IN VITRO NUCLEAR MEDICINE

Clinical Assessment of a Radioimmunoassay for Free Thyroxine Using a Modified Tracer

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A radioimmunoassay for measuring free thyroxine in plasma was introduced by Amersham using a I-125-labeled T_4 derivative that does not bind significantly to the thyroxine-binding proteins. We evaluated this RIA for its clinical utility in assessing 278 patients with thyroid and nonthyroidal diseases. The precision of the Amerlex free T_4 assay, expressed as coefficient of variation, was 20% at 0.16 ng/dl, 6.9% at 0.55 ng/dl, 4.2% at 1.08 ng/dl, 5.3% at 2.29 ng/dl, and 6.3% at 3.18 ng/dl. A reference range for free T_4 was established as 0.68–1.8 ng/dl, n = 171. The correlation coefficients (r) of a dialysis method and a free thyroxine index were 0.871 and 0.911, respectively. Free T_4 correctly classified 98% euthyroid, 92% hypothyroid, 100% hyperthyroid, 100% euthyroid with elevated TBG, and 87% of phenytoin patients. In addition, 80 patients with acute nonthyroidal illness were studied. Most of these patients have normal to low free T_4, very low T_3, and elevated rT_3. We found this free T_4 assay to be precise, easy to perform, and reliable in classifying thyroid status in most patients.

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In human plasma there exists a dynamic equilibrium between thyroxine (T_4) in the free state, thyroxine in the bound state, and three transporting proteins: thyroxine-binding globulin (TBG), thyroxine binding prealbumin (TBPA), and albumin (Alb) (*1*). The corresponding affinity constant of T_4 to TBG is 2×10^{10}/M, to TBPA is 2×10^8/M, and to albumin is 2×10^6/M (*2*). In normal healthy euthyroid subjects, only 0.03% of serum total thyroxine is "free" or not protein bound (*3*). The proportion of free T_4 varies inversely with the binding affinity and concentration of unoccupied protein binding sites, principally TBG and to a lesser extent TBPA. While it represents a small percentage of the total, it is the free fraction of T_4 that can enter the cell and bind to the intracellular receptor leading to protein synthesis and thyroid hormone action (*4*). Therefore, the concentration of free T_4 best reflects the thyroid status of the patient.

Traditionally, free T_4 has been measured by equilibrium dialysis, which is time-consuming, technically somewhat demanding, and not widely used in routine clinical laboratories. Several dialysis methods have been published (*5–9*), differing in the sample dilution and the method for eliminating contaminants before and after dialysis. The free-T_4 concentration measured by these procedures appeared to give different results (*10*). Nevertheless, equilibrium dialysis has been considered the reference method for free-T_4 measurement.

A free thyroxine index, the product of a total thyroxine (T_4) and a tri-iodothyronine resin uptake (T_3RU) (FTI = $T_4 \times T_3$ uptake), is often used as a substitute for the direct measurement of free T_4. However, many extrathyroidal factors such as drugs, hormones, genetic factors, pregnancy, and hepatic and renal diseases may influence the expected value for T_3RU (*11*). Recently, several investigators suggested that a free-thyroxine

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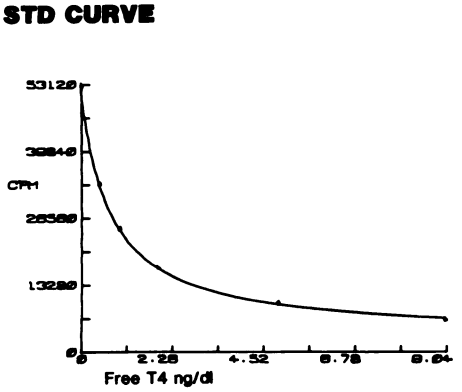

FIG. 1. Standard curve of Amerlex free T_4.

index using a TBG assay (FTI-TBG = T_4/TBG) is superior in some respects to the FTI-T_3RU (*12–14*). However, Szpunar et al. reported abnormal FTI-TBG results in a group of clinically euthyroid patients (*15*).

Commercial RIAs for the estimation of free T_4 include Corning Immuno Phase free T_4 RIA (*16*), Damon microencapsulated antibody free T_4 assay (*17*), and the Clinical Assays' Gamma coat free T_4 RIA (*18*). Controversies concerning these three assays were reported from various laboratories (*19–25*). Recently, a free T_4 RIA using a I-125-labeled T_4 derivative was introduced by Amersham as Amerlex free T_4. Since the derivatized T_4 does not bind significantly to the natural binding proteins, the perturbation of the equilibrium between free and bound T_4 is minimal. A high-affinity antibody is used in small amounts, which binds both T_4 and the T_4 derivative to provide good sensitivity.

We evaluated this method for its analytical performance and its clinical utility in the assessment of patients with hypothyroidism, euthyroidism, hyperthyroidism, abnormally elevated binding proteins, phenytoin treatment, and acute nonthyroidal illnesses. The results were also compared with conventional thyroid function tests, e.g., T_4, T_3RU, T_3, TSH, and rT_3.

MATERIALS AND METHODS

Samples for the determination of reference values were collected from 80 normal healthy volunteers during pre-employment screening, and from the 91 euthyroid patients in group 1 (see below). Six groups of patients, with a total of 278, were studied. Group 1 consisted of 92 euthyroid patients who were seen in the clinic or admitted to the hospital for nonthyroidal illness or illness not known to influence thyroid function tests. They had no evidence of thyroid disorder by physical examination and laboratory evaluation. Group 2 included 23 patients with typical manifestations of hyperthyroidism, and the diagnosis was further established by elevated serum T_4 and T_3, and Tc-99m uptake by the thyroid. They had no severe complications from thyrotoxicosis. Group 3 was comprised of 26 hypothyroid patients whose clinical diagnosis was further documented by a low or borderline-low serum FTI and high TSH. All of them were judged to have primary hypothyroidism. Group 4 was comprised of 80 patients who were admitted to intensive care unit of our hospital because of serious illnesses such as sepsis, shock, severe renal, hepatic, or cardiac failure. They were usually receiving several drugs while studied. Any patients with known history of thyroid or pituitary disease were not included in this study. Patients who had received drugs that are known to influence thyroid function were also excluded from this group. Group 5 consisted of 20 individuals with elevated TBG as a result of estrogen therapy. Group 6 consisted of 37 epileptic patients, treated with phenytoin, and 10 control patients who were treated with other antiepileptic drugs.

TABLE 1. PRECISION OF THE AMERLEX FREE T_4

	N	Mean ng/dl	s.d.	CV
Hypothyroid	30	0.16	0.031	20%
Hypothyroid	22	0.55	0.038	6.9%
Euthyroid	30	1.08	0.045	4.2%
Hyperthyroid	30	2.29	0.121	5.3%
Hyperthyroid	23	3.18	0.20	6.3%

The methods used in this study were: T_4 RIA*, T_3 Resin uptake†, TSH‡, T_3 RIA§, reverse T_3¶, TBG RIA‡, Free T_4‖, and Free T_4 dialysis.** The reference ranges as determined by our laboratory were: T_4 RIA = 5–12 μg/dl, T_3 RU = 0.8–1.2, FTI = 5–12, TSH = 0–7.0 mIU/L, T_3 RIA = 90–200 ng/dl, rT_3 = 80–350 ng/l, free T_4 = 0.68–1.8 ng/dl (by Amerlex), free T_4 = 1.0–2.3 ng/dl (by dialysis), and TBG RIA = 13–30 mg/l.

RESULTS

Analytical assessment of Amerlex free T_4. The characteristic of the standard curve is shown in Fig. 1, derived from a data-reduction program fitting three parameters. A large signal difference, 54.8%, was generated between the maximal binding of 62% at B_0 and 7.5% at the highest standard (9 ng/dl). The minimum detectable concentration of free T_4 was less than 0.1 ng/dl as calculated from two standard deviations and the mean from 20 determinations of the zero standard. The reproducibility of the assay is summarized in Table 1. This was done by analyzing three patient pools and two controls with every run. All determinations were done in duplicate. The coefficients of variation (CV) for the hypothyroid range were 20% at 0.16 ng/dl and 6.9% at 0.55 ng/dl; for the euthyroid range 4.2% at 1.08 ng/dl; for the hyperthyroid range 5.3% at 2.29 ng/dl and 6.3% at 3.18 ng/dl.

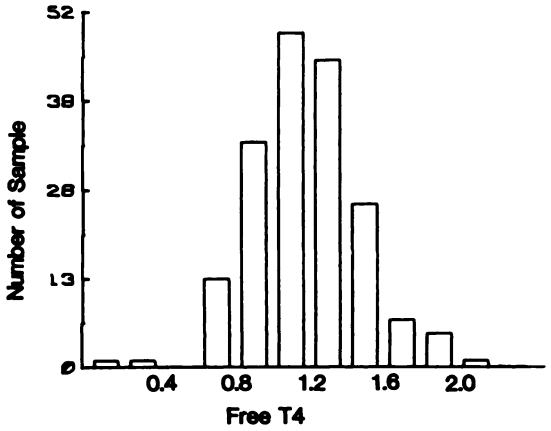

FIG. 2. The "normal" distribution of free T_4.

Reference range. The reference values for free T_4 determined by the Amerlex method were done on samples from defined euthyroid patients and normal subjects during pre-employment screening for syphilis, the values being calculated by the mean and two standard deviations. The range was 0.68–1.8 ng/dl, with a mean of 1.20 ng/dl on 171 samples. The distribution of free T_4 is shown in Fig. 2.

Method correlation. The correlation of Amerlex free T_4 with equilibrium dialysis for free T_4 was done on 75 patients including hypothyroid, euthyroid, hyperthyroid, and patients on phenytoin (Fig. 3). The coefficient of correlation was 0.871. The least-squares analysis gave a slope of 0.74 and an intercept at 0.023 ng/dl. For patients with nonthyroidal illness, the comparison of free T_4 by Amerlex and by equilibrium dialysis showed a coefficient of correlation of 0.489, a slope of 0.35, and an intercept at 0.19 (Fig. 4).

The correlation of Amerlex free T_4 with free T_4 index on 186 patient samples showed a coefficient of correlation of 0.911 (Fig. 5). In calculating the coefficient of correlation (r value), results were excluded if they exceeded the highest standard of one or both assay procedures. The correlation of 76 patients with nonthyroidal illness showed a coefficient of correlation of 0.675 (Fig. 6).

TABLE 2. CLINICAL CORRELATION OF AMERLEX FREE T_4

Patient	Amerlex Free T_4 (%) <0.68 ng/dl	0.68–1.80 ng/dl	>1.80 ng/dl	Total number
Hypothyroid	92%	8%	0	26
Euthyroid	1%	98%	1%	92
Hyperthyroid	0	0	100%	23
Contraceptives	0	100%	0	20
Phenytoin	13%	87%	0	37

Clinical correlation. Amerlex free T_4 correctly classified 92% of hypothyroid patients, 98% euthyroid patients, 100% hyperthyroid patients, 100% of patients on contraceptive, and 87% of phenytoin patients (Table 2). The euthyroid patients taking contraceptive had a mean TBG concentration of 39.8 mg/l (reference range 13–30 mg/l). The mean free T_4 was 1.01 ng/dl with a standard deviation (s.d.) of 0.19 and a range of 0.67 to 1.32 ng/dl. Patients treated with phenytoin had serum phenytoin concentrations of 5 to 224 mg/l. Their mean level of free T_4, 0.94 ± s.d. 0.26 ng/dl, did not differ statistically (by t-test) from the 0.97 ± 0.21 ng/dl for the ten control patients treated with other antiepileptic drugs.

For comparison, the FTI-RIA correctly classified all hypothyroid, euthyroid, and hyperthyroid patients, all patients on contraceptive, and 68% of patients on phenytoin (Table 3). On the other hand, the dialysis free T_4 correctly classified 90% hypothyroid, 92% euthyroid, and all hyperthyroid patients and patients on phenytoin (Table 4).

Patients with nonthyroidal illness (NTI) were separated into three groups for comparison: $T_4 > 5$ μg/dl, T_4 between 3–5 μg/dl, and $T_4 < 3$ μg/dl. The Amerlex free T_4 showed 91% in the euthyroid range for $T_4 > 5$ μg/dl,

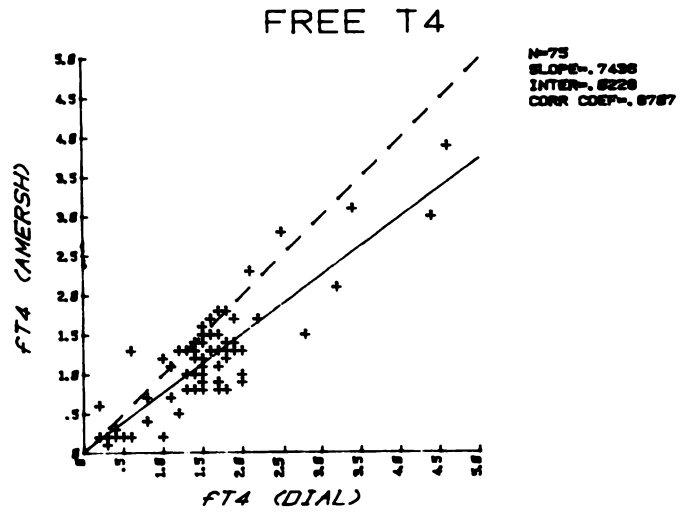

FIG. 3. Patient correlation between Amerlex free T_4 and equilibrium dialysis free T_4. Amerlex = 0.74 (dialysis) + 0.02, n = 75, r = 0.871.

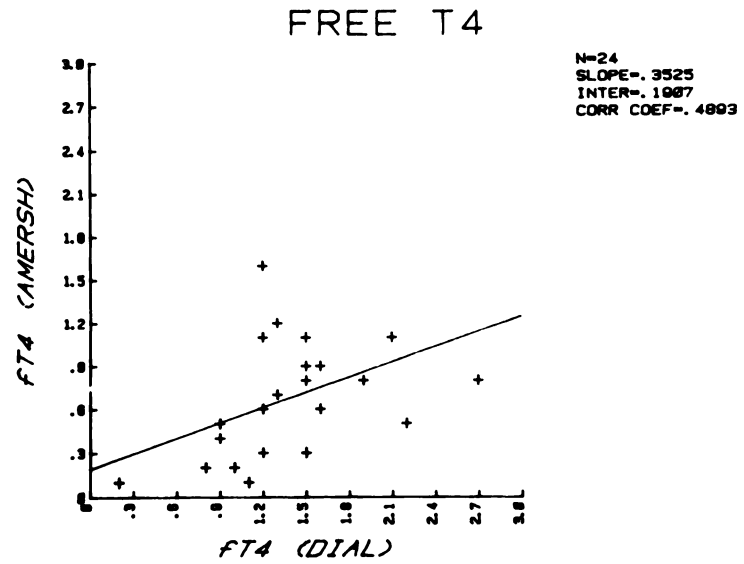

FIG. 4. NTI patient correlation between Amerlex free T_4 and equilibrium dialysis free T_4. Amerlex = 0.35 (dialysis) + 0.19, n = 24, r = 0.489.

72% in the euthyroid range for T_4 between 3–5 μg/dl, and 100% in the hypothyroid range for T_4 < 3 μg/dl (Table 5). For comparison, TSH concentrations were within the reference range for 41 of 48 samples with T_4 > 5 μg/dl, 12 of 13 samples with T_4 between 3–5 μg/dl, and all 4 samples with T_4 < 3 μg/dl. The mean T_3 concentration was 96 ng/dl for T_4 > 5 μg/dl, 40 ng/dl for T_4 between 3–5 μg/dl, and 15 ng/dl for T_4 < 3 μg/dl. Five patients had no measurable T_3.

The FTI showed 96% in the euthyroid range for T_4 > 5 μg/dl, 89% for T_4 between 3–5 μg/dl, and 100% in the hypothyroid range for T_4 < 3 μg/dl (Table 6). Dialysis free T_4 showed 89% in the euthyroid range for T_4 > 5 μg/dl, 89% in the euthyroid range for T_4 between 3–5 μg/dl, and 76% in the euthyroid range for T_4 < 3 μg/dl (Table 7).

Discussion. The Amerlex free T_4 standards were calibrated by an equilibrium dialysis method that was different from the Bioscience dialysis method used in this study. The reference ranges for Amerlex free T_4 (0.68–1.80 ng/dl) were different from the dialysis method (1.0–2.3 ng/dl). These differences are probably reflected by the slope of the regression line, 0.74. Of the 75 patients, five were discordant between these two methods in classifying patients according to their clinical diagnosis. For the Amerlex free T_4, two hypothyroid patients were classified as euthyroid and a euthyroid patient was classified as hyperthyroid. Dialysis free T_4 misclassified a hypothyroid patient in the euthyroid range and a euthyroid patient in the hyperthyroid range.

The correlation coefficient of the Amerlex FT_4 with the FTI was 0.911. The main area of poor correlation was in the hyperthyroid region of both assays. The relatively low r value probably resulted because both methods were at the upper limit of the useful range of the standard curves (Fig. 1), so that the coefficients of variation are probably higher for both assays. The clinical correlation of both the FT_4 and the FTI was excellent in this region, with both methods correctly

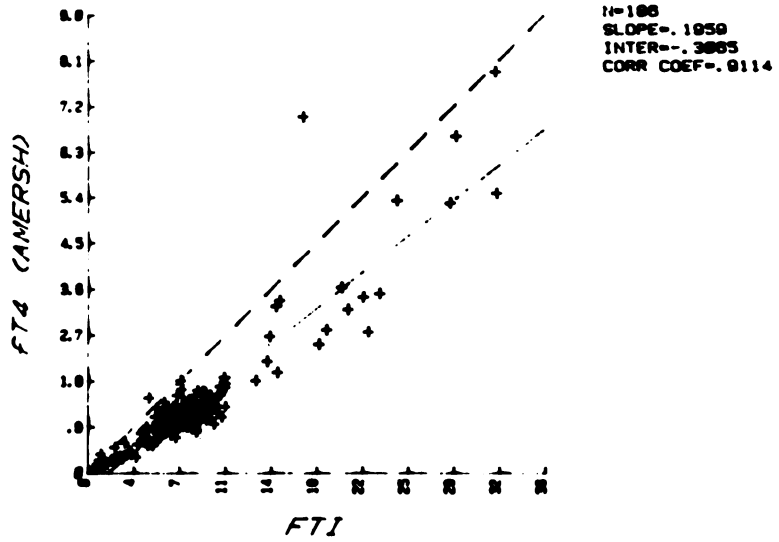

FIG. 5. Correlation of Amerlex free T_4 with free T_4 index for all patients except NTI. Amerlex = 0.19 (FTI) − 0.31, n = 186, r = 0.911.

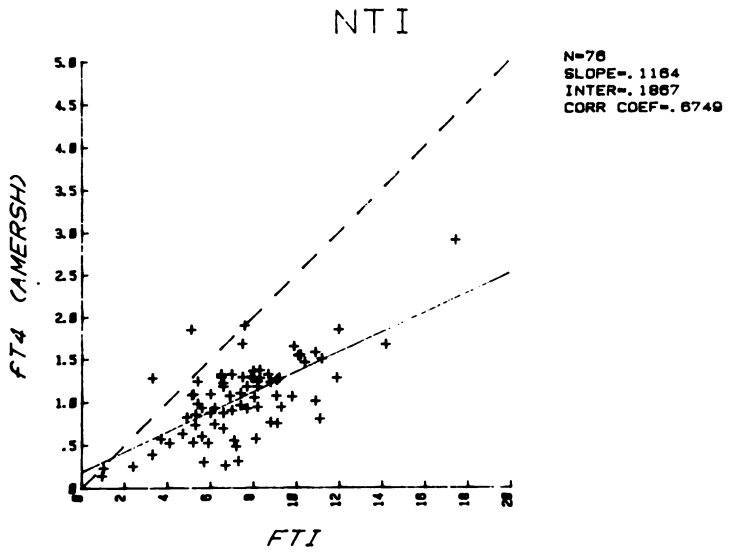

FIG. 6. Correlation of Amerlex free T_4 with free T_4 index for patients with nonthyroidal illness. Amerlex = 0.12 (FTI) + 0.187, n = 76, r = 0.675.

identifying 23/23 clinically hyperthyroid patients (Group 2).

In the euthyroid group of patients (Group 1) both the Amerlex FT_4 and the FTI correlated well with the clinical findings. These procedures correctly classified euthyroidism in 90/92 and 86/86 patients, respectively. Using total T_4 alone, 88/92 patients were correctly classified. However, this euthyroid group did not include those individuals with either increased or decreased TBG. In such patients it was found to be essential that either the FTI or FT_4 be used as a screening procedure (see below).

In the hypothyroid group of patients (Group 3) the Amerlex FT_4 method successfully classified 24/26 patients. The FTI correctly classified all 26. When a patient had a borderline low-normal FTI with an elevated TSH, the classification became dependent on clinical signs or symptoms. Low-normal FTI with an elevated TSH is a finding that in practice may indicate a patient with a limited thyroid reserve, a partially treated hypothyroid patient, or a patient with impending hypothyroidism. Furthermore, there was some overlap of the normal range with the hypothyroid range in both the FT_4 and the FTI. In routine use as a thyroid screen, the lower limit of normal might well be set at a higher value of the FTI, thereby including all patients suspected of hypothyroidism.

In addition to correctly classifying patients with abnormal thyroid function, it is also important that an assay should accurately classify patients with abnormal thyroid parameters due to nonthyroidal factors. We have investigated the use of the Amerlex FT_4 method in patients on phenytoin, patients with severe nonthyroidal illness, and patients with elevated TBG.

It has been reported that the in vivo effect of phenytoin on thyroid economy is to produce lower total T_4, lower free T_4, and normal T_3 (*21*). We have found the free T_4 concentration as measured by the Amerlex method was not statistically different between patients treated with this drug (Group 6) and the control group.

The evalution of thyroid status in patients with critical nonthyroidal illnesses (NTI) is very difficult. In a variety of severe NTI, total T_4 and T_3 are greatly depressed, to the range seen in patients with severe primary hypothyroidism, but TSH is not elevated (*26*). It was suggested that the mechanism may be an abnormality of pituitary and/or hypothalamic TSH regulation (*27*) or defective binding of T_4 to serum proteins (*28*).

We have observed similar decreases in total T_4 and T_3 in patients with severe nonthyroidal illness (Group 4).

TABLE 3. CLINICAL CORRELATION OF FTI

Patient	FTI (%) <5 ng/dl	5–12 ng/dl	>12 ng/dl	Total number
Hypothyroid	100%	0	0	26
Euthyroid	0	100%	0	86
Hyperthyroid	0	0	100%	23
Contraceptives	0	100%	0	20
Phenytoin	24%	68%	8%	38

TABLE 4. CLINICAL CORRELATION OF EQUILIBRIUM DIALYSIS FREE T_4

Patient	Equilibrium dialysis (%) <1.0 ng/dl	1.0–2.3 ng/dl	>2.3 ng/dl	Total number
Hypothyroid	90%	10%	0	20
Euthyroid	0	92%	8%	26
Hyperthyroid	0	0	100%	12
Phenytoin	0	100%	0	17

TABLE 5. CLINICAL CORRELATION OF AMERLEX FREE T_4 IN NTI PATIENTS

NTI patient	Amerlex Free T_4 (%) <0.68 ng/dl	0.68–1.80 ng/dl	>1.80 ng/dl	Total number
$T_4 > 5$ μg/dl	7%	91%	2%	54
T_4 3–5 μg/dl	28%	72%	0	18
$T_4 < 3$ μg/dl	100%	0	0	8
Overall	21%	78%	1%	80

TABLE 6. CLINICAL CORRELATION OF FTI IN NTI PATIENTS

NTI Patient	FTI (%) <5	5–12	>12	Total number
$T_4 > 5$ μg/dl	2%	96%	2%	54
T_4 3–5 μg/dl	11%	89%	0	18
$T_4 < 3$ μg/dl	100%	0	0	8
Overall	14%	85%	1%	80

TABLE 7. CLINICAL CORRELATION OF EQUILIBRIUM DIALYSIS IN NTI PATIENTS

NTI patient	Equilibrium dialysis (%) <1.0 ng/dl	1.0–2.3 ng/dl	>2.3 ng/dl	Total number
$T_4 > 5$ μg/dl	0	89%	11%	9
T_4 3–5 μg/dl	0	89%	11%	9
$T_4 < 3$ μg/dl	57%	43%	0	7
Overall	16%	76%	8%	25

Of the 80 patients, 53 had normal total T_4 (i.e., >5 μg/dl) and 27 had low total T_4 (i.e., <5 μg/dl). Of the 27 patients with subnormal T_4, 18 had T_4 between 3 and 5 μg/dl and nine had T_4 less than 3 μg/dl. The mean total T_3 decreased from low normal in patients with normal T_4 to near zero T_3 in patients with $T_4 < 3$ μg/dl. This latter group of patients consistently came from intensive care units, representing those with the most severe NTI. The reduction of T_4 and T_3 in nonthyroidal illness may represent an attempt by the body to conserve energy at the time of severe illness (*29*). The extremely low T_3 concentration may be due to the inhibition of the conversion of T_4 to T_3 (the more potent thyroid hormone), thereby reducing catabolism. We also found that rT_3 was elevated in most of these patients. This is probably because the same '5-deiodinase that produces T_3 from T_4 is also involved in the metabolism of rT_3 to 3,'-3-di-iodothyronine; inhibition of this enzyme will therefore result in low T_3 and high rT_3.

We have found that the majority of patients with NTI and normal T_4 have normal FT_4. A decrease in both FT_4 and FTI was observed in the group of patients with total $T_4 < 3$ μg/dl. Kaptein et al. (*30*) reported that free T_4 in serum was normal in five and above normal in the remaining five of ten NTI patients who had normal total T_4. They also found that free T_4 was subnormal in two patients, above normal in two, and normal in the remaining five among nine NTI patients with total T_4 less than 3 μg/dl. Chopra et al. (*24*), using a dialysis method, reported significantly elevated free T_4 in NTI patients with normal total T_4. In a group of 11 patients with low total T_4 in Chopra's series, they found free T_4 to be high in six, normal in three, and low in the remaining two. It was proposed that such unexpectedly elevated free T_4 may be due to the presence of a serum inhibitor for thyroid-hormone binding (*28*). An alternative explanation is that these elevated free T_4 values may be due to nonlinearity of the percent dialyzed in the presence of low TBG, as observed by Witherspoon et al. (*31*). This nonlinearity may result in the elevation of the calculated value of free T_4. It is difficult to assess the thyroid status of these patients clinically, especially those with extremely severe illness. Further clinical studies using the TRH stimulation test, and the evaluation of thyroid function during the illness and recovery phases, will enable us to gain additional insight into thyroid function in patients with severe NTI.

Based on our findings, we believe that either the FTI or the Amerlex FT_4 method is suitable for the routine measurement of thyroid function in a variety of types of patients. The FTI, however, requires two tests (T_4RIA, T_3RU) per patient, whereas the FT_4 requires only one. We note that Daniels and Henry (*32*) have pointed out that a T_3RU is not routinely needed in all patients, since a T_4RIA will suffice for the majority of them. Using a procedure similar to theirs, we found that only 30% of patients need a T_3RU. This 30% represented a group of hospital patients with borderline total T_4 (11–12 or 5–6 μg/dl) or abnormal total T_4. Therefore, the FTI as used in our institution requires approximately 1.3 tests per patient. The use of the FT_4 may still constitute a saving in time and resources relative to the use of FTI.

The use of the FTI does have some advantages, however, in that it may be important to document TBG changes at some point in a patient's course. This is especially true in patients with increased TBG and a corresponding elevation in both total T_4 and T_3. It is also of some importance in distinguishing low TBG states (congenital deficiency, NTI) from hypothyroidism.

FOOTNOTES

* E. R. Squibb & Sons, Inc., Princeton, NJ 08540.
† Mallinckrodt, Inc., St. Louis, MO 63134.
‡ Clinical Assays, Cambridge, MA 02139.

§ Corning Medical, Medfield, MA 02052.
¶ Serono Laboratories, Inc., Braintree, MA 02184.
‖ Amersham Corp., Arlington Heights, IL 60005.
** Bioscience Laboratories, Van Nuys, CA 91405.

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REFERENCES

1. ROBBINS J: Thyroxine-binding proteins. In *Methods in Investigative and Diagnostic Endocrinology 1*. Chap. 13, Part 1. S. Berson, Ed. North-Holland Amsterdam, 1972, pp 241-254
2. ROBBINS J, CHEN S-V, GERSHENGORN MC, et al: Thyroxine transport proteins of plasma. Molecular properties and biosynthesis. *Recent Progr Horm Res* 34:477-519, 1978
3. WOEBER K: Tests of thyroid hormone transport. In *The Thyroid*. 4th ed., Chap 17. Werner S, Ingbar S, Eds. Harper & Row, Hagerstown Maryland, 1978, pp. 338-344
4. STERLING K: Thyroid hormone action at the cell level. *N Engl J Med* 300:117-123, 1979
5. OPPENHEIMER JH, SURKS MI: Determination of free thyroxine in human serum: a theoretical and experimental analysis. *J Clin Endocr* 24:785-793, 1964
6. STERLING K, BRENNER M: Free thyroxine in human serum: simplified measurement with the aid of magnesium precipitation. *J Clin Invest* 45:153-163, 1966
7. LEE NP, PILEGGI VJ: Measurement of "free" thyroxine in serum. *Clin Chem* 17:166-173, 1971
8. FINUCANE JF, GRIFFITHS RS: A rapid and simple method for simultaneous measurement of serum free thyroxine and triiodothyronine fractions. *J Clin Path* 29:949-954, 1976
9. EKINS R, ELLIS S: The radioimmunoassay of free thyroid hormones in serum. In *Thyroid Research: Proceedings of the Seventh International Thyroid Conference*. Robbins J, Braverman L, Eds. Elsevier (Excerpta Medica), New York, 1976, pp 597-600
10. JIANG NS, TUE KA: Determination of free thyroxine in serum by radioimmunoassay. *Clin Chem* 23:1679-1683, 1977
11. SISSON JC: Principles of and pitfalls in thyroid function tests. *J Nucl Med* 6:853-901, 1965
12. CHAN DW, PERLSTEIN MT, STEM J, PARRA KA: Three approaches for measuring thyroxine-binding globulin. *Clin Chem* 26:1070, 1980
13. ALBERTSON DF, RYBICKI SA: Which free thyroxine index is best? An in vitro study. *Clin Chem* 26:1067-1068, 1980
14. BURR WA, EVANS SE, LEE J, et al: The ratio of thyroxine to thyroxine-binding globulin in the assessment of thyroid function. *Clin Endocrinol* 11:333-342, 1979
15. SZPUNAR WE, STOFFER SS, BEDNARZ MN: Clinical evaluation of a thyroxine-binding globulin assay in calculating a free thyroxine index. *J Nucl Med* 22:793-795, 1981
16. HERTL W, ODSTRCHEL G: Kinetic and thermodynamic studies of antigen-antibody interactions in heterogeneous reaction phases. I. L-thyroxine(T_4) with specific antibody immobilized on controlled pore glass. *Mol Immunol* 16: 173-178, 1978
17. HALPERN EP, BORDENS RW: Microencapsulated antibodies in radioimmunoassay. II. Determination of free thyroxine. *Clin Chem* 25:1561-1563, 1979
18. BAYER MF, MCDOUGALL IR: Radioimmunoassay of free thyroxine in serum: comparison with clinical findings and results of conventional thyroid-function tests. *Clin Chem* 26:1186-1192, 1980
19. PERLSTEIN M, HERNER A, ROCK R, et al: Drug interference and protein matrix effects upon measurement of free thyroxine in serum. *Clin Chem* 25:1104, 1979
20. EKINS R: Commercial radioimmunoassay for free thyroxine. *Lancet* 1:1190-1191, 1979
21. CAVALIERI RR, GAVIN LA, WALLACE A, et al: Serum thyroxine, free T_4, triiodothyronine, and reverse-T_3 in diphenylhydantoin treated patients. *Metabolism* 28:1161-1165, 1979
22. ROCK R, PERLSTEIN M, CHAN DW: Estimation of free thyroxine concentration in serum: effects of protein matrix and interferences from drugs. *Am J Clin Path* 73:297, 1980
23. BRAVERMAN LS, ABREAU CM, BROCK P, et al: Measurement of serum free thyroxine by RIA in various clinical states. *J Nucl Med* 21:233-239, 1980
24. CHOPRA IJ, VAN HERLE JA, CHUA TECO GN, et al: Serum free thyroxine in thyroidal and nonthyroidal illness: a comparison of measurements by radioimmunoassay, equilibrium dialysis and free thyroxine index. *J Clin Endocrinol Metab* 51:135-143, 1980
25. OBREGON MJ, KURTZ A, EKINS R, et al: Evaluation of free and total L-thyroxine in serum by a commercial procedure. *Clin Chem* 27:149-152, 1981
26. CHOPRA IJ, SOLOMON DH, HEPNER GW, et al: Misleadingly low free thyroxine index and usefulness of reverse triiodothyronine measurement in nonthyroidal illnesses. *Ann Int Med* 90:905-912, 1979
27. KAPTEIN EM, SPENCER CA, KAMIEL MB, et al: Prolonged dopamine administration and thyroid hormone economy in normal and critically ill subjects. *J Clin Endocrinol Metab* 51:387-393, 1980
28. CHOPRA IJ, CHUA TECO GN, NGUYEN AH, et al: In search of an inhibitor of thyroid hormone binding to serum proteins in nonthyroid illnesses. *J Clin Endocrinol Metab* 49:63-69,1979
29. CHOPRA IJ, SOLOMON DH, CHOPRA U, et al: Pathways of metabolism of thyroid hormones. *Rec Prog Horm Res* 34:521-567, 1978
30. KAPTEIN EM, MACINTYRE SS, WEINER JM, et al: Free thyroxine estimates in nonthyroidal illness: comparison of eight methods. *J Clin Endocrinol Metab* 52:1073-1077, 1981
31. WITHERSPOON LR, SHULER SE, GARCIA MM, et al: An assessment of methods for the estimation of free thyroxine. *J Nucl Med* 21:529-539, 1980
32. DANIELS DL, HENRY HW: Selective performance of the T_3 uptake: a practical approach to thyroid function studies: concise communication. *J Nucl Med* 22:606-609, 1981