91.3</td>
<td>88.3</td>
<td>0.917 38.3</td>
</tr>
<tr>
<td>7.0</td>
<td>91.5</td>
<td>91.3</td>
<td>0.934 42.2</td>
</tr>
<tr>
<td>8.0</td>
<td>112.2</td>
<td>90.3</td>
<td>0.941 49.9</td>
</tr>
<tr>
<td>9.0</td>
<td>105.2</td>
<td>92.1</td>
<td>0.934 44.5</td>
</tr>
<tr>
<td>10.0</td>
<td>70.7</td>
<td>94.9</td>
<td>0.856 19.0</td>
</tr>
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</table>

a Values were obtained by linear regression.

Table 4. Cross-Reactivity of Some Compounds in T3 Immunoassay

<table>
<thead>
<tr>
<th>Compound</th>
<th>Cross-reactivity</th>
</tr>
</thead>
<tbody>
<tr>
<td>L-Triiodothyronine</td>
<td>100%</td>
</tr>
<tr>
<td>L-Thyroxine (Cyclo)</td>
<td>33.5</td>
</tr>
<tr>
<td>L-Thyroxine (Sigma)</td>
<td>0.20</td>
</tr>
<tr>
<td>3'-Isopropyl-L-diodothyronine</td>
<td>0.122</td>
</tr>
<tr>
<td>3,5'-Diiodothyronine</td>
<td>0.17</td>
</tr>
<tr>
<td>Diiodotyrosine</td>
<td>0.014</td>
</tr>
<tr>
<td>Moniodotyrosine</td>
<td>0.010</td>
</tr>
<tr>
<td>Triiodothyroacetic acid</td>
<td>29.4</td>
</tr>
</tbody>
</table>

equilibrium had not been established. The N values did not vary significantly.

Maximum binding. Table 3 shows that the binding was maximum at pH 8.0. The N values decreased progressively from pH 6.0 to pH 10.0. The coefficient of correlation did not vary significantly with pH.

Specificity was measured by studying the ability of compounds related to T3 to compete with [125I]-T3 for the binding to the T3 antibody. This was determined by measuring the amount of analog required to cause a 50% decrease in the binding of [125I]-T3 and comparing it to the amount of L-T3 (the standard T3 used) required to produce the same decrease in the binding of labeled T3 (Table 4). Most of the binding of T3 to the antibody was due to the presence (detected chromatographically) of T3 in the commercial preparations, especially in the Cyclo preparation. Triiodothyroacetic acid was the best competitor tested.

Incubation at 4 °C and at pH 8.0 for 72 h gave a curve similar to that obtained at 37 °C and at pH 8.0 for 30 min (Figure 1).

Reproducibility was assessed by repeated assays. The intra-assay variability was 7.2 ± 2.7 (SD) % (n = 8). Eight sera were assayed on six different occasions and the interassay variability was found to be 7.9 ± 2.5 (SD) % (n = 6).

Accuracy was determined by analysis of quality-control samples (500 pg/tube). The mean value was 480 ± 50 (SD) pg/tube (n = 6); the coefficient of variation was thus 10%. A further indication of accuracy was obtained by making serial dilutions of serum from a hyperthyroid patient. The standard curve obtained was compared to that using standard solutions of T3 (Figure 2).

Clinical values. Sera from patients seen in the Clinical Research Center at Children’s Hospital in Buffalo were analyzed for T3. The T3 values in different clinical conditions are shown in Table 5. The T3 values in samples of cord blood were in the hypothyroid range, with five additional values in the range of 1.5-3.0 ng/ml. The distribution of T3 in 135 samples of cord serum is shown in Figure 3.

Discussion

Production of T3 antibody of high specificity by use of conjugated proteins has been reported by several workers (13-15, 17-20). The published molar ratios of T3 to the proteins vary tremendously. Liebleh et al. (17) analyzed the final product of conjugation reactions—for protein by the Lowry et al. (22) method, for protein iodide by the AutoAnalyzer technique, and ultimately in T3 radioimmunoassay—and found the calculated T3 to BSA molar ratios for T3-BSA conjugates ranged from 1.82-6.78:1. Using [125I]-T3, Larsen (18) found that 20% of the radioactive T3 remained in the protein solution. The quantity of residual unbound [125I]-T3 was obtained and by using the original specific activity of [125I]-T3 and information on the protein concentration it was calculated that 1-2 moles of T3 were covalently linked per mole of albumin in the supernatant fluid of the suspension. Earlier, Chopra et al. (23) had reported
T₃:BSA molar ratios of 12:1, T₃:polylysine ratios of 204:1, and T₃:polyglutamic acid ratios of 97:1. However, in the last two cases precipitates observed during dialysis were not analyzed.

Gharib et al. (14) found by spectrometry that 17 molecules of T₃ are incorporated into one molecule of HSA. However, using the same procedure we found only six moles of T₃ conjugated to one mole of HSA. The difference may be ascribed to exclusion of the precipitate that forms during dialysis, which was isolated and found to be protein-free T₃.

A modification of the method of Gharib et al. (14) was used to calculate the molar ratios, because it was found that T₃ also absorbs at 280 nm. To determine the contribution of T₃ absorption at 280 nm, we calculated the ratio of absorbances at 280-320 nm and found it to be 0.770. Calculation of the molar ratio of T₃/HSA is as follows:

\[
A_{HSA}^{320\text{ nm}} + A_{T₃}^{320\text{ nm}} = \Sigma A^{320\text{ nm}}
\]

\[
A_{HSA}^{280\text{ nm}} + 0.770 A_{T₃}^{320\text{ nm}} = \Sigma A^{280\text{ nm}}
\]

Since \(A_{HSA}^{320\text{ nm}}\) is approximately zero,

\[
T₃ = \frac{\Sigma A_{T₃}^{320\text{ nm}} \times \epsilon_{HSA}^{280\text{ nm}}}{(\epsilon T₃^{320\text{ nm}} \times \Sigma A^{280\text{ nm}} - 0.770 \Sigma A_{T₃}^{320\text{ nm}})}
\]

where \(\epsilon\), the molar extinction coefficient, is \(3.6 \times 10^4\) liter/mol cm for HSA at 280 nm and \(4.5 \times 10^3\) liter/mol cm for T₃ at 320 nm in distilled de-ionized water.

However, it appears that the antigenicity of the conjugated proteins does not depend critically on the number of molecules of T₃ conjugated to each molecule of protein because in all cases cited above, antibodies were produced.

Any system used in RIA is composed of several components that should be in equilibrium: the labeled hormone, the unlabeled hormone, the protein antibody, and the complexes between the two forms of the hormones and the antibody. Variation in concentration of any of the elements can alter the equilibrium and so change the absolute values of unlabeled ligand obtained. In addition, a number of variables—such as temperature, time, and pH—markedly affect the equilibrium. The responsiveness of the assay was evaluated experimentally for all cases from the slope of the rectilinear plot of \(B/B₀\) vs. hormone concentration for the range of 0.00-500 pg, because beyond 500 pg the curves became nonlinear. The time, temperature, and pH that gave the steepest slope were adopted for the assay system: 30 min, 37 ºC, and pH 8.0.

Incubation is usually done at 4 ºC for days, because in our case the slope decreased after 30 min, we concluded that, for maximum precision, time and temperature had to be controlled. The temperature instability in the assay system above 37 ºC might be the result of distinction of binding proteins by proteolytic enzymes, or changes in protein conformation producing a change in affinity constant(s). The flatter slopes below 37 ºC and 30 min, with a tendency to poorer coefficient of correlation, could be attributed to failure to attain equilibrium. After incubation at 37 ºC, there is a 10-min incubation in an ice-water bath. This is sufficient to estab-
lish a new equilibrium, which is identical to that obtained by incubating for three days at 4 °C (Figure 1). The equilibrium constants at 37 °C (followed by 10 min at 4 °C) and 4 °C were 6.3 × 10^6 and 5.9 × 10^8 liter/mol, respectively. This again shows that both approaches arrive at the same end-point.

It is well known that pH's on either side of the optimum pH promote dissociation of antigen-antibody complexes. This might be the cause of the observed low $B_o/T$ values at high pH despite low blank values. The values were also decreased at lower pH because of the subtraction of a high blank value. The decrease in nonspecific binding with increasing pH has been observed before (26).

We considered the possibility that the presence of TBG and other T3-binding proteins in the serum might influence T3 values. Such a phenomenon might influence the assay in that $^{125}$I-T3 could become bound to TBG and thus be unavailable to bind to the antibody. This would result in an overestimation of serum T3 values. Binding of T3 to TBG was inhibited by using ANS at a concentration of 25 mg/100 ml of buffer (62.5 ng of ANS per 500 μl of incubation mixture) as compared to a concentration of 50 μg/100 ml of buffer in the assay for T4 (27). ANS has recently been used by Chopra et al. (20) in RIA of T3 (21). Their final concentration of 250 μg per incubation mixture is much higher than ours, and that high an ANS concentration produced a significant displacement of $^{125}$I-T3 from the antibody. The differences in ANS concentration may be attributable to the antibodies used. T3 concentrations measured about 20% higher if ANS was omitted.

Other compounds have been used to prevent T3 from binding to TBG and at the same time displace it from T3-binding proteins in the serum. These include tetrachlorothyronine (15) and T4 (16), which have been replaced by ANS (20), diphenylhydantoin (17), sodium salicylate (18), and merthiolate (28), which were inadequate in our system.

It is known that polyethylene glycol can precipitate α-globulins when human serum is added as a carrier initially or immediately before the separation step (29). Desbuquois and Aurbach (26) exploited this fact to separate free and antibody-bound peptide hormones. Polyethylene glycol also worked well for T3, thus eliminating the need for the second antibody (30). The presence of T3 in the carrier protein added after the incubation step did not affect results as compared to the use of serum deficient in T3.

Sensitivity and reproducibility were increased appreciably if pipetting was done at 0–4 °C as compared to room temperature. This would be expected, because the affinity of the antibody for T3 increased as the temperature decreased. This in turn is reflected in a decreased rate of dissociation, thus enhancing reproducibility.

Intra-assay variability was found to be 7.2 ± 2.7 (SD) %, as measured by comparing duplicates within assays. The interassay variability was studied by comparison of T3 concentration in eight sera measured in duplicates in different assay and a value of 7.9 ± 2.5 (SD) % was obtained. In assessing accuracy, T3 was added to T3-free serum and 96% of the added hormone was recovered.

The cross-reactivity between L-T4 and T3 antibody was largely due to the presence of T3 in commercial preparations of T4. Even a preparation of T4 that cross-reacted with the antibody to the extent of 0.2% was shown by paper chromatography to contain T3; the amount of T3 was not quantitated, however. Because of the spontaneous de-iodination of T3 to T3, it is very difficult to assign an exact value of the cross-reactivity of T4 with T3 antibody, but we believe that it is about 0.1% for this antibody preparation. While others have reported similar values (14, 17), it does not follow that every antiserum will have this degree of specificity.

The cross-reactivities of the other compounds reported in Table 4 have no practical importance, as none of them are present in blood in appreciable concentration. Even the T4 contributed only about 6% of the T3 values.

With this simplified assay, 50 samples can be processed in one working day. Manual pipetting takes about 60 min, the incubation time is 30 min at 37 °C plus 10 min at 0–4 °C, and separation of bound from unbound T3 requires about 50 min.

The values obtained at 37 °C are comparable to those other people obtained at 4 °C. The values obtained with this system are 1.46 ± 0.25 ng/ml for euthyroids. The values for other workers are Gharib et al. (14), 2.18 ± 0.55 ng/ml; Mitsuma et al. (15), 1.38 ± 0.23 ng/ml; Lieblich and Utiger (17), previously 1.45 ± 0.25 ng/ml and recently 0.7–1.5 ng/ml (31); Chopra et al. (16), previously 1.0–1.7 ng/ml and recently 1.13 ± 0.33 ng/ml (20); and Larsen (18), 1.1 ± 0.25 ng/ml.

Obviously many factors still exist that must be resolved before a normal range is established. To say that there is a general agreement that normal levels of T3 are between 1.0 and 1.5 ng/ml is still questionable (31). When technical variability was evaluated, values ranging from 0.56–1.63 ng/ml were obtained on the same serum sample (28). It has been shown that T values depend on the specificity and sensitivity of the antibody used (21, 31). However, the methodological differences existing might be due to the fact that different antibodies are used by different laboratories, and that these antibodies have varying specificity and sensitivity. Another factor that could cause differences is use of different compounds in the assay systems to prevent T3 from binding to TBG. Perhaps, the use of one compound universally will minimize this.

There is still a possibility that the high values obtained by Gharib et al. (14) could be attributed to relatively low iodine intake in Minnesota (28) that tends to increase serum T3 concentrations and T3/T4 ratios (33, 34).

The values for cord serum were mostly in the hypothyroid range, with five additional values in the
range of 1.5–3.0 ng/ml. Low values in the cord serum have been documented (18, 31, 32). Most of the T₃ values in the patients with chronic thyroiditis were in the normal range with an average value of 1.20 ± 0.81.

We conclude that our method is more rapid than that previously reported, in which incubation was at 37 °C (35). The T₃ values obtained are in agreement with those obtained by longer procedures. Great rapidity is frequently important in clinical laboratories.

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