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EVALUATION OF A RAPID AND SIMPLE TECHNIQUE FOR THE RADIOIMMUNOASSAY OF TRIIODOTHYRONINE (T_3)

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This report compares T_3 measurements obtained on the same serum samples by a resin-strip technique and by another existing radioimmunoassay method. The samples analyzed were obtained from a total of 90 subjects who were clinically categorized as hypothyroid, normal, hyperthyroid, or euthyroid while taking estrogen-containing compounds or while pregnant. The correlation coefficient for all 90 sera with these two different techniques was 0.94. All subjects who were clinically euthyroid (32) had a normal serum T_3 concentration by the resin-strip technique. Similarly, 23 clinically hyperthyroid patients had elevated serum T_3 concentrations and 15 of 17 clinically hypothyroid patients had decreased serum T_3 levels.

Recent improvements in the techniques of measuring triiodothyronine (T_3) have established its importance in the thyroidal economy of health and disease (*1*). Although T_3 assays utilizing sensitive and specific radioimmunoassays are becoming accessible, the development of an accurate commercial kit to measure T_3 would permit broader clinical application of T_3 determinations and would aid in the diagnosis and treatment of patients with thyroidal abnormalities. The purpose of the present study was to evaluate a new commercial T_3 assay that utilizes a resin strip to separate free radioactive tracer from that bound by antisera (RIA-MAT^{tm} Circulating T_3 ^{125}I Kit, Mallinckrodt Inc., St. Louis, Mo.). The results of T_3 measurements obtained by use of this kit were compared with those obtained by another existing T_3 radioimmunoassay which employed a double-antibody technique in the separation procedure. Sera were analyzed from normal subjects as well as from patients who were clinically hyperthyroid, hypothyroid, and euthyroid while taking estrogen-containing agents or while pregnant.

MATERIALS AND METHODS

Triiodothyronine assays. *Commercial kit: test procedure.* Reaction vials, standards, and T_3 antiserum were stored at −10°C; veronal buffer (0.05 *M* sodium barbital) and the resin strips were stored at 5°C as were the T_3 standards after the initial thaw. Each reaction vial contained approximately 25–50 pg ^{125}I-T_3 to allow an approximate counting rate per vial of 10,000 cpm. The specific activity of the ^{125}I-T_3 was approximately 600 μCi/μg and the isotope was prepared at Mallinckrodt Inc. All samples and standards were analyzed in triplicate. Immediately prior to performing an assay, a 1-min count was obtained on each reaction vial. One hundred microliters of each T_3 standard prepared in hypothyroid sera prior to shipment was added to the reaction vials to give a final T_3 concentration of 0, 50, 100, 200, and 600 ng/100 ml; 100 μl of the patients' serum was added to the remaining vials; and then 100 μl T_3 antiserum was added to all reaction vials. The vials were mixed for 1 min and were then placed in a 37°C water bath for 1 hr. The vials were then removed from the water bath and 1.0 ml veronal buffer was added to the vials. A resin strip was added and all vials were rotated at 12–14 rpm for 1 hr at 32°C. At the end of this time, the resin strip was removed and 1 min counts for ^{125}I were performed on the fluid remaining in the vials. Since the resin strip removes the unbound or free radioactive T_3, the radioactive tracer that is bound to antibody remains in the vial. The percent of radioactive tracer that was bound by antibody was calculated by dividing the postcount by the precount. The percent bound or bound over free ratio (B/F) was then plotted as a function of

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concentration of T_3 standard expressed as ng/100 ml. The concentration of T_3 in unknown sera was determined by extrapolation from the standard curve obtained. Monitrol I and II were employed as normal and high T_3 controls, respectively. Pooled plasma obtained from the blood bank was also employed as a normal control.

The goat anti-T_3 antibody employed in the resin-strip technique was used in final concentration of approximately 1:150,000. The antibody was diluted so that 50–75% of tracer ^{125}I-T_3 was bound in the absence of unlabeled T_3; 8-anilino-1-naphthalene sulfonic acid (ANS) was used to compete with T_3 for binding sites on thyroxine-binding proteins and was placed in each reaction vial in an approximate concentration of 250 μg per vial. In previous experiments performed at the Mallinckrodt Laboratories, it was found that the resin strip removed essentially all of the free T_3 when added to tubes not containing antibody. Cross reactivity with T_4 or other thyroid congeners was less than 0.3% in experiments that had also been performed at the Mallinckrodt Laboratories.

Reference radioimmunoassay. The radioimmunoassay of T_3 was performed by the method of Chopra, et al (*2*) at the Nichols Institute for Endocrinology, San Pedro, Calif. This assay utilizes 250 μl unknown sera or diluted standards, 100 μl ANS (2.5 mg/ml), 100 μl diluted rabbit anti-T_3 serum, and 15–25 pg ^{125}I-T_3. Total incubation volume is 1.0 ml and the buffer employed is 1% normal rabbit serum in barbital buffer. This reaction is incubated for at least 24 hr at 4°C; antibody-bound hormone is separated from free hormone by a double-antibody technique. In this study, all samples were run in duplicate.

CLINICAL EVALUATION

Sera were obtained from 32 normal subjects, 17 hypothyroid, and 23 hyperthyroid patients, and from 18 patients taking conjugated estrogen, birth control pills, or who were pregnant. Patients were assigned to one of the above categories based on their clinical status, serum thyroxine (T_4), and resin T_3 uptake, as well as on their serum thyrotropin (TSH) and 24-hr radioactive iodine uptake when performed. The sera obtained from each patient was divided into two aliquots, one to be used for T_3 analysis with the resin-strip technique and another to be used for analysis by Nichols Institute. Sera was stored at −20°C until measured.

RESULTS

Resin-strip. The correlation coefficient for all 90 sera analyzed by the two different techniques was 0.94 (Fig. 1). When the various groups of patients were examined separately, however, the correlation coefficient comparing the two techniques was 0.23 for the normal group, 0.61 for the hypothyroid group, 0.59 for the patients taking estrogen-containing medications, and 0.90 for the hyperthyroid patients. For statistical purposes, T_3 concentrations less than 25 ng/100 ml were assigned the value of 25 ng/100 ml.

Table 1 shows the range, mean, and standard deviation for the T_3 concentrations measured by both the resin-strip technique and by the method of Chopra, et al (*2*). Mallinckrodt Laboratories has determined the normal T_3 range to be 72–214 ng/100 ml by the resin-strip technique. The normal range referred to in this report, however, was derived from our 32 euthyroid individuals and was found to be 78–170 ng/100 ml (mean ± 2 s.d.). All individuals who were considered to be clinically normal and to have a normal serum T_4 also had a normal T_3 by the resin-strip technique. Two patients who were clinically hypothyroid had normal serum T_3 values and 7 of 17 clinically hypothyroid patients had undetectable T_3 values as defined below. All patients who were clinically hyperthyroid had elevated serum T_3 values. All patients who were clinically euthyroid and taking estrogen-containing compounds had normal serum T_3 values.

Sensitivity. The percent binding of ^{125}I-T_3 to T_3 antiserum was 69.0% ± 1.0 (mean ± s.e.m.) in 22 separate assays when no standard was added (Trace-binding tube). The mean actual percent binding of ^{125}I-T_3 in 22 separate assays was 64.4% ± 1.0 (mean ± s.e.m.) when the standard had a final T_3 concentration of 50 ng/100 ml. The mean percent binding with a final concentration of 100 ng/100 ml T_3, 200 ng/100 ml T_3, and 600 ng/100 ml T_3 was 60.0% ± 1.0, 52.9% ± 1.0, and 37.4% ± 0.6 (mean ± s.e.m.), respectively, in 22 separate assays (Fig. 2). Statistical analysis of the 22 standard curves by paired t-test revealed that the binding of ^{125}I-T_3 to antibody (mean ± s.e.m.) in the absence of unlabeled T_3 (trace binding, 69% ± 1) was significantly different ($p < 0.001$) from the binding of ^{125}I-T_3 in the presence of a final concentration of 50 ng/100 ml unlabeled T_3 (64.4% ± 1). The minimal detectable T_3 concentration of these assays was determined by extrapolation from the standard curve and by rejecting binding values that fell within two standard errors of the mean of trace binding. Defined in this way, the minimal detectable concentration of T_3 by this radioimmunoassay technique was 25 ng/100 ml. Statistical analysis by paired t-test also reveals that each concentration employed in the standard curve was significantly different from that immediately preceding it ($p < 0.001$).

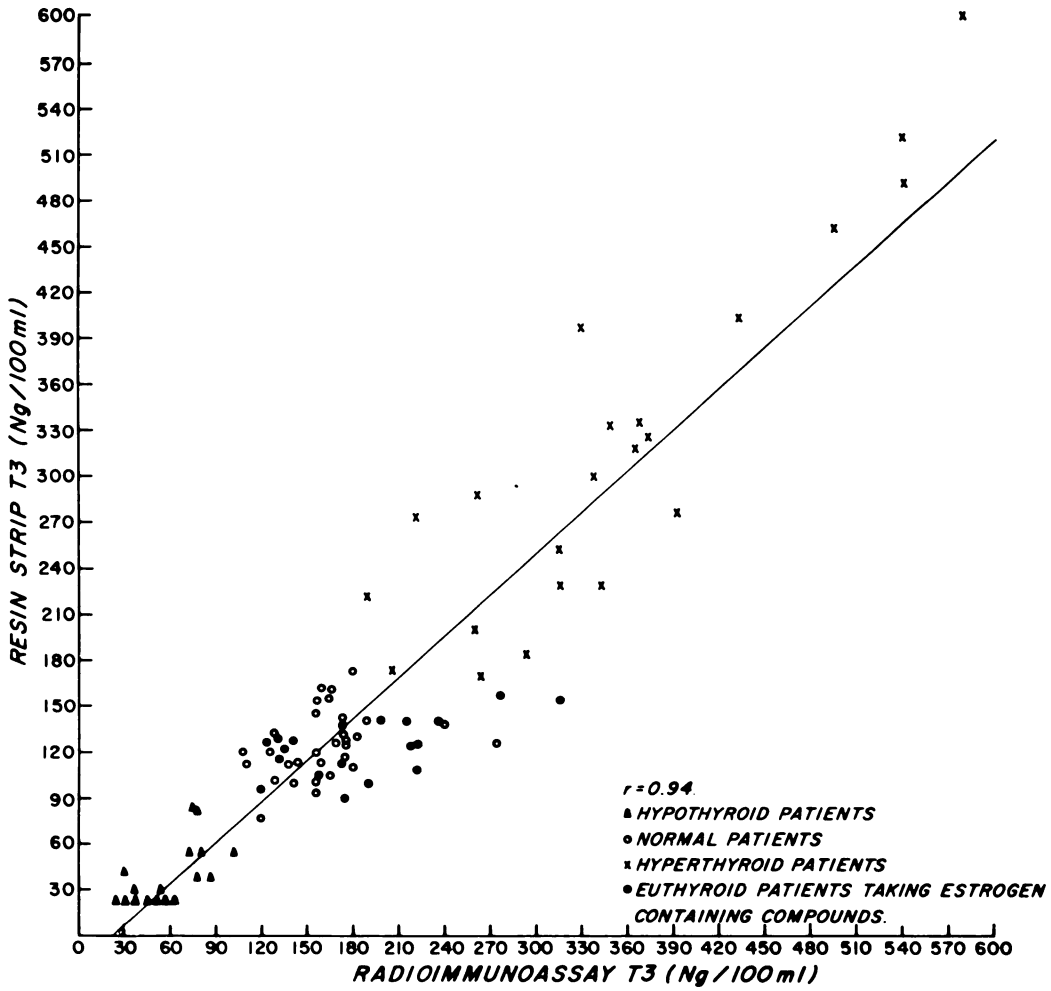

FIG. 1. Comparison of T_3 values obtained on same serum samples by two different techniques.

Reproducibility. *Interassay variability.* Monitrol I (normal control) was analyzed in 13 consecutive assays and had a coefficient of variation of 21.8%. Monitrol II (high control) in 13 consecutive assays had a coefficient of variation of 27.2%, while pooled plasma (normal control) analyzed in 13 consecutive assays had a coefficient of variation of 18.7%.

Intra-assay variability. To determine the average coefficient of variation, one sample was analyzed twice (each analysis in triplicate) in a particular assay. In this way, 11 such samples were analyzed and the coefficient of variation was 9.1%.

DISCUSSION

In order to assess the usefulness and accuracy of a new resin-strip technique to measure T_3, the same serum samples were analyzed by this method and by a method which utilizes a second antibody to separate antibody-bound hormone from free hormone. Comparative analysis of all 90 samples measured by the two methods revealed a correlation coefficient of 0.94. The correlation coefficients for individual clinical subgroups by the two methods ranged from 0.23 in normal subjects to 0.90 in hyperthyroid patients. Patients who were either clinically euthyroid or hyperthyroid had normal or elevated serum T_3 concentrations, respectively, when determined by the resin-strip technique. Two of 17 hypothyroid patients had normal serum T_3 concentrations. Previous reports suggest that normal serum T_3 concentrations are noted in approximately 19% of hypothyroid patients (*1*).

The principal difference between these two assay systems relates to how bound radioactive tracer is separated from unbound. The resin strip is an anion-exchange resin that binds and removes radioactive tracer that is unattached to antibody, whereas the other T_3 radioimmunoassay as described by Chopra, et al (*2*) employs a goat anti-rabbit antibody to precipitate the radioactive tracer that is antibody bound. The latter technique requires at least 12-hr

TABLE 1. T_3 MEASUREMENTS (ng/100 ml) OBTAINED IN VARIOUS CLINICAL CATEGORIES

Method	Number	Range	Mean ± s.d.
Resin-strip T_3			
Normal	32	76–174	124 ± 23
Hypothyroid	17	<25–81*	41 ± 20
Hyperthyroid	23	182–>600†	328 ± 113
Estrogen or pregnant	18	95–159	127 ± 17
Chopra, et al (2)			
Normal	32	110–240	163 ± 34
Hypothyroid	17	24–104	59 ± 24
Hyperthyroid	23	206–780	382 ± 141
Estrogen or pregnant	18	120–318	189 ± 55

* Serum T_3 levels < 25 were designated at 25 ng/100 ml for the purpose of statistical analysis.

† Values measured as > 600 were designated as 600 ng/100 ml for the purpose of statistical analysis.

incubation whereas the resin strip can perform this function in 1 hr, thus accounting for the more rapid performance time of the resin-strip technique.

Samples analyzed by the method of Chopra, et al tended to have a higher serum T_3 concentration than the same samples assayed by the resin-strip method. Gharib, et al (*3*) observed that variations in sensitivity and specificity of antisera as well as the degree of inhibition of T_3 binding to thyroid-binding globulin could account for different serum T_3 values on the same sample in two different assay systems. As long as these factors remain constant in a given technique, however, normal ranges can be obtained and deviations from this range may provide useful information.

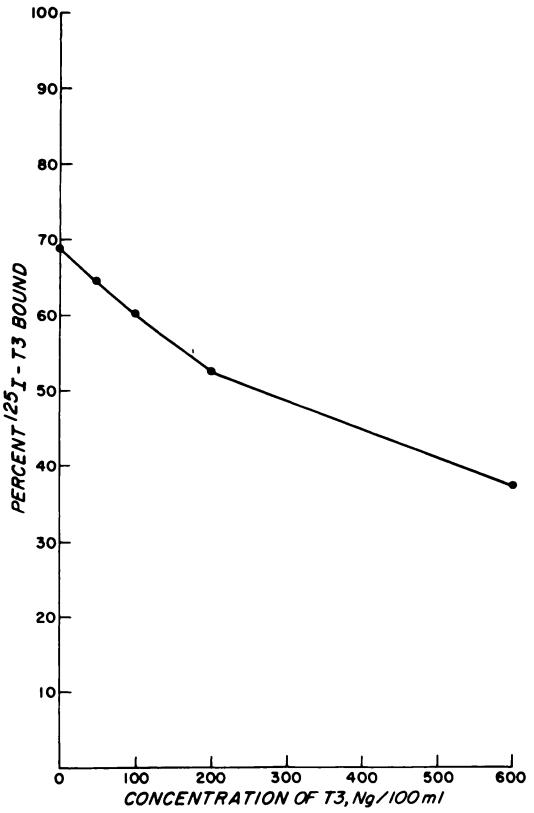

FIG. 2. Standard curve for measuring T_3 by resin-strip technique obtained from mean actual percent binding observed for each standard in 22 separate assays.

A favorable aspect of the resin-strip technique was the speed of the procedure which allowed the analysis in triplicate of T_3 concentrations in 35 unknown samples in 1 day. One unfavorable aspect of the resin-strip technique was an interassay variability that was slightly above the desired range; three different control sera were analyzed and the coefficients of variation ranged from 18.7% to 27.2%. Although attempts were not made to improve the sensitivity of the assay, the zero standard could be differentiated from the 50 ng/100 ml T_3 standard. Conceivably, a slight modification in the antibody titer might alter favorably the characteristics of the standard curve to make the assay even more sensitive. As employed, the standard curve resulted in a mean decrease in actual percent binding of 4.6% between the zero and 50 ng/100 ml standard, which permitted a lower limit of detectability of 25 ng/100 ml. Another desirable modification of this assay procedure would be the addition of nonimmune tubes so that the effectiveness of the resin strip in removing free radioactive T_3 in the absence of antibody could be analyzed separately in each assay.

In short, measurements of T_3 obtained by the resin-strip technique compared favorably with measurements obtained by the method of Chopra, et al. We believe that the resin-strip technique can be utilized effectively to aid in the clinical diagnosis and management of patients with thyroid disorders.

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