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Evaluation of an Immunoextraction Procedure for the Estimation of Free Thyroxine Concentration

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We have examined the performance of a commercial free-thyroxine assay in which a radiolabeled T_4 derivative permits the competitive quantitation of extracted T_4 in the presence of serum proteins.* After the total T_4 pool had been radiolabeled with either I-125 T_4 or I-131 T_4, the solid-phase antibody was found to be associated with 4–8% of the total T_4 present in the assay tube. Of this, 15–60% was displaceable (antibody-bound). The assay estimated free T_4 to be 0.6–1.8 ng/dl in euthyroid patients, and distinguished them from hyperthyroid (sensitivity 91%) and hypothyroid patients (sensitivity 91%) without apparent TBG dependence. In patients with severe nonthyroidal illnesses, the assay correctly quantitated a reduced extracted mass in some. In other patients, however, the assay results were inappropriately lower than the actual extracted mass, in agreement with the FTI but not with the measurements of free T_4 by dialysis. This assay appears to produce clinically appropriate results in most patients. In some nonthyroidally ill patients however, the indicated free T_4 is spuriously low.

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Recently, free-thyroxine (FT_4) assays relying on the antibody extraction of a portion of the total serum-thyroxine (T_4) mass have been proposed for the estimation of free T_4 (*1–3*). The antibody-extracted mass has been calculated as the fraction of total mass extracted times total T_4, or by competitive immunoassay after separation of competing serum binders. We report here our experiences with an immunoextraction assay* in which the extracted mass of T_4 is quantitated using a radiolabeled T_4 derivative that permits competitive immunoassay in the presence of serum thyroxine-binding proteins (*4*).

MATERIALS AND METHODS

Laboratory measurements. Measurements used to characterize reagents or patients' serum samples and reference ranges established in our laboratory are:

Total serum thyroxine (T_4)†: 4.5–11.5 ng/dl.

Tri-iodothyronine (T_3) uptake†: 35–45%.

Free-thyroxine index (FTI) calculated as total T_4 times T_3 uptake: 1.6–5.2.

Thyroid-stimulating hormone (TSH)‡: ≤7 μU/ml.

Total 3,5,3′-tri-iodothyronine (T_3)§: 70–210 ng/dl.

Thyroxine-binding globulin (TBG)§: 12–30 μg/ml.

Reverse 3,3′,5′-tri-iodothyronine (rT_3)¶: 8–35 ng/dl.

Free thyroxine (FT_4) by equilibrium dialysis as previously described (*3,5*): 1.2–3.6 ng/dl.

All assays using commercial reagent kits were performed according to the manufacturer's instructions.

Free T_4 assay. Reagent kits* are used. In this assay system, patient serum is incubated with polymer particles coated with sheep antithyroxine antibody. Thyroxine present in the serum sample is distributed among all binders (antibody, serum thyroxine-binding proteins) depending upon their affinities for thyroxine and their concentrations. Thyroxine bound by the antibody is quantitated by competitive binding with a radiolabeled

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derivative that purportedly has the properties of being bound by the antibody with an affinity similar to that of T_4 but of not being bound by any of the serum thyroxine binders. This property permits the simultaneous addition of antibody and I-125-labeled derivative to the serum specimen. After incubation at 37°C for 1 hr, the antibody-bound fraction is separated from the free fraction by centrifugation. The mass of antibody-bound derivative is inversely proportional to the mass of T_4 extracted from patient serum. This extracted mass is related to free T_4 concentration by reference to a similarly obtained calibration curve. Human serum calibrators are assigned free T_4 concentrations by Amersham using equilibrium dialysis. FT_4 estimates were obtained by following the manufacturer's protocol without modification.

Reaction kinetics. We examined the percentage of labeled derivative bound to antibody over time for the zero calibrator, for the 0.95 ng/dl calibrator, for serum pooled from hypothyroid and hyperthyroid patients, and for serum from euthyroid patients with elevated and low TBG concentrations. Each serum pool was made from several samples of patient serum to obtain the volume necessary to accomplish this study. Antibody, assay tracer, and aliquots from the calibrators or each serum pool were added to replicate tubes and incubated at 37°C. The reaction was interrupted in duplicate tubes at intervals by centrifugation. The antibody-bound counts were expressed as the fraction of total added activity. We estimated nondisplaceable (nonspecific) binding of derivative by adding 0.5 mg thyroxine to a series of similarly treated tubes.

Extraction experiments. The apparent mass of thyroxine extracted from patient serum by the assay's solid-phase antibody was quantitated independently of the assay both with and without the assay tracer in the reaction tube.

1. Without assay tracer. Serum (1.4 ml) was incubated for 30 min at 37°C with 0.01 ml I-125 thyroxine of high specific activity (>1200 μCi/μg; ~2 ng thyroxine/0.1 ml*). To duplicate tubes we added:

0.1 ml patient serum labeled with I-125 thyroxine,
0.5 ml tracer buffer (0.1*M* phosphate-buffered saline)*,
0.5 ml anti-T_4 antibody solution*.

Tubes were incubated at 37°C for 0.5, 1, 2, and 3 hr. The reaction was interrupted in duplicate tubes after each time interval by centrifugation. Activity (I-125 thyroxine from patient serum) found in the pellet was counted and expressed as the percent of total added activity (percent extracted). The mass of T_4 extracted is the fraction extracted times total T_4 in the tube. Four separate extraction experiments were done using three different lots of I-125 thyroxine. Twenty-one serum pools were studied, derived from hyperthyroid (4), hypothyroid (4), and euthyroid patients with different concentrations of TBG (13).

2. With assay tracer. Three milliliters serum were incubated for 30 min at 37°C with 0.025 ml I-131 thyroxine of high specific activity (233 μCi/μg or 177 μCi/μg; ~150 ng thyroxine/ml). To duplicate tubes we added:

0.1 ml patient serum labeled with I-131 thyroxine,
0.5 ml I-125-labeled thyroxine derivative*,
0.5 ml anti-T_4 antibody solution*.

Tubes were incubated at 37°C for 0.5, 1, 2, and 3 hr. The reaction was interrupted in duplicate tubes after each time interval by centrifugation. Activity (I-131 thyroxine from patient serum and I-125 derivative) was quantitated in a dual-channel gamma counter with both an I-125 window (15–75 keV) and an I-131 window (320–400 keV). The tube for total activity of I-131 thyroxine was counted with both windows, and the percentage of I-131 counts observed in the I-125 window was used to correct counts in the I-125 window in assay tubes containing both isotopes.

Free T_4 was calculated from the corrected I-125 counts bound after 1 hr by reference to concurrently incubated calibrators.* The percentage of the total serum T_4 found in the pellet was calculated from the I-131 counts expressed as a percentage of the total added I-131 activity. The mass of T_4 extracted is the fraction extracted times total T_4 in the tube. Two separate extraction experiments were done using two different lots of I-131 thyroxine. Ten of the same pools used in the extraction experiments without assay tracer were used.

Nonspecific binding. To assess the degree of nonspecific binding in our extraction experiments (presumably by adsorption of thyroxine to the polymer particles and/or tube walls), four serum samples were extracted without assay tracer for 1 hr as described above. To a second set of tubes was added 0.5 mg carrier thyroxine diluted in the 0.5 ml assay buffer (i.e., about 5×10^5 times the mass of extracted thyroxine).

Precision. Variation within same assay was calculated as the coefficient of variation (CV) for data obtained from 20 replicate measurements of three serum pools. We calculated between-assay precision (CV) from duplicate measurements of three serum pools measured in 13 free-T_4 assays.

We subjected the labeled derivative* to dialysis against phosphate buffer, precipitation with 10% trichloroacetic acid (TCA), and chromatography on Sephadex G-25 columns.

Patients studied. Serum samples were selected for study from patient samples submitted to the laboratory for measurement of total serum thyroxine concentration and T_3 uptake, thus permitting calculation of a free-thyroxine index (total T_4 times T_3 uptake). Samples were selected from hypothyroid, hyperthyroid (both endogenously and iatrogenic), and euthyroid patients, including some patients receiving replacement thyroxine

and some with elevated or low TBG concentrations. A group of patients severely ill with nonthyroidal illnesses was also studied. The medical records of all patients were examined. We excluded patients receiving medications that could potentially interfere with thyroid function testing and those with thyroid disease whose functional status was unclear. A total of 221 patient samples were selected for measurement of free T_4 using the free-T_4 kit*.

Hypothyroid patients (N = 34). All patients studied had primary hypothyroidism with abnormally elevated basal TSH concentrations. Two patients were receiving inadequate replacement doses of synthetic thyroxine (FTI 1.6, 2.2). In the other 32 patients the FTI was 1.6-2.4 in 7 and <1.6 in 25.

Hyperthyroid patients (N = 34). All endogenously hyperthyroid patients (N = 19) had abnormally elevated FTIs and T_3 concentrations and had clinical signs and symptoms consistent with that diagnosis. All iatrogenically hyperthyroid patients (N = 15) were receiving synthetic thyroxine and all had an abnormally elevated FTI (one patient 5.3, 14 patients 6.2-9.6). The total T_3 concentration was elevated in five and normal in ten. These patients either had had thyroid carcinoma and were receiving suppressive doses of thyroxine (evidenced by suppressed TSH response to thyrotropin-releasing hormone) or were receiving excessive doses of replacement medication.

Euthyroid patients (N = 137). All of these were outpatients who had no significant intercurrent illness. We studied 17 euthyroid patients who were receiving physiologic amounts of synthetic thyroxine. Thirteen patients (three of whom were receiving thyroxine) were taking estrogen-containing medications. None was pregnant.

Patients with nonthyroidal illnesses (N = 14). Sera from 14 hospitalized patients severely ill with nonthyroidal illnesses were studied. These had total T_4s of 2.6-6.4 μg/dl, T_3 uptakes of 35-56%, and FTIs of 1.2-2.9. One patient was receiving thyroxine (FTI = 1.2). None had elevated TSH concentrations. These patients had a variety of illnesses including sepsis, cardiovascular disease (2), malignancy (5), renal disease (2), gastrointestinal diseases (2), arthritis, and psoriasis. Four died during the current hospital admission.

Extraction of thyroxine from the serum of patients undergoing renal dialysis. Antibody extraction of thyroxine was studied using ten serum samples obtained from patients on chronic hemodialysis for chronic renal failure, who were not included in the above studies. The serum specimens were carefully obtained immediately before a hemodialysis session and before the administration of heparin. We followed the protocol described above for extraction experiments without assay tracer. These patients had a mean total T_4 of 3.7 μg/dl (range 2.5-5.4), T_3 uptake 42% (38-48), FTI 1.6 (1.2-2.3), TBG 17 μg/ml (14-22), T_3 66 ng/dl (34-122), rT_3 16 ng/dl (3-44), FT_4 (by dialysis) 2.1 ng/dl (1.2-4.2), and FT_4* 0.5 ng/dl (0.4-0.6).

RESULTS

Displacement that we observed in the assay calibration curve* was similar to that reported by Chan et al. (*6*). Intraassay precisions, expressed as CVs within the same assay, were 10% (0.11 ng/dl), 4% (1.4 ng/dl), and 6% (2.5 ng/dl). Interassay precisions were 20% (<0.1 ng/dl), 12% (1.2 ng/dl) and 9% (3.6 ng/dl). Assay sensitivity (B/B_0 = 90%) was ~0.2 ng/dl.

Assay quantification of extracted thyroxine is plotted against time in Fig. 1. The 1-hr recommended incubation time resulted in 73-83% of the antibody binding that was observed after 6 hr of incubation of the patients' serum samples. The zero calibrator represents the maximum observed binding. Bound counts in the zero calibrator relative to total activity at 1 hr were 60%. These data typify the performance of the assay; label is only slightly displaced from antibody by the T_4 present in hypothyroid sera and is largely displaced by the T_4 present in hyperthyroid sera, while binding is apparently not affected by TBG concentration. Nondisplaceable binding of assay tracer was ~3% (2.9-4.1%) of the total activity after 1 hr of incubation in all samples, and increased little with time.

Quantitation of antibody-extracted T_4, and characteristics of the serum pools studied are shown in Table 1. Results representative of the serum pools studied are shown in Fig. 2. The greatest percent of total T_4 is extracted from serum samples from hypothyroid patients and from serum samples with reduced concentrations of TBG, whereas the smallest percent of total T_4 is extracted from sera from hyperthyroid patients and from serum samples with increased TBG concentrations. When the fraction of total T_4 extracted is multiplied by

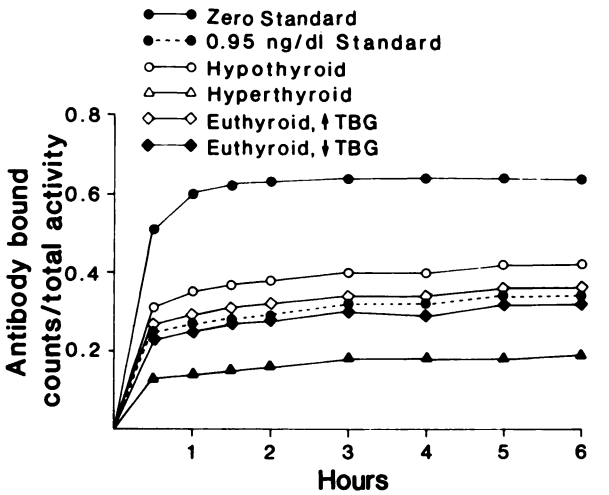

FIG. 1. Observed counts bound by solid-phase antibody, expressed as fraction of total added activity, plotted against time.

TABLE 1. CHARACTERISTICS OF SERUM SAMPLES USED IN EXTRACTION EXPERIMENTS

Pool label	T_4 (μg/dl)	T_3 uptake (%)	FTI	TBG (μg/ml)	Equilibrium dialysis: Percent dialyzed fraction (× 10^2)	Equilibrium dialysis: FT_4 (ng/dl)	Amersham FT_4 (ng/dl)	Extraction (3 hr): % of total	Extraction (3 hr): Total mass (ng)	Extraction (3 hr): NSB (%)	Tracers (I-125 or I-131 thyroxine or both)
Euthyroid											
6B	2.4	62	1.7	9.3	—	—	2.0	8.0	0.25	—	I-125
2C	6.2	51	3.1	13	—	—	1.3	6.1	0.38	—	Both
2B	3.9	49	1.9	15	4.9	1.9	1.1	7.0	0.29	—	Both
2A	4.6	47	2.2	16	3.3	1.5	1.2	8.0	0.37	—	I-125
2D	5.3	46	2.5	16	2.8	1.4	0.9	7.2	0.38	42, 44	I-125
5C	8.7	38	3.3	19	—	—	0.9	6.8	0.59	—	Both
5B	7.6	40	3.1	20	3.3	2.5	1.4	7.0	0.50	—	Both
5A	11	40	4.4	23	2.2	2.5	1.5	5.7	0.63	—	I-125
1B	12	31	3.7	35	2.9	3.5	1.2	5.0	0.66	—	Both
1C	13	29	3.8	39	—	—	1.0	5.5	0.72	—	Both
7B	11.9	25	3.0	43	—	—	0.8	6.1	0.73	—	I-125
1D	15	26	3.9	43	1.3	1.9	0.8	3.8	0.59	78, 65	I-125
1A	14.7	28	4.1	51	1.5	2.2	0.8	6.4	0.95	—	I-125
Hypothyroid											
3B	2.6	35	0.9	22	3.1	0.8	0.2	8.5	0.22	—	Both
3A	3.2	38	1.2	25	2.2	0.7	0.5	8.4	0.27	—	I-125
3C	2.4	37	0.9	22	—	—	0.2	8.6	0.21	—	Both
3D	3.8	33	1.2	28	1.8	0.7	0.3	8.4	0.32	44, 46	I-125
Hyperthyroid											
4B	19.9	53	10.6	19	4.1	8.2	4.3	5.6	1.1	—	Both
4A	14.3	48	6.9	22	2.8	4.0	3.5	6.0	0.86	—	I-125
4C	23.1	54	12.5	15	—	—	4.9	4.4	1.0	—	Both
4D	21	46	9.8	23	2.8	6.0	4.0	7.2	1.5	85, 76	I-125

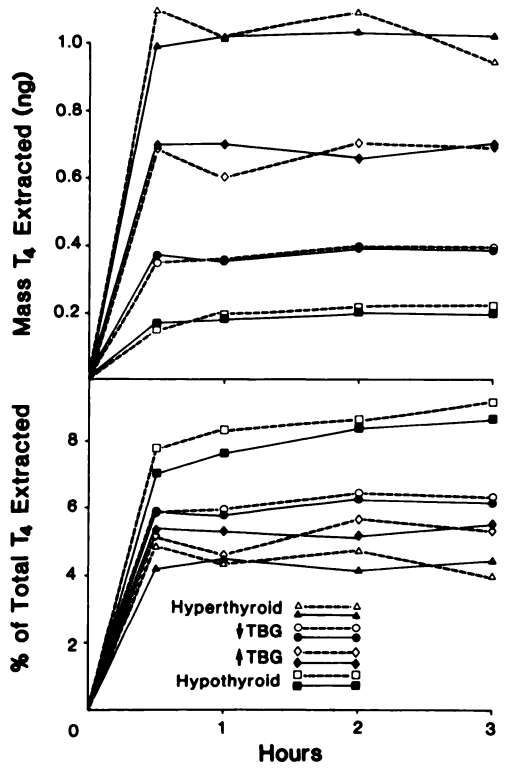

FIG. 2. Percentage of total T_4, and apparent mass of T_4, extracted by Amersham antibody from various serum pools against incubation time. Dashed lines show when total serum T_4 pool was labeled with I-131 T_4; all assay components including I-125-labeled derivative are present in assay tubes. Solid lines show when total serum T_4 pool was labeled with I-125 T_4. Assay tracer is omitted from assay tubes. Data are shown for pools 1C (↑ TBG), 2C (↓ TBG), 3C (hypothyroid), 4C (hyperthyroid). See Table 1 for pool data.

the total T_4, the extracted mass thus found is elevated in hyperthyroidism, decreased in hypothyroidism, and intermediate in euthyroid patients, increasing with increases in TBG concentration. These results in euthyroid patients differ from the FT_4 estimates obtained with the assay,* which bear no obvious relationship to the TBG concentrations (Fig. 5).

As shown in Fig. 2, we observed no differences, either in the percent of total T_4 extracted or the apparent mass extracted, between those pools that included the assay tracer and those that did not. This was true of all ten serum pools spiked with I-131 thyroxine. The results shown in Fig. 2 are not corrected for nonspecific binding but represent total extracted mass—both specific antibody and nondisplaceable. We found that 40–45% of the activity associated with the polymer pellets obtained from samples with low total T_4 concentration (hypothyroid, low TBG) was not displaced by the addition of 0.5 mg of carrier T_4. From samples with elevated total T_4 (hyperthyroid, increased TBG), 65–80% of the pellet-associated activity was not displaced. If the results shown in Fig. 2 are corrected for this nondisplaceable activity, then the T_4 that is extracted by the antibody from the samples with elevated and low TBG concentrations is similar—a result in agreement with the apparent free-T_4 estimates made using the assay* for these same serum specimens. We were able to rinse a substantial amount of this nondisplaceable activity from the pellet, but obtained less consistent results if we rinsed the pellets before applying this correction.

We found that 15–20% of I-125 activity associated

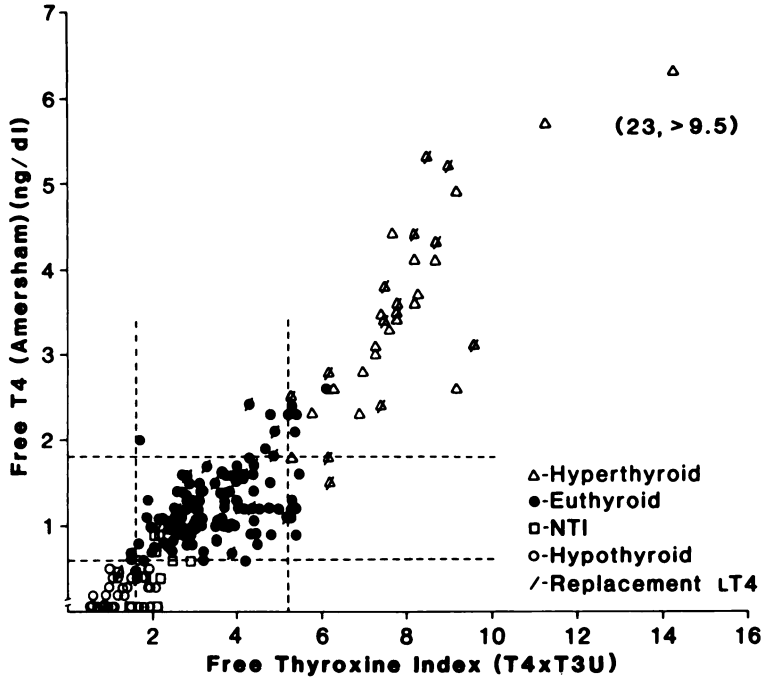

FIG. 3. Correlation between free-thyroxine index and free T_4 for serum specimens studied. NTI = nonthyroidal illness. / indicates patient was receiving synthetic thyroxine orally. Excluding patients receiving replacement therapy, regression line is y = 0.43x − 0.24, correlation coefficient r = 0.93, standard error of estimation = 0.44.

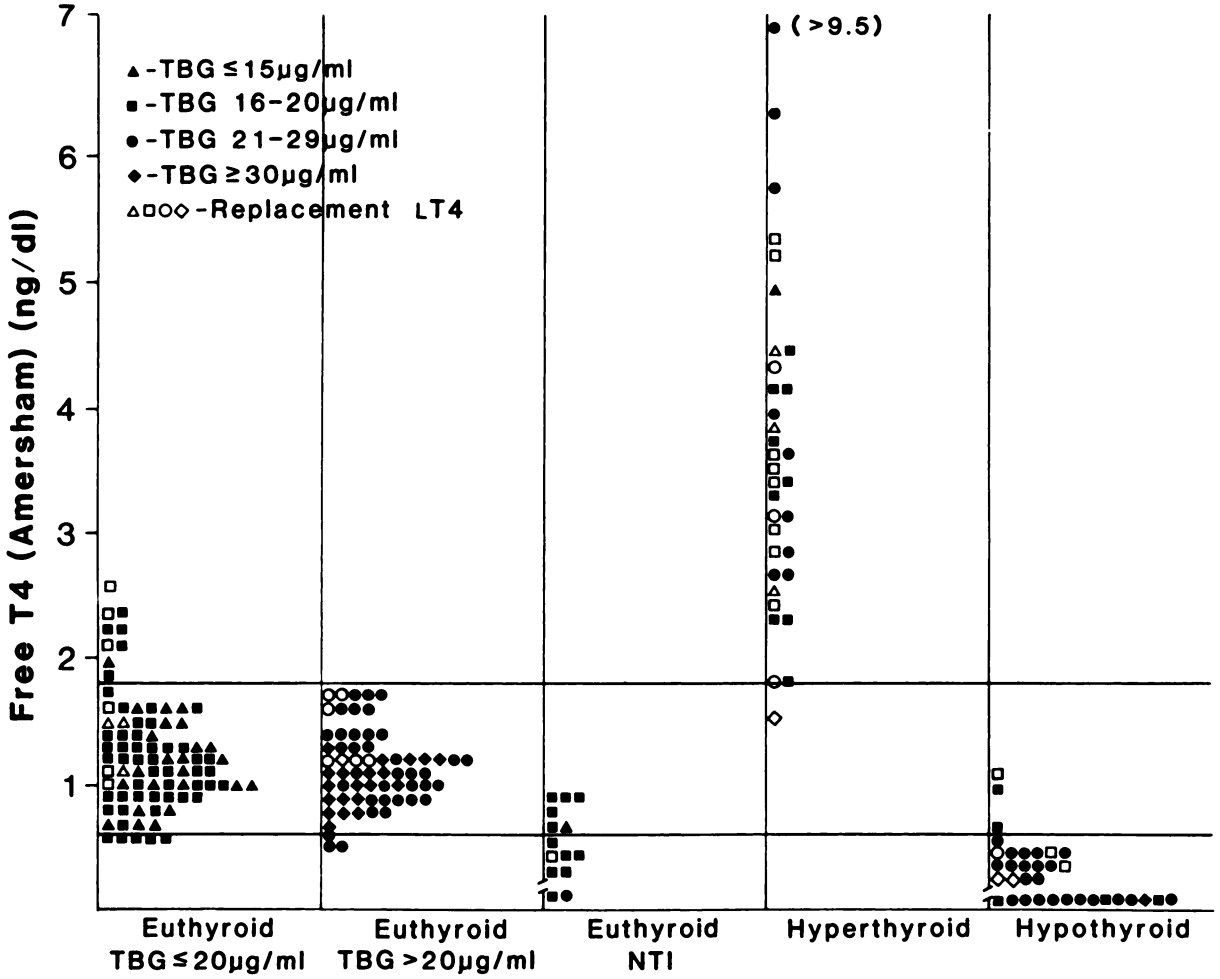

FIG. 4. Free T_4 related to clinical thyroid functional status.

with the tracer* dialyzed across a semipermeable membrane. About 80% of the activity was precipitated by TCA, and the tracer largely eluted immediately after the void volume from G-25 columns.

Figure 3 shows the correlation between free thyroxine index and free T_4,* measured in all the samples of patient serum studied. Figure 4 shows the free-T_4 concentration* as related to the clinical functional status of these patients. Figure 5 shows the lack of correlation between TBG concentration and serum free-T_4 concentration as measured by the assay* in all the euthyroid patients we studied. Results obtained from hyper- and hypothyroid patients, and those without thyroid disease, are not included.

Figure 6 shows the anti-T_4 antibody extraction of T_4* from serum samples obtained from patients on chronic hemodialysis for chronic renal failure. A hypothyroid sample is included for reference. Although the assay* estimated free-T_4 concentration in these samples to be 0.4–0.6 ng/dl, relatively more T_4 was extracted from at least some of the samples studied. Although the nondisplaceable (nonspecific) activity was not quantified for these patients, we have found it to be low (<40%) in samples with low total T_4s obtained from other patients with nonthyroidal illnesses.

DISCUSSION

In immunoextraction assays developed to quantify free T_4, patient serum may be enriched with radiolabeled T_4 before exposure to solid-phase anti-T_4 antibody. The mass of extracted T_4 is calculated as the fraction of total T_4 extracted times total T_4 present in the specimen. This approach is the basis of the assay that was initially commercially available§ (*1*), and we have used it to evaluate other immunoextraction assays (*3*). This approach is valid only if nonspecific binding is uniform and is a relatively small percentage of the total extracted thyroxine. The immunoextraction assay* uses a radiolabeled T_4 derivative that is purported to compete with endogenous T_4 for antibody but not for endogenous serum protein binding. This might be accomplished by preparing either a protein or a smaller molecular conjugate of T_4 itself that has the property of being recognized and bound by antibody but not by serum protein. Midgley and Wilkins, in a United States patent describe

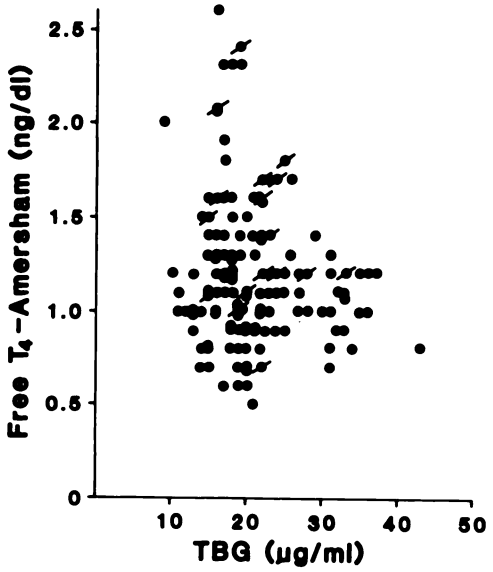

FIG. 5. Relationship between TBG concentration and free T_4 in 139 euthyroid patients. / indicates patient was receiving synthetic thyroxine orally. There is no significant correlation.

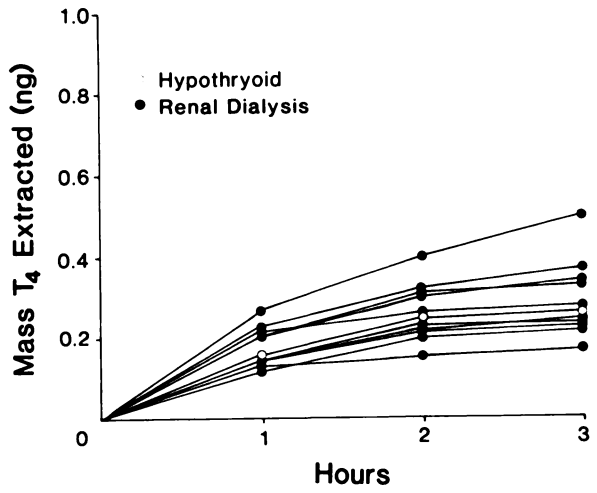

FIG. 6. Apparent mass of T_4 extracted by Amerlex antibody from sera obtained from ten patients on chronic hemodialysis due to chronic renal failure (●). Extraction of T_4 from serum from hypothyroid patient (O) is shown for reference.

such modifications of thyroxine (*7*). Binding of T_4 to TBG and TBPA apparently depends upon the configuration of the alanine side chain (*8*). Modification of the alanine side chain would therefore be expected to interfere with TBG and TBPA binding of thyroxine. Binding by anti-T_4 antibodies that recognize a determinate elsewhere in the molecule would be little affected by such substitutions. Although the actual derivative used in the assay is proprietary,* Midgley and Wilkins illustrate these principles using I-125-labeled N-acetyl-thyroxine-methyl ester and N-acetyl-thyroxine-dimethylamide (*7*). Nonspecifically adsorbed T_4 is apparently not quantitated by this approach—only the specific antibody-bound (displaceable) T_4.

Immunoextraction for the estimation of free ligand concentration depends upon the partition of ligand between endogenous binders present in the patient's sample and specific antibody added to an aliquot of sample. The final equilibrium may favor anti-T_4 antibody rather than T_4 binders present in diluted serum. The reaction kinetics we observed (Fig. 1) suggest that after the 1-hr incubation recommended by the manufacturer, an equilibrium between the binding of T_4 by antibody and by endogenous T_4-binding protein has not been reached. There is, however, little further antibody binding after longer incubation periods, so that errors due to variation in the incubation times of individual assay tubes should be minimal. The nonspecific binding of the radiolabeled T_4 derivative* in this system is low (about 3% of the total added activity), whereas binding in the presence of sera with low free-T_4 concentrations (hypothyroid patients) exceeds this by 10–15%.

We were able to estimate the mass of T_4 extracted independently of the assay system by enriching serum samples with radiolabeled T_4 before their exposure to the solid-phase antibody.† The apparent mass extracted was calculated as the fraction extracted times total T_4. Using I-131-labeled T_4, we show that the immunoextracted fraction and mass is the same whether or not the I-125-labeled T_4 derivative in the assay is present in the reaction mixture (Fig. 2). The percent of total T_4 associated with the antibody pellet is ~4–8% of the total T_4 present in the serum specimens. This is similar to the percent immunoextraction we observed for a different FT_4 assay§ (*3*). In contrast to this assay, however, a large fraction of the pellet-associated activity in the assay reported here is not displaced by the addition of a marked excess of carrier T_4. Because of the large mass of T_4 nonspecifically associated with the pellet in our extraction experiments, the mass of T_4 extracted (calculated by fraction extracted times total T_4) overestimates the mass quantified in the assay.

We found that the nondisplaceable fraction varied between approximately 40% and 85%, depending upon the total T_4 concentration in the specimen (Table 1). Specimens with low total-T_4 concentrations—such as those obtained from hypothyroid patients or from patients with low concentrations of binding protein—were associated with lower nondisplaceable fractions, whereas those obtained from patients with elevated total-T_4 concentrations were associated with higher fractions. Much of this activity can be removed by rinsing the pellet. When the apparent mass extracted illustrated in Fig. 2 is corrected for the nonspecifically bound activity, the estimated mass obtained from both high and low TBG concentrations is similar. This result is in agreement with estimates made by the assay itself.

We did not see the expected inverse relationship between TBG concentration and the percent of total mass

extracted (*3*) (Table 1). We believe this is explained by the large, variable fraction of T_4 nonspecifically associated with the antibody's solid phase.

We found an excellent overall correlation between the FT_4 estimates and the FTIs in hypo- and hyperthyroid patients and in patients with elevated or low TBG concentrations (Fig. 3). While the FTI becomes elevated in patients with marked elevations in TBG concentration, due to the inability of the T_3 uptake test to reflect these elevations adequately (*9*), we did not observe any apparent TBG concentration effects in the results (Fig. 5). These findings are consistent with those of Wellby et al. (*10*), Wilke (*11*), and Chan et al. (*6*).

We observed below-normal results in patients who had severe nonthyroidal illnesses but who were apparently euthyroid (Fig. 3). These results are similar to the FTI results in these same patients, and lower than free-T_4 estimates made by equilibrium dialysis. In other studies of nonthyroidal illness, the best agreement with dialysis estimates of free T_4 was seen with the Clinical Assays "two step" assay, in which serum is separated from the antibody-extracted T_4 before its quantification (*12–14*). In this assay,* patient serum (diluted 1:11) is present during the competitive quantification of antibody-extracted T_4. We attempted to discover whether the low free-T_4 estimates in these patients reflect the actual immunoextracted mass of T_4 or whether they might be due to some interference in the assay quantification of the extracted mass. The mass of T_4 extracted from sera obtained from patients on renal dialysis (calculated as the fraction extracted times total T_4) was unusually low in some patients but not in all (Fig. 6), whereas the assay results were uniformly low. This suggests that while the assay system does accurately quantify the mass of T_4 extracted by the antibody in some patients, the actual mass extracted is underestimated in others. In sera from these patients, as with the hypothyroid sera, nondisplaceable binding was about 40% of the total. Stockigt et al. (*15*) have presented evidence that the labeled derivative is normally bound by prealbumin. Low serum prealbumin concentrations with less-than-normal label sequestration may in part explain the low results in some nonthyroidally ill patients—a suggestion contested by Midgley and Wilkins (*16*). Amino et al. (*17*) found that the Amerlex labeled derivative is sequestered by albumin, suggesting that hypoalbuminemia may result in greater antibody binding of label in patients with nonthyroidal illnesses. Stockigt et al. (*18*) report low FT_4 estimates using the Amerlex reagents in patients with analbuminemia, consistent with decreased tracer-albumin binding relative to the albumin binding of tracer in the assay calibrators.

Although acknowledging the importance of TBG and TBPA binding of the thyroxine derivative, Midgley and Wilkins postulate that albumin association is unimportant because albumin is relatively unimportant as a T_4 binder (*7*). While this may be true, the importance of albumin as a tracer binder in the assay itself cannot be ignored. We found that the Amerlex labeled derivative was largely nondialyzable, was precipitated by TCA, and eluted immediately after the void volume from Sephadex columns, suggesting that their derivative is largely protein-associated. The only protein demonstrably present in the tracer preparation is albumin. We suggest that the results we observed in nonthyroidally ill patients are best explained by less-than-usual binding of tracer by serum components in these sera, with resultant greater antibody binding of tracer relative to the "normal" serum calibrators and thus an erroneously low apparent free T_4. A more rigorous understanding of the labeled derivative's potential serum protein-binding properties would be reassuring before the Amerlex FT_4 assay is more widely applied in clinical practice.

FOOTNOTES

* Amerlex, Amersham Corporation, Arlington Heights, IL 60005.
† Nuclear Medical Laboratories, Irvine, TX 75061.
‡ Beckman Instruments, Brea, CA 92621.
§ Corning Medical, Medfield, MA 02052.
¶ Serono Laboratories, Inc., Braintree, MA 02184.

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