RAPID RADIOIMMUNOASSAY OF TRIIODOTHYRONINE ON SEPHADEX G-25 BY THE AMES KIT

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A rapid kit method for the determination of serum triiodothyronine levels is described. The method was satisfactory in cases of thyrotoxicosis, but its lower sensitivity in euthyroid patients gave higher levels (range 2.3–3.3 nM/liter) than normally found. The kit would be of value as a rapid method (little over 3 hr) to screen patients suspected of early or T_3 thyrotoxicosis.

The direct radioimmunoassay of triiodothyronine (T_3) in unextracted serum is recognized as a valuable and established thyroid function test. One technique uses Sephadex columns (Pharmacia Fine Chemicals, Sweden) to separate the iodothyronine from plasma (*1*). The adsorbed T_3 and thyroxine (T_4) are eluted from the Sephadex and the final radioimmunoassay is carried out in a liquid phase. The Ames Company (Miles Laboratory, Elkhart, Indiana) has developed a simpler, but less sensitive variant in which the entire procedure is carried out at room temperature on disposable Sephadex columns. We have compared results for the Ames T_3 kit with those obtained with a modification (*2*) of a standard radioimmunoassay method (*3*).

MATERIALS AND METHODS

Basic principles. The iodothyronines T_3 and T_4 are extracted from serum by "alkaline stripping" (*4*) into 0.1 *M* sodium hydroxide solution enriched with a tracer amount of ^{125}I-T_3, followed by adsorption onto a Sephadex column. Denatured serum proteins are washed off the column with phosphate–EDTA buffer at pH 7.0. A T_3-specific antibody is added to the column and allowed to react with the adsorbed T_3 for at least 2 hr. Antibody-bound T_3 is removed from the Sephadex by a second wash with the phosphate–EDTA buffer. The percent residual ^{125}I-T_3 is measured by direct counting of the Sephadex column.

Ames T_3 assay kit. The kit consists of the following:

1. 20 Sephadex G-25 columns with alkaline buffer, resembling those in the Ames Trilute and Tetralute Kits (*5*);
2. 3.6 gm of 0.1 *M* Na_2HPO_4–EDTA buffer at pH 7.0 (to be made up with 200 ml of distilled water);
3. 2.5 μCi of ^{125}I-T_3 in 0.1 *M* NaOH (12 ml);
4. 6 ng of T_3 standard, lyophilized in bovine albumin (to be made up with 2 ml of distilled water);
5. 30 mg of lyophilized rabbit antibody to T_3 (to be made up with 15 ml of phosphate–EDTA buffer). Ames states without evidence that the antibody is specific for T_3.

T_3 reference method (*2*). Total serum T_3 was measured on unextracted serum using 8-anilino-1-naphthalene sulfonic acid (ANS) to block T_3 binding to thyroxine-binding globulin. Cross reactivity with T_4 is 0.1% and with mono- and diiodotyrosine is less than 0.1%. The within-batch variation coefficient is 6.4% and that between batches is 8.5%. The first incubation for the assay lasts 1.5 hr; it is completed in the afternoon and counting can be done overnight. The normal range of 0.9–3.0 n*M*/liter for total serum T_3 represents 90% confidence limits derived from the cumulative distribution function, which agreed closely with that derived from the ln values for T_3 (in normal subjects the distribution of serum T_3 is log-normal).

Clinical evaluation. Sera were obtained from 15 euthyroid, 14 hyperthyroid, and 5 hypothyroid patients, and also from 17 pregnant euthyroid women and 6 euthyroid women taking oral contraceptive

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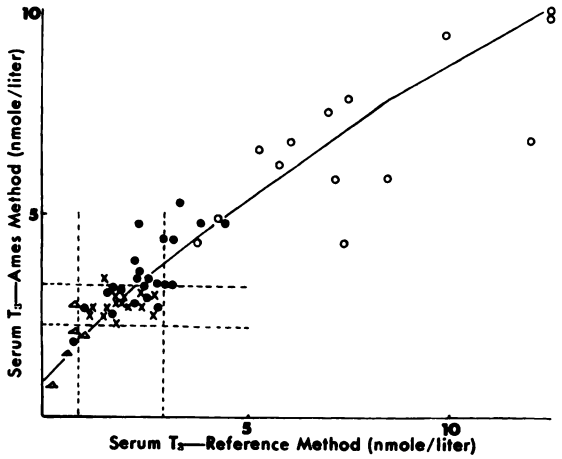

FIG. 1. Results of total serum T_3 assay by Ames kit plotted against those by reference radioimmunoassay method. Line shown is that predicted by analysis of variance for second-degree polynomial. Normal ranges are indicated by dotted lines. Open circles, thyrotoxic subjects; crosses, normal euthyroid subjects; closed circles, euthyroid subjects with raised TBG; triangles, hypothyroid subjects.

tablets. The thyroid status of the patients was established clinically using such tests as the serum T_4/T_3-uptake ratio (*6*) and, in some, serum thyroid-stimulating hormone and pertechnetate scanning.

RESULTS

The mean determinations of total serum T_3 for the 57 patients are shown in Fig. 1. Analysis for polynomial regression (BMD05R) gave a linear correlation coefficient of $r = 0.922$; the line in the figure is for a second-degree polynomial: intercept: —0.826; regression coefficients ± standard error: (1) 0.861 ± 0.319, (2) 0.0439 ± 0.0282.

The 95% confidence range for the Ames kit was calculated as the mean ± 2 s.d. In the 15 euthyroid patients it was 2.8 ± 0.52 n*M*/liter; in the 14 hyperthyroid patients it was 7.0 ± 3.75 n*M*/liter; and in women with raised thyroxine-binding globulin (TBG) it was 3.5 ± 1.6 n*M*/liter. The working precision of the T_3 kit was assessed by analysis of the differences in duplicates. The within-batch variation coefficient was 4.3% and that between batches was 7.5%. The standard curve was linear over the range 1–9 n*M*/liter.

DISCUSSION

The biggest difference between the two methods was in the results for the euthyroid patients, regardless of raised TBG, i.e., the normal range with the Ames kit (2.3–3.3 n*M*/liter) is higher than those found with more sensitive radioimmunoassay tests (*1,3,7*). The mean result in euthyroid patients was 29% higher with the Ames kit than with the reference method. The means in hyperthyroid patients, however, were 11.5% lower with the kit ($t = 0.98$, not significant). The mean T_3 level in euthyroid women with raised TBG was about 1 s.d. above the mean level in normal euthyroid patients, as has been noted previously (*7,8*). The Ames kit was able to discriminate adequately between hyperthyroid patients and euthyroid patients with normal TBG.

If six Sephadex columns are used to plot the standard curve, a batch of seven sera can be analyzed in duplicate in about 3 hr. Thus, the Ames kit could be usefully employed as a rapid screening test for patients suspected of early or T_3 thyrotoxicosis, provided that the serum TBG is not raised. The kit is not suitable for T_3 determination in hypothyroidism or for screening for hyperthyroidism in women with raised TBG. Patients having a raised T_3 according to the Ames kit (above 3.5 n*M*/liter) should then be given a more sensitive radioimmunoassay test.

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