Free Thyroxine Assessed with Three Assays in Sera of Patients with Nonthyroidal Illness and of Subjects with Abnormal Concentrations of Thyroxine-Binding Proteins

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Three methods for estimating free thyroxine (FT4) in serum were studied: equilibrium dialysis, the SPAC-ET FT4 radioimmunoassay, and the Amerlite MAB FT4 luminometric assay. Serum samples from 10 subjects with above-normal thyroxine-binding globulin (TBG), 6 with low TBG, 30 with familial dysalbuminemic hyperthyroxinemia (FDH), 13 with nonesterified fatty acids (NEFA) concentrations in serum >1.0 mmol/L, and 178 patients with various degrees of nonthyroidal illness (NTI) were measured and compared with samples from 42 euthyroid blood donors. The Amerlite MAB FT4 assay compared well with equilibrium dialysis, whereas the SPAC-ET assay averaged 40% lower. All three assays were not influenced by changes in TBG and showed no or only little changes in the presence of NEFA. Mean FT4 values in the FDH samples were somewhat higher than in controls when measured with the SPAC-ET assay, about equal with equilibrium dialysis, and somewhat below the mean control value with the Amerlite MAB FT4 assay, although individual results were within the control reference range. In NTI patients, no FT4 values were below the control reference range by the Amerlite MAB FT4 assay, 4 of 178 were below this range by equilibrium dialysis, and 1 of 178 was below this range by the SPAC-ET assay. In all assays a large proportion of the NTI samples showed FT4 values above the control reference range, a result that will interfere with the efficacy of these assays for assessing thyroid function in NTI patients.

Indexing Terms: intermethod comparison · variation, source of · thyroid status · familial dysalbuminemic hyperthyroxinemia

Besides the measurement of serum thyrotropin (TSH), the estimation of the free thyroxine (FT4) concentration in serum samples is often performed in the evaluation of thyroid function.4 However, in patients with acute or chronic illness without apparent thyroid disease [nonthyroidal illness (NTI)] or in starvations, the estimated FT4 values are not always within the reference range, rendering evaluation of thyroid function in these situations sometimes difficult. The frequency of this phenomenon appears to be related to the severity of the underlying illness (1). There exists controversy with regard to the true FT4 concentration in patients with NTI. Melmed et al. (2) showed decreased FT4 by equilibrium dialysis in 43% of patients admitted to an intensive-care unit, whereas by commercially available FT4 kits, between 30% and 100% of these samples were subnormal, depending on the kit used. Similarly, Wang et al. (3), using ultrafiltration and RIA methods, reported decreased FT4 values in critically ill patients. However, Surks et al. (4) showed with both ultrafiltration and equilibrium dialysis of undiluted serum that FT4 was normal in all 30 intensive-care patients studied. Finally, we have shown that FT4 by equilibrium dialysis was above normal in 54% of a group of 504 patients with mild to severe NTI (1), although this percentage was much lower (24%) in the most severely ill group. Therefore, the differences in the reported FT4 concentrations in NTI patients may be attributed to discrepancies in patient selection and (or) in methodology. A further explanation involves the finding that some NTI patients possess a circulating inhibitor of thyroxine (T4) binding to serum proteins (5, 6), and thus exhibit interference in the measurement of FT4, depending on the method used.

Recently, Amerlite Diagnostics Ltd., formerly The Clinical Reagents Division of Amersham International, developed a new kit (Amerlite MAB FT4) to measure FT4 in patients' serum samples. The kit uses a horseradish peroxidase-labeled anti-T4 monoclonal antibody and triiodothyronine (T3)-coated wells and—according to the description accompanying the kit—should be free from the discrepancies seen with analog tracer assays, similar to the previously supplied Amerlex-M FT4 kit, which used an analog of T4 as tracer. This analog was designed to bind to the antisera used in the FT4 RIA, but not to serum binding proteins present in the incubation mixtures. However, it appeared that, especially in the case of large changes in albumin concentrations, or in the presence of familial dysalbuminemic hyperthyroxinemia (FDH) (7; for a review, see 8), binding to albumin did occur, leading to falsely increased values for FT4 (9). Also the Amerlite FT4 kit, which was introduced thereafter and uses a horseradish peroxidase (EC 1.11.1.7)-conjugated T4 instead of an analog, appeared not to be completely free of these artifacts caused by FDH albumin (10). However, this new Amerlite MAB FT4 kit is still an analog method, using T3 coupled to the wall of the incubation wells as the analog. Any

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3 Nonstandard abbreviations: FT4, free thyroxine; NTI, nonthyroidal illness; T4, thyroxine; T3, 3,3',5-triiodothyronine; FDH, familial dysalbuminemic hyperthyroxinemia; rT3, reverse T3 (3,3',5'-triiodothyronine); TBG, thyroxine-binding globulin; NEFA, nonesterified fatty acids; TSH, thyrotropin.

Received June 29, 1992; accepted February 22, 1993.
serum constituent that binds to this solid-phase analog will interfere with the binding of the labeled antibody to the wall of the wells. Therefore, there is no reason, in principle, why a labeled antibody method relying on a solid-phase analog should avoid the problems caused by binding of serum proteins to the analog, although in practice, binding of serum proteins to this solid-phase T₃ seems to be effectively inhibited, even in the case of FDH albumin.

To evaluate the new Amerlite MAB FT₄ kit, we paid special attention to samples from subjects with euthyroid or nonthyroidal illness (NTI). Results for these and other kinds of sera were compared with those measured in parallel by equilibrium dialysis and with the SPAC-ET FT₄ kit.

Materials and Methods

Methods

The Amerlite MAB FT₄ kit, supplied by Kodak Clinical Diagnostics Ltd. (Cardiff, Wales, UK), was used as described in the product information supplied by the manufacturer. The measuring principle is based on an equilibrium of a fixed amount of monoclonal anti-T₄ antibody labeled with horseradish peroxidase between a fixed amount of T₄ coupled to the walls of a 96-well microtiter plate and the FT₄ in the sample. Finally, the amount of enzyme bound to the wall of the well is measured by generating a chemiluminescent signal from a luminol substrate. The luminescence measurements are performed with a luminometer supplied by the manufacturer of the kit. In the assay, 25 μL of sample is incubated for 30 min at 37 °C with 100 μL of anti-T₄ antibody solution per well, giving a final dilution of the sample of fivefold.

SPAC-ET FT₄ was supplied by Byk-Sangtec BV (Zwolle, The Netherlands). The dialyzable fraction was measured according to the instructions of the manufacturer. The measuring principle is based on the observation that the amount of T₄ bound to a fixed amount of monoclonal anti-T₄ antibody coated to the tube wall depends on the FT₄ concentration. After incubation of serum samples (50 μL) with labeled T₄ in buffer (950 μL) in antibody-coated tubes for 2 h at 37 °C, the tubes are washed and the amount of T₄ bound to the tube is counted. For each sample and the reference standard a response parameter R is calculated:

\[ R = C_r / [(C_T - C_b) (P - TT_4 C_r / C_T)] \]

where \( C_r \) = counts taken up from tracer in pure buffer, \( C_b \) = counts bound from serum corrected for tracer binding from serum to tubes without coating (i.e., blanks), \( P \) = binding capacity of the tube coating (data supplied by the manufacturer), and \( TT_4 \) = total T₄ in the sample divided by the dilution factor. From this response parameter, the percent dialyzable fraction is calculated as \( \text{FT}_4\%_{\text{unknown}} = \text{FT}_4\%_{\text{reference}} \cdot R_{\text{unknown}} / R_{\text{reference}} \) \( (11) \). Therefore, the assay is standardized with only one serum sample with a known dialyzable fraction, as estimated by the manufacturer. To calculate the serum FT₄ from the dialyzable fraction, we used the serum T₄ measured with our in-house total T₄ RIA. The final dilution of the sample was 20-fold. Lower final dilutions can be obtained by introducing more serum into the incubation mixtures, taking into account in the calculations of the results the greater total amount of T₄ introduced in the system by this change of sample size.

Equilibrium dialysis was carried out as described by Sterling and Brenner \( (12) \). Each batch of \( ^{125i}\)T₄ (Amersham International, Aylesbury, Bucks, UK) was checked by HPLC \( (13) \) for radiolabeled impurities on arrival. Besides \( ^{125i} \), which is removed before use by chromatography on Sephadex LH-20 as described for T₃ \( (14) \), only one other peak of radiolabeled T₄ was detected. We dialyzed 1 mL of diluted serum [diluted 10-fold with phosphate-buffered saline (150 mmol/L NaCl, 50 mmol/L NaH₂PO₄, pH 7.4)] against 4 mL of potassium phosphate buffer (75 mmol/L, pH 7.4) for 20 h at 37 °C. Therefore, the final dilution of the sample was 50-fold. In the dilution studies, we varied the dilution factor by changing the start dilution of the serum.

T₄, T₃, and reverse T₃ (rT₃) were measured with our in-house double-antibody RIAs, with commercially available antibodies, tracers, and unlabeled hormone, and with 8-anilinonaphthalene sulfonic acid to block the binding of the iodothyriones to the serum proteins.

All estimations were performed in duplicate.

Samples

Serum pools with low, medium, and high total or free thyroid hormone concentrations (different sets for each assay) were constructed by mixing patients' sera sent to the routine laboratory for assessment of thyroid hormone parameters. Sera were allotted to the different pools on the basis of the results of these measurements.

Sera from 42 normal blood donors (12 men, 30 women) served as controls. Sera from 29 FDH subjects (members of seven different families), 10 subjects (4 men and 6 women taking oral contraceptives) with above-normal serum TBG, and 6 subjects with low or absent serum TBG were investigated. We also assessed 13 sera from euthyroid hyperlipemic subjects with nonesterified fatty acids (NEFA) >1.0 mmol/L and normal concentrations of serum TSH.

We investigated 178 sera from NTI patients, newly admitted to the departments of internal medicine or to the intensive-care unit of the hospital. Samples with normal serum TSH values were divided into four groups on the following basis:

Group I: T₄, T₃, and rT₃ within the reference range of the 42 euthyroid controls (60–145, 1.1–3.1, and 0.15–0.38 mmol/L, respectively)

Group II: T₄ and T₃ within the reference range, rT₃ above the reference range

Group III: T₄ within the reference range, but T₃ below and rT₃ above the reference range

Group IV: T₄ and T₃ below the reference range, rT₃ above the reference range

This type of grouping indicates, as previously shown \( (1) \), increasing severity of illness from group I to group

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IV, as reflected in an increase in mortality rate—group I, 0.8%; group II, 8%; group III, 22%; and group IV, 30%—and in median hospitalization time of the surviving patients—12, 15, 19, and 29 days, respectively.

For each group, the mean FT₄ obtained with the different methods was compared with the mean FT₄ of the control group, obtained with the same method, by use of Student’s t-test. We also used an orthogonal Deming-debiased regression (15, 16), using the variance of repeated measurements in the x and y variables as shown in Table 1, for analysis of the correlation between the results of the various assays within the different groups. This method reduces the residuals perpendicular to the line, rather than reducing only the vertical y residuals, as occurs by least-squares regression. This accounts for the variability in both the x and y measurements. Regression of y on x or x on y gives the same line by Deming regression. A small range of values is not as great a source of distortion as in least-squares analysis.

Finally, we measured serum samples from four control subjects, six subjects from group IV, and from two pools of four sera from group IV patients in different dilutions (final dilutions, 5- to 100-fold). In all cases, the procedures followed were in accordance with the Helsinki Declaration of 1975 and the 1983 revision thereof.

Results

Table 1 shows the reproducibility of the assays used in this study. Three different serum pools (with low, medium, and high total and free thyroid hormone concentrations, one set for each assay) were measured in 10 consecutive assays on different days. The low coefficients of variation (CVs) indicate that the run-to-run stability was good.

Table 2 shows the mean total thyroid hormone concentrations and the mean results of the three FT₄ assays in the sera from 42 controls, used in this study, and the reference values for the different assays, as estimated in a much larger group (n = 200) of blood donors. Figure 1 shows the relation between the Amerlite MAB FT₄ and the equilibrium dialysis results for the 42 control subjects. The correlation between both assays is good (r = 0.92), with a slope near unity.

Changes in TBG binding did not significantly alter FT₄ concentrations measured with the three assays tested (Table 3). In the case of changes in albumin binding, as found in samples of subjects with FDH, the results depended on the assay used (Table 3). Results obtained with equilibrium dialysis were not significantly different from those for controls (0.1 < P < 0.2), and the mean result of the SPAC-ET assay was significantly higher (P < 0.001) than the control mean, although the individual values were all within the control reference range. The mean result for FDH subjects by the Amerlite MAB FT₄ assay was significantly lower (P < 0.001) than in the controls, although here also all

Table 1. Results for Pooled Control Sera in 10 Consecutive Assays

<table>
<thead>
<tr>
<th>Assay</th>
<th>Low</th>
<th>Medium</th>
<th>High</th>
</tr>
</thead>
<tbody>
<tr>
<td>FT₄ SPAC-ET, pmol/L</td>
<td>10.6 ± 0.9 (8.3)</td>
<td>15.0 ± 1.1 (7.3)</td>
<td>21.1 ± 1.4 (8.7)</td>
</tr>
<tr>
<td>FT₄ Amerlite MAB, pmol/L</td>
<td>6.8 ± 0.35 (5.1)</td>
<td>18.2 ± 0.63 (3.5)</td>
<td>48.7 ± 0.91 (1.9)</td>
</tr>
<tr>
<td>FT₄ dialysis, pmol/L</td>
<td>27.3 ± 1.6 (6.0)</td>
<td>89 ± 3.1 (3.5)</td>
<td>143 ± 3.7 (2.6)</td>
</tr>
<tr>
<td>T₂ RIA, nmol/L</td>
<td>41 ± 3.1 (7.6)</td>
<td>1.7 ± 0.13 (7.6)</td>
<td>4.4 ± 0.24 (5.4)</td>
</tr>
<tr>
<td>T₂ RIA, nmol/L</td>
<td>0.17 ± 0.01 (7.8)</td>
<td>0.23 ± 0.02 (6.6)</td>
<td>0.39 ± 0.03 (6.8)</td>
</tr>
</tbody>
</table>

Data are presented as * Mean ± SD (and CV, %). Each assay was tested with a separate set of pooled sera.

Table 2. Results for Total T₄, T₃, rT₃, and FT₄ Assays in Control Subjects

<table>
<thead>
<tr>
<th>Assay</th>
<th>A*</th>
<th>B*</th>
</tr>
</thead>
<tbody>
<tr>
<td>FT₄ SPAC-ET, pmol/L</td>
<td>10.9 ± 2.1</td>
<td>11.6 ± 2.4</td>
</tr>
<tr>
<td>FT₄ Amerlite MAB, pmol/L</td>
<td>17.7 ± 3.4</td>
<td>19.0 ± 4.0</td>
</tr>
<tr>
<td>FT₄ dialysis, pmol/L</td>
<td>18.7 ± 3.7</td>
<td>19.0 ± 4.0</td>
</tr>
<tr>
<td>T₂ RIA, nmol/L</td>
<td>102 ± 24</td>
<td>102 ± 21</td>
</tr>
<tr>
<td>T₂ RIA, nmol/L</td>
<td>2.0 ± 0.5</td>
<td>2.1 ± 0.5</td>
</tr>
<tr>
<td>rT₃ RIA, nmol/L</td>
<td>0.26 ± 0.06</td>
<td>0.30 ± 0.06</td>
</tr>
</tbody>
</table>

* 42 control subjects (this study).

Laboratory reference values estimated from a group of 200 normal blood donors.

Fig. 1. Correlation between Amerlite MAB FT₄ and equilibrium dialysis FT₄ as measured in 42 controls: r = 0.92, slope = 0.92
Table 3. Results for Sera with Abnormalities in T₄-Binding Proteins or with High NEFA Concentrations

<table>
<thead>
<tr>
<th>T₄, nmol/L</th>
<th>SPAC-ET MAB</th>
<th>Dialysis</th>
</tr>
</thead>
<tbody>
<tr>
<td>nT₄</td>
<td>SPAC-ET</td>
<td>Amerlite MAB</td>
</tr>
<tr>
<td>TBG increase</td>
<td>10</td>
<td>149 ± 40</td>
</tr>
<tr>
<td>TBG deficiency</td>
<td>6</td>
<td>31 ± 16</td>
</tr>
<tr>
<td>FDH</td>
<td>30</td>
<td>192 ± 37</td>
</tr>
<tr>
<td>NEFA&lt;sup&gt;a&lt;/sup&gt;</td>
<td>13</td>
<td>81 ± 21</td>
</tr>
</tbody>
</table>

*From seven different families.
<sup>b</sup>Significantly different from the mean of the control group estimated with the same assay: *P < 0.001, †P < 0.005.

Fig. 2. Influence of dilution on the FT₄ results: mean ± SD for controls (solid symbols) and NTI sera (individual and pooled sera) (open symbols). (II, III, Amerlite MAB FT₄ (△, △), equilibrium dialysis FT₄ (Θ, Θ), SPAC-ET FT₄)

Fig. 3. Thyroid hormone measurements in 178 patients with nonthyroidal illness

Table 4. Influence of Nonthyroidal Illnesses on Thyroid Hormones

<table>
<thead>
<tr>
<th>Assay</th>
<th>I (n = 48)</th>
<th>II (n = 38)</th>
<th>III (n = 57)</th>
<th>IV (n = 35)</th>
</tr>
</thead>
<tbody>
<tr>
<td>T₄ RIA, nmol/L</td>
<td>85 ± 19</td>
<td>98 ± 30</td>
<td>83 ± 21</td>
<td>45 ± 13</td>
</tr>
<tr>
<td>T₃ RIA, nmol/L</td>
<td>1.9 ± 0.4</td>
<td>1.4 ± 0.3</td>
<td>0.8 ± 0.4</td>
<td>0.6 ± 0.3</td>
</tr>
<tr>
<td>rT₃ RIA, nmol/L</td>
<td>0.28 ± 0.06</td>
<td>0.56 ± 0.15</td>
<td>0.66 ± 0.18</td>
<td>0.63 ± 0.17</td>
</tr>
<tr>
<td>FT₄&lt;sup&gt;α&lt;/sup&gt; pmol/L</td>
<td>9.9 ± 1.7&lt;sup&gt;α&lt;/sup&gt;</td>
<td>12.2 ± 3.1&lt;sup&gt;α&lt;/sup&gt;</td>
<td>14.3 ± 4.4&lt;sup&gt;α&lt;/sup&gt;</td>
<td>12.0 ± 2.7&lt;sup&gt;α&lt;/sup&gt;</td>
</tr>
<tr>
<td>Amerlite MAB</td>
<td>18.7 ± 2.9</td>
<td>22.0 ± 5.1&lt;sup&gt;α&lt;/sup&gt;</td>
<td>25.0 ± 7.6&lt;sup&gt;α&lt;/sup&gt;</td>
<td>21.9 ± 7.9&lt;sup&gt;α&lt;/sup&gt;</td>
</tr>
<tr>
<td>Dialysis</td>
<td>18.9 ± 2.9</td>
<td>24.1 ± 9.0&lt;sup&gt;α&lt;/sup&gt;</td>
<td>29.8 ± 11.1&lt;sup&gt;α&lt;/sup&gt;</td>
<td>21.6 ± 5.8&lt;sup&gt;α&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Significantly different from the mean of the control group estimated with the same assay: *P < 0.05; †P < 0.025; ‡P < 0.01; ††P < 0.001.
Table 5. Number of NTI Samples with FT₄ Values outside the Estimated 95% Reference Interval

<table>
<thead>
<tr>
<th>NTI group</th>
<th>I</th>
<th>II</th>
<th>III</th>
<th>IV</th>
</tr>
</thead>
<tbody>
<tr>
<td>FT₄ assay</td>
<td>Below</td>
<td>Above</td>
<td>Below</td>
<td>Above</td>
</tr>
<tr>
<td>SPAC-ET</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>7</td>
</tr>
<tr>
<td>Amerlite MAB</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>10</td>
</tr>
<tr>
<td>Equilibrium dialysis</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>11</td>
</tr>
</tbody>
</table>

n = for each group as in Table 4.

Discussion

The data presented in Table 1 indicate that the reproducibility of the different assays is adequate. Most of the CVs were around 7% (range 1.9–9.6%). Of the FT₄ assays tested, the FT₄ Amerlite MAB had the lowest CVs and the FT₄ SPAC-ET the highest, although the differences are only marginal.

Sera from 42 control subjects were used to establish a normal reference range for the different FT₄ assays tested here. Although measurement of 42 control sera is not enough to establish a reference range (17), the values found were not significantly different from our laboratory reference values estimated in a group of 200 blood donors (Table 2). Therefore, we think the use of this small control group in this study is justified, because it appears to be a representative sample of the healthy population. The mean value measured by the Amerlite MAB FT₄ was not significantly different from the value obtained by equilibrium dialysis. The mean value found with the SPAC-ET was significantly lower: the values measured by the SPAC-ET assay were ~60% of those measured by equilibrium dialysis. This difference is caused by the standardization of this assay. If the standard supplied by the manufacturer is measured with our in-house dialysis technique, and the resulting value is used instead of the one proposed by Byk-Sangtec, the values obtained are similar to those by equilibrium dialysis.

Neither increases nor deficiencies of serum TBG concentration significantly affected the final FT₄ concentration in the three assays tested (Table 3), although there seems to be a small positive correlation between TBG concentration and FT₄ by equilibrium dialysis, and a negative correlation for the other two assays. Similar results were reported by Csako et al. (18), but other authors (19, 20) deny the existence of such a correlation between the TBG concentration and the FT₄ concentration estimated by equilibrium dialysis. In the group of FDH subjects, the FT₄ concentrations obtained by equilibrium dialysis are not significantly different from those for the control group, whereas mean FT₄ by the SPAC-ET assay was significantly higher in the FDH group than in the controls; however, all individual values were still within the control reference range. In contrast to the analog-based FT₄ assays, which produce FT₄ values in FDH sera in the hyperthyroid range (9), measurement of FT₄ in FDH samples with the Amerlite MAB FT₄ assay results in values somewhat lower than those in controls; i.e., the mean FT₄ of the FDH samples...
is significantly lower than the control mean, even though all individual values except one were within the control reference range. Therefore, we see no risk that the use of this assay will lead to a wrong diagnosis of hyperthyroidism.

Some authors claim that the changes in FT₄ seen in NTI patients are caused by the presence of one or more so-called inhibitors (5, 21), which are supposed to interfere with the binding of T₄ to serum binding proteins. Others, however, could find no evidence for the presence of such compounds (22). Some reports in the literature (23, 24) state that the changes in FT₄ in NTI patients are caused by circulating NEFA as inhibitors of serum T₄ binding. Therefore, we tested whether the FT₄ assays were influenced by high concentrations of NEFA. We measured FT₄ in sera from euthyroid hyperlipemic subjects with high NEFA (Table 3), i.e., NEFA concentrations higher than normally found in sera of NTI patients with markedly increased FT₄ concentrations (0.46-1.74 mmol/L) (22). The mean FT₄ as measured with all three assays was lower than the control mean, but only the value measured by the Amerlite MAB FT₄ was significantly lower (P < 0.005). Therefore, NEFA does not seem to be the cause of the increase in FT₄ observed in patients in groups II, III, and IV (see Figure 3). If other inhibitors are present, dilution of the sera will affect the measured free hormone concentration, if the affinity of the inhibitor for the T₄-binding proteins differs from that of T₄. If the affinity is lower, the inhibitory effect will be diluted out, and the final result will be a decrease of the apparent FT₄ concentration with increasing dilution. If the affinity is higher or if no inhibitor is present, dilution will not affect the measured FT₄ concentration (25, 26). The presence of a dialysis membrane in the case of equilibrium dialysis is another complicating factor. If the inhibitor is able to traverse the dialysis membrane, the above discussion applies; however, if the inhibitor is not able to traverse the membrane, then only the dilution of the serum inside the dialysis bag has to be taken into account, which is only one-fifth of the total dilution of the system. This could be an explanation for the data presented in Figure 2, from which we conclude that only the results obtained by equilibrium dialysis show a dependence of FT₄ on dilution. The large standard deviation of the data points indicates that in some NTI sera the effect of dilution is considerable, whereas others exhibit no effect at all. In general, therefore, assessment of FT₄ in NTI should be performed with minimal sample dilution, to rule out any effect caused by dilution and to reflect the in vivo situation as closely as possible. Another explanation for the fact that the results of the SPAC-ET FT₄ and the Amerlite MAB FT₄ assays are only minimally affected by dilution could be that, in both assays, the putative inhibitor of the serum T₄ binding inhibits to a similar extent T₄ binding to the T₄ antibodies used in these assays.

It is generally accepted that evaluation of thyroid function in patients with nonthyroidal illness is difficult. Such patients develop a so-called low-T₃ syndrome with increased rT₃ concentrations (because of the decreased metabolic clearance of rT₃), lower than normal T₃ concentrations (caused by decreased peripheral production of T₃ from T₂) and, in cases of critical illnesses, also depressed total T₄ concentrations (attributable to decreased production and serum binding of T₄) (27). To investigate the performance of the Amerlite MAB FT₄ assay in NTI sera, we selected a large number of sera from newly hospitalized patients and categorized these sera into four groups on the basis of the T₄, T₃, and rT₃ concentrations (1). Measurement of FT₄ in serum samples of NTI patients by the Amerlite MAB assay gave results that were comparable with those by the equilibrium dialysis method. In both assays a large number of T₄ values were above the reference range, especially in samples from NTI groups II and III (Table 5). Of the three assays tested, the SPAC-ET FT₄ assay had the highest number of FT₄ values within its reference range (Table 5), although still 28 of 178 samples from NTI patients were outside this range. Consequently, it is often not possible to exclude hyperthyroidism in NTI patients by measuring only the FT₄ concentration. Above-normal FT₄ values in NTI patients were reported earlier (1, 28), both for the equilibrium dialysis technique and for other methods that do not rely on the use of an analog of T₄ as the tracer. On the other hand, methods that do use a labeled analog of T₄ to avoid binding of the tracer to serum binding proteins often show serum FT₄ values below the reference range, notably for samples from NTI groups III and IV (1, 28, 29).

Regression analysis within the various groups (Table 6) yielded a rather poor correlation between the FT₄ values obtained by the three assays, especially in the FDH subjects and the NTI group IV patients. Perhaps the variations are caused by changes in albumin-T₄ binding, as seen in FDH, where it is increased, and in NTI, where albumin concentrations are almost invariably depressed in moderately and severely ill patients (28, 29). At first, this seems a part of the explanation in the case of the Amerlite MAB FT₄ assay, because with this assay we found that the mean FT₄ value in the FDH group was lower, and the mean FT₄ value in the NTI group was higher than in the control group. However, it is equally possible that there is no common denominator for the observed deviations of the mean FT₄ from the control mean in the FDH and NTI groups, because the three assays all produced significantly increased mean FT₄ values in the NTI groups II, III, and IV (Table 4), whereas the change in the mean FT₄ in the FDH group was assay dependent (Table 3).

The SPAC-ET FT₄ assay, as well as the FT₄ estimated by equilibrium dialysis, is dependent on the total T₄ assay, because in fact both assays measure a free (i.e., unbound) fraction, which has to be multiplied by the total T₄ in serum to obtain the actual FT₄ value. Therefore instead of one, two measurements have to be performed to obtain the serum FT₄ concentration, which obviously increases the workload of the laboratory. The Amerlite MAB FT₄ assay, on the other hand, is easy and rapid to
perform and produces FT₄ results that are not dependent on the estimation of the total T₄ concentration.

Finally, it appears that the absolute free hormone concentrations measured with the Amerlite MAB FT₄ assay compare much better with the FT₄ concentrations found with the equilibrium dialysis method, leading to a slope of the regression line of about unity, than does the SPAC-ET FT₄ assay, which renders results that are only about 60% of the equilibrium dialysis values. This systematic difference is due to the calibration of the standard in this assay.

References