Effects of Treatment on Tyrosine Tolerance in Thyroid Disease
A Modified Tyrosine Assay

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A widely used assay technique for plasma tyrosine determination has been found to give rise to large errors. It was modified to improve accuracy, and the relationship between the values derived by the modified and unmodified procedures was studied. The plasma tyrosine concentrations in thyrotoxic and hypothyroid patients during oral tyrosine loading are reported. Tyrosine tolerance in thyrotoxicosis reverts to normal after successful treatment. In hypothyroidism, most patients had normal tyrosine tolerance before and after treatment.

Additional Keyphrases fluorometry • thyrotoxicosis • hypothyroidism • TSH

The fluorometric technique of Waalkes and Udenfriend (1) for the determination of plasma tyrosine is an extension of an earlier colorimetric procedure (2). The tyrosine-nitrosonaphthol derivative measured in this assay has an absorption spectrum with a peak of 460 nm, while in its fluorescence spectrum there is an excitation peak at this wavelength. Thus, when fluorescence measurements are made, part of the exciting energy—i.e., light at 460 nm—is absorbed. As the concentration of tyrosine derivative and the intensity of the color of the solution increases, the fluorescence yield with increasing concentration will decrease, and linearity of calibration curves may be expected only in a low-concentration range.

Interest in plasma tyrosine concentrations in thyroid disease has been aroused in recent years, after it was demonstrated that plasma tyrosine concentrations are higher than normal in cases of thyrotoxicosis both in the fasting state and after an oral load, while they tend to be lower than normal in cases of hypothyroidism. Such values are not related to age or sex, either in health or disease (3–6).

In estimating plasma tyrosine concentrations during tests of tyrosine tolerance, workers have found that, after the oral load, such concentrations were outside their calibrated range; consequently, such samples were diluted with water to various degrees (between 4- and 10-fold) at the final fluorescence stage of the assay. The readings thus obtained were compared with an undiluted standard and then multiplied by the appropriate dilution factor to give a final value (3–6).

This procedure results in large errors, as we found on studying the effect of dilution and of the relationship between data derived in this manner and the actual tyrosine concentration. We applied the results of this study to data obtained by the "dilution procedure," and subsequently modified the tyrosine assay to eliminate the errors associated with aqueous dilution.

Results obtained during tyrosine tolerance tests in thyroid disease, before and after treatment, are given.

Methods
Tyrosine Assay (A)

In the original technique (6), 1 ml of plasma or water as a reagent blank or 1 ml of standard aqueous tyrosine solution (20 μg/ml) was diluted with 3 ml of water, and then 1 ml of trichloroacetic acid solution (300 g/liter) was added. The mixture was shaken and centrifuged, and 2 ml of the supernatant fluid was pipetted into a stoppered tube. To this mixture 1 ml of nitrosonaphthol reagent (1-nitroso-2-naphthol, 1 g/liter of 95% ethanol) was added, followed by 1 ml of nitrite-nitric acid reagent [0.5 ml of NaNO₂ (25 g/liter) and 24.5 ml of dilute nitric acid (prepared by dilution of the concentrated acid fivefold with water)]. The mix-
ture was incubated in a water bath at 55°C for 30 min. After cooling, 10 ml of ethylene dichloride was added and the mixture shaken vigorously for 10 s. The ethanolic-aqueous (upper) layer was diluted fourfold with water with the exception of those from the blank, standard, and fasting samples. Fluorescence of the final solutions was read in a spectrophotofluorometer (Aminco-Bowman) with activating wavelength at 460 nm, emission at 560 nm.

Relative fluorescence readings were then compared with the undiluted 20-µg standard and multiplied by four to give a final value.

Effect of dilution. The above procedure was followed with use of aqueous standards over the range 0 to 300 µg/ml. Values obtained were compared with actual tyrosine concentrations.

Tyrosine Assay (B)

We diluted 0.2 ml of plasma or water, as a reagent blank, or aqueous tyrosine standards (50, 75, and 100 µg/ml) with 4 ml water to which was added 1 ml of trichloroacetic acid solution, 300 g/liter. The technique thereafter was as in assay A except that the final ethanolic-aqueous layer was read undiluted in all cases.

Tyrosine Tolerance Tests

Tyrosine tolerance was tested after oral administration of a tyrosine load (50 mg/kg), as described previously (7). Heparinized blood samples were taken in the fasting state and 30, 90, and 180 min after tyrosine administration.

Forty-four thyrotoxic patients (19 to 61 years old; 35 women and 14 with hypothyroidism (18 to 72 years old; 12 women) were studied before treatment. Eleven thyrotoxic and eight hypothyroid subjects were restudied 3 to 24 (mean, 9) months after treatment, at which time they were clearly euthyroid. Twenty-nine normal subjects, 18 to 45 years old, were also studied, of whom nine were women.

Treatment in the thyrotoxic group consisted of carbimazole administration followed by partial thyroidectomy (eight cases), carbimazole alone (two cases), or radioiodine therapy (one case). Hypothyroid patients were treated with thyroxine.

Because the data were not normally distributed, but skewed, they were processed by logarithmic transformation. The paired Student’s t test (two-tailed) was then applied.

Results

Figure 1 shows that, when the modified assay B was used, a calibration curve was obtained that was linear from 0 to 60 µg/ml of original standard solution (equivalent to 0–12.0 µg/ml in assay A, in terms of the total tyrosine content of the reaction tube).

Four determinations were made on the relationship between the true tyrosine concentrations and those obtained when the fluorophore was diluted, the sample range being 0–300 µg/ml. The mean results (Figure 2) show that assay A overestimates the tyrosine concentration at all concentrations.

Figure 3 shows the overestimate effected by the dilution, as a percentage of the true concentration. Similar results were found when plasma samples, rather than aqueous standards, were assayed by the two procedures.

Plasma tyrosine concentrations during oral tyrosine tolerance tests in patients with thyroid disease are shown in Table 1. Of the data, 40% were obtained by the modified assay technique. In the remainder, the original values have been corrected by use of the curve shown in Figure 2.

In the pretreatment thyrotoxic group, the mean tyrosine concentrations at all time intervals were significantly higher than normal. In 39 of 44 subjects, either the fasting concentration, the maximum postloading concentration, or both were above the normal range. After treatment, plasma tyrosine concentrations in nine of the 11 subjects who were restudied were in the normal range. The mean post-treatment levels were not significantly different from normal.

There was no difference between the mean
Table 1. Geometric Means and Ranges for Plasma Tyrosine Concentration (µg/ml) during Oral Tyrosine Tolerance Tests in Normal Subjects and in Patients with Thyroid Disease, before and after Treatment

<table>
<thead>
<tr>
<th></th>
<th>Fasting Mean</th>
<th>Fasting Range</th>
<th>30 min Mean</th>
<th>30 min Range</th>
<th>90 min Mean</th>
<th>90 min Range</th>
<th>180 min Mean</th>
<th>180 min Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal (29)*</td>
<td>12.7</td>
<td>6-18</td>
<td>21.2</td>
<td>11-32</td>
<td>26.3</td>
<td>15-46</td>
<td>27.3</td>
<td>14-37</td>
</tr>
<tr>
<td>Thyrotoxicosis</td>
<td></td>
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<tr>
<td>Before treatment (44)</td>
<td>19.9*</td>
<td>12-36</td>
<td>40.7*</td>
<td>21-92</td>
<td>47.3*</td>
<td>22-118</td>
<td>39.9*</td>
<td>23-79</td>
</tr>
<tr>
<td>After treatment (11)</td>
<td>11.8</td>
<td>8-14</td>
<td>23.3</td>
<td>12-50</td>
<td>29.5</td>
<td>21-55</td>
<td>21.5</td>
<td>12-60</td>
</tr>
<tr>
<td>Hypothyroidism</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Before treatment (14)</td>
<td>10.6*</td>
<td>7-16</td>
<td>20.5</td>
<td>7-35</td>
<td>27.8</td>
<td>17-58</td>
<td>31.0</td>
<td>20-46</td>
</tr>
<tr>
<td>After treatment (8)</td>
<td>10.8*</td>
<td>9-13</td>
<td>17.9</td>
<td>10-31</td>
<td>24.1</td>
<td>14-37</td>
<td>25.5</td>
<td>16-44</td>
</tr>
</tbody>
</table>

* Numbers in parentheses are number of individuals.
° Significantly different from normal group (p < 0.001).
* Significantly different from normal group (p < 0.05). For other values, p > 0.05.

Fig. 2. Apparent tyrosine concentration obtained with unmodified aqueous dilution technique as a function of the actual tyrosine concentration. Means of four duplicate determinations are shown (±2 sd). Mean cv, 5.2%

The broken line indicates the previously assumed relationship among fasting concentrations among the hypothyroid patients before and after treatment, although these values were lower than those found for the control group, the difference just reaching the 5% level of significance. In the hypothyroid group, all concentrations after loading were within the normal range.

Discussion

Clearly, it is inadvisable to compare solutions of the fluorescent tyrosine-nitrosonaphthol derivative that have been diluted with water with standards not similarly diluted, and data derived in this manner need reassessment.

To bring all plasma tyrosine readings within the calibrated and linear range, the initial sample size has been decreased, thus eliminating a final dilution and the associated errors.

Most plasma tyrosine concentrations encountered during tyrosine tolerance tests in normal subjects and in patients with thyroid disease fall within the range 7-100 µg/ml, and 0.2 ml of undiluted plasma may be used in the modified assay. However, in cases of liver disease, in which concentrations may be greater than this, the plasma
must be more highly diluted before the determination.

In a preliminary study, we found that the fluorescence yield of the fluorophore at 560 nm is appreciably increased by a slightly increased pH. Dilution with water increases the pH, and this effect may well contribute to the errors involved.

Conclusions reached in an earlier report on tyrosine tolerance in thyroid disease (6) have been borne out despite correction of the absolute values of tyrosine concentrations and increase in the size of the experimental group. After treatment, the tyrosine tolerance curve for patients with thyrotoxicosis was normal in all but two of the 11 cases. These patients had been treated with carbimazole for 12 months and were euthyroid by other criteria. In the hypothyroid group, tyrosine concentrations were barely affected either in the fasting state or after a tyrosine load.

Tyrosine tolerance tests may be of value in the diagnosis of hyperthyroidism but not hypothyroidism, because normal and abnormal ranges overlap too greatly. As an index of the effectiveness of treatment, the value of the test is limited to situations in which other thyroid-function tests are inapplicable, such as pregnancy.

References