A SINGLE METHOD FOR MEASURING TOTAL THYROXINE AND FREE THYROXINE INDEX IN SERUM

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A simple, two-stage modification of a competitive protein-binding technique for measuring T_4(D) is described which permits measurement of both T_4(D) and, indirectly, free T_4 (FT_4). The method employs small Sephadex columns and ^{125}I-T_4 as described previously (JCEM 32: 487, 1971) and a final elution of the column with 0.02 ml of the patient's serum. In 68 euthyroid subjects, values for T_4(D) were 7.0 ± 1.6 μg% (mean ± s.d.) and for indirect FT_4, 0.80 ± 0.18; in 21 pregnant patients T_4(D), 10.8 ± 1.9 μg% and indirect FT_4, 0.80 ± 0.19; in 9 euthyroid subjects with low or absent TBG T_4(D), 2.9 ± 0.8 μg% and indirect FT_4, 0.91 ± 0.17; in 22 hypothyroid patients T_4(D), 1.7 ± 0.7 μg% and indirect FT_4, 0.28 ± 0.09; in 24 hyperthyroid T_4(D), 20.9 ± 5.5 μg% and indirect FT_4, 3.04 ± 0.75. The indirect measurement of FT_4 is similar to the T_4-RT_3 index but more accurately reflected thyroid status in this series. The present method is rapid, accurate, and reproducible.

It is generally agreed that the single most useful test of thyroid function is the serum concentration of free or nonprotein-bound thyroxine (FT_4) (*1*)*. This test, in contrast to the total serum T_4 concentration and methods which measure the percent free T_4 (% FT_4) in serum, remains normal in euthyroid individuals in spite of abnormalities in the T_4 binding affinity of the serum-binding proteins, especially the thyroxine-binding globulin (TBG) (*2*). The FT_4 is calculated as the product of the total serum T_4 concentration and the % FT_4 measured by prolonged dialysis of ^{131}I- or ^{125}I-labeled T_4 enriched serum. Since the latter technique is not readily available in most laboratories, an indirect measurement of percent FT_4 by the resin triiodothyronine uptake test (RT_3U) has been substituted and the thyroxine-resin T_3 index (T_4-RT_3 index) used as an indirect measurement of FT_4. More recently, we and others, have described single procedures for indirectly measuring FT_4, but none of these methods allow for a quantitative assessment of total serum T_4 concentration (*3–6*).

The present report describes a method which combines the previously described methods for directly measuring serum T_4 concentration by isotopic displacement T_4(D) (*7*) and indirectly measuring FT_4 (*6*) into a single two-step procedure for determining both parameters of thyroid function.

MATERIALS AND METHODS

Test procedure. All reactions are carried out in small plastic columns containing Sephadex G-25 equilibrated with 0.1 *N* NaOH providing a pH greater than 11. The direct measurement of T_4(D) using ^{125}I-labeled T_4 is then carried out as described previously, unknown sera being compared with a standard curve (*7*)*. After completing this method which requires two determinations of radioactivity on the column (initial or pre-elution and second or post-elution), the bottom of the column is capped. A small quantity of the patient's serum (0.02 ml) and 0.5 ml of barbital buffer, pH 8.6 [same buffer used in measuring T_4(D)] is then added to the same

* Nomenclature for tests of thyroid hormones in serum is adopted from a committee report of the American Thyroid Association (*1*).

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* Tetralute and Trilute kits kindly supplied by Morton Alpert, Ames Co., Division Miles Laboratory, Inc., Elkhart, Ind.

column used for the patient's T_4(D) determination, gently swirled, and allowed to drain for 3 min. Thus, the quantity of T_4 remaining on the column which is eluted will be directly proportional to the T_4 binding affinity of the patient's TBG. The column is then washed with 4-ml buffer, recapped, and counted again. This final count divided by the initial count gives the percent retention of ^{125}I-T_4 and is compared with either a concurrently run normal serum pool or with the 5 μg% T_4 standard used in the standard curve for measuring T_4(D). The indirect measurement of FT_4 is then calculated by dividing the final percent retention of the patient's serum by the final percent retention of the normal serum pool or standard. The total time required to run 22 serum samples for both T_4(D) and indirectly FT_4 is approximately 90 min.

The principle of the test is outlined in the following example. In pregnant patients or subjects receiving estrogen, serum TBG is increased, resulting in an elevated total serum T_4 which is measured in the first part of the present method. A greater quantity of ^{125}I-T_4 remains on the column (second count). Since the patient's TBG is increased, when a small quantity of serum is then added to the column, more of the retained ^{125}I-T_4 will be eluted than would occur if TBG were normal. Thus, the final column count will be similar to that obtained in a normal serum in which T_4(D) is normal as reflected in fewer counts on the column before the final elution, with less being then eluted since TBG is normal. In contrast, in thyrotoxicosis, T_4(D) is also elevated, but since TBG is either normal or slightly low, far less of the ^{125}I-T_4 retained on the column is eluted in the final step and the indirect FT_4 is elevated.

All tests were run in duplicate, and results of the indirect FT_4 were compared with those obtained for the T_4-RT_3 index calculated as the product of T_4(D) and RT_3U ratio (Trilute). Since the present method for measuring T_4(D) is not as accurate when the standard curve is above 16.5 μg%, those thyrotoxic sera with values above this limit were rerun for T_4(D), using 0.05 ml rather than 0.1 ml serum and an appropriate correction made for the smaller volume of serum used.

Clinical evaluation. Sera were obtained from 68 (25 male and 43 female) clinically evaluated euthyroid healthy or hospitalized subjects, 24 hyper- and 22 hypothyroid patients, 21 pregnant women, 3 euthyroid females receiving estrogen, 9 euthyroid subjects with hereditary absence of or low TBG_{cap}, and 3 females receiving replacement or suppressive therapy with 0.2 to 0.3 mg L-T_4.

RESULTS

Serum T_4(D) concentration. Values for T_4(D) concentration in the euthyroid subjects averaged 7.0 ± 1.6 μg% (mean ± s.d.) with a range of 4.6–11.1 μg% (Table 1). All values for serum T_4(D) were elevated in the hyperthyroid subjects (20.9 ± 5.5 μg%) and decreased in the hypothyroid patients (1.7 ± 0.7 μg%). Values for T_4(D) in the pregnant patients (10.8 ± 1.9 μg%) were significantly increased when compared with the euthyroid subjects ($p < 0.01$) and were above the normal range in 9 of the 21 patients. In the three estrogen-treated subjects, serum T_4 was in the high normal range. In all nine subjects with absent or low serum TBG_{cap}, serum T_4(D) concentration was decreased, averaging 2.9 ± 0.8 μg%. In the three subjects receiving

TABLE 1. VALUES FOR THYROID FUNCTION TESTS IN NORMAL SUBJECTS, PATIENTS WITH THYROID DYSFUNCTION, AND PATIENTS WITH ABNORMALITIES IN SERUM THYROXINE BINDING

Group	No. subjects	T_4(D) (μg%)	RT_3U (%)	T_4-RT_3 Index	Indirect FT_4
Euthyroid	68	7.0 ± 1.6* (4.6–11.1)†	48.4 ± 6.0 (26.9–60.2)	6.7 ± 1.5 (4.8–10.9)	0.80 ± 0.18 (0.54–1.31)
Hyperthyroid	24	20.9 ± 5.5 (11.9–31.6)	69.2 ± 8.8 (50.2–87.2)	30.1 ± 9.3 (15.1–51.7)	3.04 ± 0.75 (1.98–4.83)
Hypothyroid	22	1.7 ± 0.7 (0.8–2.9)	34.2 ± 5.5 (21.4–43.1)	1.1 ± 0.5 (0.6–2.0)	0.28 ± 0.09 (0.12–0.43)
Pregnant	21	10.8 ± 1.9 (6.8–14.4)	24.3 ± 4.7 (16.9–34.4)	5.4 ± 1.3 (3.3–8.8)	0.80 ± 0.19 (0.49–1.13)
Estrogen	3	10.6 ± 0.6 (9.9–11.1)	32.5 ± 0.9 (32.0–33.6)	7.2 ± 0.6 (6.6–7.8)	0.99 ± 0.05 (0.94–1.06)
Absent or low TBG	9	2.9 ± 0.8 (1.7–4.1)	86.5 ± 11.4 (62.8–95.9)	4.8 ± 1.2 (2.8–6.7)	0.91 ± 0.17 (0.63–1.17)
L-T_4 therapy	3	11.2 ± 0.4 (10.9–11.7)	59.2 ± 6.2 (52.6–65.0)	13.8 ± 1.7 (11.9–15.0)	1.82 ± 0.08 (1.73–1.89)

* Mean ± s.d.
† Range.

exogenous L-T_4 therapy, serum T_4(D) was either slightly elevated or at the upper limit of normal range.

T_4-RT_3 index. As expected, the RT_3U was increased in most of the hyperthyroid patients (69.2 ± 8.8%), all the subjects with absent or low serum TBG (86.5 ± 11.4%), and in two of the three subjects receiving exogenous L-T_4 therapy and decreased in most of the hypothyroid patients (34.2 ± 5.5%) and in all the pregnant subjects (24.3 ± 4.7%) and patients receiving estrogen therapy (32.5 ± 0.9%). The T_4-RT_3 index averaged 6.7 ± 1.5 in the euthyroid subjects with a range of 4.8–10.9. The index was within the normal range in the 3 subjects receiving estrogen, in 15 of the 21 pregnant subjects, in 3 of the 5 subjects with absent TBG_{cap}, and in 2 of the 4 subjects with low TBG_{cap}. The abnormal values for the T_4-RT_3 index in these ten subjects were slightly decreased but not as low as those found in hypothyroidism. Values were elevated in all hyperthyroid patients (30.1 ± 9.3) and decreased in all hypothyroid patients (1.1 ± 0.5). The T_4-RT_3 index was slightly elevated in the three subjects receiving exogenous L-T_4.

Indirect FT_4. Values obtained in the euthyroid subjects averaged 0.80 ± 0.18 with a range of 0.54–1.31. The indirect FT_4 was within the normal range in the 3 subjects receiving estrogen (0.99 ± 0.05), in 19 of the 21 pregnant subjects (0.80 ± 0.19), and in all 9 subjects with low or absent TBG_{cap} (0.91 ± 0.17). Indirect FT_4 was elevated in all hyperthyroid patients (3.04 ± 0.75), decreased in all hypothyroid patients (0.28 ± 0.09), and slightly increased in the three subjects receiving exogenous L-T_4. Comparison of the T_4-RT_3 index and the indirect FT_4 in individual patients revealed a highly significant correlation coefficient ($r = 0.95$, $p < 0.001$).

DISCUSSION

The present method for measuring serum T_4(D) and indirectly FT_4 is rapid, reproducible, requires only 0.12 ml serum, and does not require extraction of serum, centrifugation, use of a rotary mixer, or dialysis. All steps are carried out within a small Sephadex column and only requires pipetting and counting of the columns for ^{125}I. Other single methods for indirectly measuring serum FT_4 have been described but do not have the advantage of permitting a direct measurement of total serum T_4 concentration (*3–6*). The present method for indirectly assessing FT_4 also eliminates the necessity of carrying out two separate tests as is required in calculating the T_4-FT_3 index. Finally, there is a close correlation between the indirect FT_4 and the T_4-RT_3 index but fewer abnormal values in euthyroid patients with serum TBG abnormalities occurred by the present method.

ADDENDUM

At the completion of the present study, a similar method was described in abstract form (*8*).

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REFERENCES

1. SOLOMON DH, BENOTTI J, DEGROOT LJ, et al: A nomenclature for tests of thyroid hormones in serum: Report of a committee of the American Thyroid Association. *J Clin Endocr* 34: 884–890, 1972

2. OPPENHEIMER JH: Role of plasma proteins in the binding, distribution and metabolism of the thyroid hormones. *New Eng J Med* 278: 1153–1162, 1968

3. MINCEY EK, THORSON SC, BROWN JL: A new in vitro blood test for determining thyroid status—the effective thyroxine ratio. *Clin Biochem* 4: 286–291, 1971

4. ASHKAR FS, BEZJIAN AA: Use of the normalized serum thyroxine (T_4N). A new approach to thyroid hormone measurement. *JAMA* 221: 1483–1485, 1972

5. Free Thyroxine by TBG Compensated Competitive Protein Binding (Isotopic.) Curtis Nuclear Corp

6. ABREAU CM, AZIZI F, VAGENAKIS AG, et al: Clinical evaluation of a new indirect indicator of serum-free thyroxine concentration. *J Nucl Med* 14: 159–163, 1973

7. BRAVERMAN LE, VAGENAKIS AG, FOSTER AE, et al: Evaluation of a simplified technique for the specific measurement of serum thyroxine concentration. *J Clin Endocr* 32: 497–502, 1971

8. SUNG LC, CAVALIERI RR: A method for determining both total serum thyroxine and free thyroxine index in a single procedure. *Clin Res* 21: 205, 1973