The Upper Limit of Normal for Thyrotropin Is 3 or 4 milli-int. units/L

A. Paul Durham

Recently Musto et al. (Clin Chem 30: 329–330, 1984) noted that, despite the clinical importance of accurate measurement of thyrotropin at low concentrations, the upper limit of normal is well below the stated upper limit of normal of most commercial tests. Here I amplify their statement.

Additional Keyphrases: reference interval · "kit" methods · pituitary hormones

In a recent discussion of thyrotropin (thyroid-stimulating hormone) measurement, Musto et al. (1) draw attention to the clinical importance of good performance at low concentrations. A thyrotropin assay that has adequate reproducibility throughout the "normal" range and is essentially free of nonspecific matrix effects can be expected to confer two benefits. First, it will minimize the number of borderline results that will require the thyroliberin (thyrotropin-releasing factor, TRF) test as followup. Second, it will provide more meaningful baseline values for comparison with values obtained after TRF stimulation.

The high upper limit of normal that is characteristic of most commercially available thyrotropin assays is symptomatic of their inability to provide precise and accurate measurements of thyrotropin at low concentrations. Musto et al. (1) state without documentation that, according to the literature, the true upper limit of normal for thyrotropin is less than 5.0 milli-int. units/L. Clinicians who regularly encounter only results for thyrotropin generated by commercially available kits may well be inclined to doubt this assertion. But the point made by Musto et al. is an important one, and this Note is intended to document its truth.

Studies aimed at determining the reference interval for normal individuals can provide important insights into the acceptability of an assay system. This is especially true of assays for thyrotropin, where an upper limit of normal significantly higher than those reported for fully optimized radioimmunoassays may well be symptomatic of shortcomings that can seriously compromise clinical efficacy (1).

There is, in fact, a striking contrast between most but not all thyrotropin kits now on the market and the several "research" radioimmunoassays (2–6) tabulated in Table 1. The latter characteristically show an upper limit of normal of approximately 3 or 4 milli-int. units/L. The stated normal range for most kits, on the other hand, has until recently extended up to 10 milli-int. units/L.

While there has been an encouraging trend towards more accurate thyrotropin kits, an upper limit of 7 milli-int. units/L represents, even today, the norm for nearly all commercially available assays. This shows that the great majority of thyrotropin kits still err, on borderline samples, by a factor of two relative to more definitive assay systems. Indeed, it is rare to encounter any thyrotropin procedure with a reasonable assay time (same-day or overnight) that measures up to standards set by the research assays for accuracy and precision at low concentrations (7, 8).

Research Assays vs Kit Methods
The systems here designated as "research" assays have strong claims to being regarded as reference methods for thyrotropin.

First, they were established more than a decade ago, and since then have contributed significantly to our understanding of the physiology and clinical relevance of this pituitary hormone. This is reflected not only in countless journal articles but also in standard medical textbooks and reviews, where values ranging from 3.0 to 5.0 milli-int. units/L are usually quoted as the accepted upper limit of normal (1, 5, 9–11).

Second, these research assays resemble the typical thyrotropin kit of today in being nonequilibrium, competitive, double-antibody radioimmunoassays. They differ principally in the degree to which they have been optimized for sensitivity and specificity. Comparisons between the research assays and the kit methods are therefore in order.

Characteristically, the research assays involve use of meticulously purified tracer, incubation times of six days or more, and standard curves prepared with human serum. These features can be expected to minimize nonspecific "matrix effects," resulting in lower and more accurate values throughout the normal reference interval (12).

Some of these features appear to be essential for meaningful results, particularly the steps taken to purify the tracer and to eliminate matrix differences between standards and unknowns. It has been shown repeatedly that neither buffered human serum albumin nor an animal serum matrix is an adequate substitute for a human serum matrix (8, 12–15).

Further corroboration for an upper limit of approximately 3 or 4 milli-int. units/L comes from studies performed with various "nonstandard" methodologies (16–20), including immunoassays based on chemiluminescence, separation by wick chromatography, or sample concentration, as well as the modern cytochemical bioassay (Table 1).

In addition, experiments in which samples were collected

Table 1. Upper Limits of Normal in Research Assays for Thyrotropin

<table>
<thead>
<tr>
<th>Assay (and ref.)</th>
<th>Thyrotropin, milli-int. units/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>Radioimmunoassays</td>
<td></td>
</tr>
<tr>
<td>Melbourne, Australia (2)</td>
<td>3.1</td>
</tr>
<tr>
<td>Newcastle, U.K. (3)</td>
<td>2.8</td>
</tr>
<tr>
<td>Munich, F.R.G. (4)</td>
<td>3.8</td>
</tr>
<tr>
<td>Boston, MA (5)</td>
<td>3.0</td>
</tr>
<tr>
<td>Los Angeles, CA (6)</td>
<td>3.5</td>
</tr>
<tr>
<td>Other methods</td>
<td></td>
</tr>
<tr>
<td>Wick chromatography/RIA (16)</td>
<td>3.0</td>
</tr>
<tr>
<td>Adsorption to concanavalin A/RIA (17)</td>
<td>4.0</td>
</tr>
<tr>
<td>Immunochemiluminescence assay (18)</td>
<td>4.5</td>
</tr>
<tr>
<td>Cytochemical bioassay (19, 20)</td>
<td>2.4, 3.7</td>
</tr>
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</table>

Diagnostic Products Corp., 5700 West 96th St., Los Angeles, CA 90045.

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from normal adults and assayed by radioimmunoassay, both before and after suppression by treatment with thyroid hormones, provide evidence that the true median value for circulating thyrotropin is barely 1 milli-int. unit/L \( (21) \).

Clinical Significance

From studies performed with the research assays, a great deal has been learned as a result of optimizing for sensitivity and specificity, thanks to the superior delineation of the normal reference interval that this entails. Thus, we now know that thyrotropin exhibits a circadian rhythm, with lower values in the morning and higher values near midnight \( (15, 22) \). We know more exactly the pattern exhibited by thyrotropin during pregnancy \( (16, 23) \). We know also that there are significant age- and sex-related variations, with an increased prevalence of moderately increased thyrotropin concentrations in ostensibly normal elderly women \( (24-26) \). The very concept of "subclinical hypothyroidism"—a condition characterized by "normal" concentrations of thyroid hormones in the presence of an increased thyrotropin concentration—had to await the development of fully optimized radioimmunoassays for thyrotropin \( (27) \).

Of even greater clinical significance is the fact that improvements in thyrotropin methodology have reduced the need for TRF tests to clarify borderline results. Optimizing a thyrotropin assay not only tends to lower values throughout the reference interval for normal, it also tends to improve the precision and discrimination that can be achieved in this region, rendering more meaningful the comparison of basal values with those measured after TRF stimulation \( (14, 28, 29) \). Indeed, Bigos et al. \( (30) \) showed excellent correlation between such values, as measured with one of the research assays. Wide and Dahlberg \( (29) \) also showed improved correlation when steps were taken to optimize their thyrotropin assay for low-end sensitivity. Furthermore, although perfect discrimination of hyperthyroid samples is not yet possible, in well-optimized thyrotropin assays a basal value exceeding the median for normal persons renders a diagnosis of hyperthyroidism highly unlikely \( (31) \).

Practical Considerations

Three major practical considerations bear on the interpretation and annotation of thyrotropin results at the stage of reporting them to physicians.

First, reference intervals for thyrotropin that are derived from blood donors or laboratory volunteers are clearly inappropriate for newborns and may be inappropriate for certain other groups as well. In particular, they should not be blindly adopted for the interpretation of results on samples from a maternity ward \( (16, 23) \) or a geriatric unit \( (25, 26) \). Thus, depending on the reference group, 3 or 4 milli-int. units/L may not represent the true upper limit of normal. Accordingly, each laboratory should establish by experiment its own expected values \( (32) \).

Moreover, clinical decision limits should respect the distribution of values and disease in the local patient population. Decision limits designed to optimize the predictive value of a test may not correspond to conventional reference interval limits based on 95% coverage for normal individuals. Use of the thyrotropin assay as a primary screen for congenital hypothyroidism provides a classic illustration of this point \( (33) \).

Finally, one must remember that in some contexts a thyrotropin result that is only slightly above the reference interval for normal individuals may not by itself warrant taking any corrective action \( (27) \).

In deciding whether to accept or reject a thyrotropin kit, an important criterion at the stage of method evaluation ought to be its ability to yield values for a normal adult population that are similar to values found by well-established, fully optimized reference methods for thyrotropin \( (1) \). The true upper limit of normal for young men and nonpregnant women in good health is close to 3 or 4 milli-int. units/L, according to the literature summarized in this report.

References

8. Wood WG. A "same day" TSH radioimmunoassay kit with acceptable precision and accuracy. Nucl Compact 11, 60-63 (1980).
Preparation of Urine Samples for Liquid-Chromatographic Determination of Catecholamines: Bonded-Phase Phenylboronic Acid, Cation-Exchange Resin, and Alumina Adsorbents Compared

Alan H. B. Wu and Terrie G. Gornet

We compared results for the liquid-chromatographic determination of free norepinephrine and epinephrine in urine after purifying the catechols by the following methods: (a) acid-washed alumina, (b) weak cation-exchange resin (WCX), (c) a combination of weak cation-exchange resin followed by alumina (WCX-alumina), and (d) commercially available phenylboronic acid adsorbent. We evaluated analytical specificity, sensitivity, recovery, and turnaround time. The WCX-alumina combination produced the most sensitive and specific chromatograms for urinary catecholamines; the other methods took less processing time. Neither WCX nor alumina alone was suitable for routine work because of chromatographic interferences in a significant proportion of urines. The phenylboronic acid method is adequately sensitive and specific for norepinephrine and epinephrine, and samples can be assayed faster. Thus, it provides a compromise between the high analytical performance of the WCX-alumina method and the speed of the WCX and alumina methods.

Additional Keyphrases: norepinephrine, epinephrine, cancer, pheochromocytoma, neuroblastoma, screening

"High-pressure" liquid chromatography is widely used for measuring total and free urinary catecholamines with either electrochemical or fluorometric detectors. In most electrochemical methods, the amines are isolated by a two-step procedure before injection into the chromatograph. Materials used for this include alumina, cation-exchange resins, and Sephadex (1-4). With each of these techniques the catecholamines are adequately separated from other urine components, but each is time consuming.

Boric acid gels, which adsorb compounds containing cis-diol groups, have been used recently to isolate urinary catecholamines (2, 5). Although these gels are highly specific towards catecholamines, they cannot be connected to a source of low pressure for rapid isolation and elution.

We evaluated a simultaneous dual-step purification procedure involving the use of chemically bonded materials (6) (ion-exchange and phenylboronic acid adsorbents: Bond Elut; Analytichem International, Harbor City, CA 90710) for rapidly isolating free catecholamines and compared results by this method with results obtained on using weak cation-exchange resin and alumina.

Materials and Methods

Standards and reagents. Norepinephrine (NE), epinephrine (E), dopamine, and the internal standard, 3,4-dihydroxybenzylamine, were from Sigma Chemical Co., St. Louis, MO 63178, as were all of the compounds in the interference study.1 Acid-washed alumina (Al2O3), prepared by the method of Anton and Sayre (7), was from Bioanalytical Systems, West Lafayette, IN 47905. The weak cation-

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1 Nonstandard abbreviations: NE, norepinephrine; E, epinephrine; WCX, weak cation-exchange; PBA, phenylboronic acid; PSA, primary and secondary amine ion-exchange.

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