EVALUATION OF THE RESIN STRIP TECHNIQUE FOR DETERMINING SERUM T_3 BINDING CAPACITY AND SERUM THYROXINE

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The principal radionuclide *in vitro* tests for evaluating thyroid function are the T_3 test for indirect estimation of thyroid binding capacity of serum protein and the T_4 test for direct determination of serum thyroxine (T_4) by competitive protein-binding analysis (CPBA). There are several commercially available methods for determining T_3 values (*1*), but until recently there has been only one method for determining T_4 by CPBA (*2*). In 1968 a new type of resin strip procedure was introduced for both the T_3 (*3*) and the T_4 test (*4*). The resin strip techniques require fewer steps than other methods, thus significantly reducing performance time. The purpose of this paper is to record an evaluation of the new resin strip method for both tests.

MATERIALS AND METHODS

All T_3 and T_4 kits were used according to the procedures recommended by the manufacturers. The same technician analyzed all samples in a consistent manner, thus reducing variations due to individual differences in technique.

T_3 determinations. We compared the resin strip T_3 method (Res-O-Mat), with respect to the reproducibility of the technique and to accepted normal values, with the Tresitope kit (*5*), which was currently being used in our laboratory for routine T_3 resin uptakes.

The Tresitope technique requires 1 ml serum and uses an ion-exchange resin powder as the T_3 uptake medium which must be washed when incubation is complete. Tresitope results are expressed as percent resin uptake after appropriate corrections have been made for any temperature or time variation from 24°C and 1 hr incubation.

The Res-O-Mat procedure, which requires 0.5 ml serum, uses a resin strip and the washing step is eliminated although the incubation period is 2 hr. Control serum and a normalizing factor are supplied with each lot, and thus the T_3 values require no correction for time or temperature. With each batch of patient sera the control serum was analyzed in duplicate. Res-O-Mat T_3 values are expressed as a T_3 binding capacity (TBC) index. For comparison with Tresitope values, each TBC index was converted to relative percent uptake by means of the conversion table supplied by the manufacturer.

T_4I determinations. Since 1968 this laboratory has used the Tetrasorb-125 commercial kit (*2*) for all determinations of thyroxine iodine (T_4I). Correlation with clinical findings and with protein-bound iodine (PBI) determinations has been satisfactory (*6*). The Tetrasorb procedure is a resin-sponge modification (*7*) of the anion exchange resin technique (*8*). We undertook a comparison of the Tetrasorb and resin strip (Res-O-Mat) T_4 kits to determine if the Res-O-Mat procedure was satisfactory with respect to reproducibility and acceptable T_4I values.

The standard curve supplied with each different Tetrasorb lot was checked in duplicate at four points when that particular batch of ^{125}I-thyroxine binding globulin (TBG) was first used. Each time after that ^{125}I-TBG solution was used, the percent resin sponge uptake was checked in duplicate at one point (10 μg% T_4).

The Res-O-Mat kit uses a resin strip instead of a sponge, and the evaporation, icebath, and washing steps of the Tetrasorb method are eliminated. The manufacturers recommend determining the precount for one T_4 solution vial. On 10 occasions we determined precounts, each for an average of four vials and except for two cases found the variations between vials to be less than statistical counting inaccuracies. With each batch of patient sera a calibration line (bound fraction of labeled $T_4)^{-1}$ versus time, was determined in duplicate from two supplied reference standards, 0 and 15 μg% T_4.

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The mean T_4 extraction efficiency using absolute alcohol was 81% with a standard deviation of 6% when measured 16 times on two occasions. All T_4 results were corrected by this factor and then multiplied by 0.653 to give T_4I.

Serum samples. Samples of serum were obtained from patients referred, in most cases, to the nuclear medicine laboratory of the University of Florida College of Medicine and, in a few cases, to the radioisotope service of the Gainesville Veterans Administration Hospital between November 1969 and March 1970.

All serum samples received were divided immediately into the quantities required for T_3 and T_4 determinations using the four kits described here. If there was sufficient serum, requisite amounts were apportioned for duplicate determinations and then all samples were refrigerated for 1 day or frozen up to 4 days until used. Each sample of serum was defrosted only once, just before use.

Reproducibility. To determine the within-batch reproducibility of the T_3 and T_4 kits, serum samples were analyzed in duplicate using each method. The serum samples for duplicate determinations were not chosen specially and they therefore include other than euthyroid samples.

Quality control. To test the between-batch reproducibility of the T_3 and T_4 kits, samples of Hyland and Versatol control serums were analyzed as a quality control over a period of several weeks with each batch of patient sera.

Comparison of variances. Variances (σ^2) were tested in pairs for significance using the F-distribution (*9*). The test quotient for the hypothesis ($\sigma_1^2 = \sigma_2^2$) is σ_1^2/σ_2^2, where $\sigma_1^2 > \sigma_2^2$. If it is smaller than the significance limit F (for degrees of freedom $\nu_1 = N_1 - 1$ and $\nu_2 = N_2 - 1$) then it can be assumed that $\sigma_1^2 = \sigma_2^2$. If the test quotient attains or exceeds F, then $\sigma_1^2 \neq \sigma_2^2$.

Paired determinations. One-hundred thirty-two serum samples that were not selected specially were analyzed in pairs for T_3 uptake using the Tresitope and Res-O-Mat techniques and in pairs for T_4I using the Tetrasorb and Res-O-Mat techniques. Some of the 132 samples were analyzed in duplicate with one or more of the techniques, and these duplicate determinations are included in the section on the reproducibility of the various methods. For the purpose of one-to-one comparison between paired sample determinations using both T_3 or both T_4 kits, the mean value was used if one of the determinations was made in duplicate.

RESULTS

Reproducibility of T_3 test. The results of duplicate determinations of T_3 percent uptake are given in Table 1. The range of differences between duplicate determinations and the standard deviation of the differences are greater for the Tresitope kit than for the Res-O-Mat kit. Res-O-Mat T_3 duplicates have a variance significantly lower ($p < 0.01$) than that of Tresitope T_3 duplicates.

Quality control of T_3 test. Table 2 gives the results of T_3 determinations for Hyland and Versatol control serums. Each Hyland lot has an assigned T_3 value and acceptable range, and both the Tresitope and Res-O-Mat kits give T_3 values within this range.

TABLE 1. DUPLICATE DETERMINATIONS OF T_3 UPTAKE

T_3 kit	Tresitope	Res-O-Mat
Number of duplicate samples	29	45
Ranges of differences between duplicates (% uptake)	0.1–8.2	0–3.9
Mean difference (% uptake)	1.3	1.1
Variance of differences σ^2	2.3	0.69
Standard deviation σ	1.5	0.8
Mean of all values	31.8	28.0
σ as a fraction of the mean	0.047	0.030

TABLE 2. BETWEEN BATCH REPEAT DETERMINATIONS OF % T_3 UPTAKE FOR HYLAND AND VERSATOL CONTROL SERUMS USING TWO T_3 KITS

T_3 kit:	Tresitope				Res-O-Mat		
Control Serum:	Hyland		Versatol		Hyland	Versatol	
Lot:	a	b	c	d	b	c	d
Assigned T_3 value (by resin sponge)	30	32			32		
Acceptable range	27–33	29–35			29–35		
No. of determinations	4	17	11	6	17	11	6
Range of % T_3 uptake	29.5–32.2	30.6–39.7	33.7–42.6	36.5–38.5	27.7–32.7	26.9–31.2	28.8–30.4
Mean % T_3 uptake	31.0	35.4	39.1	37.5	30.6	29.6	29.5
Standard deviation σ	1.4	3.1	2.9	0.8	1.3	1.4	0.5
σ as a fraction of the mean	0.045	0.088	0.074	0.021	0.042	0.048	0.019

Versatol standard serum is not supplied with an assigned T_3 value but the range and standard deviation of the Versatol T_3 values using both kits may be compared. It is apparent that the Tresitope kit results in a surprisingly high Versatol T_3 uptake and that the standard deviations using the Tresitope method are larger than corresponding Res-O-Mat values.

The variances in Table 2 were tested in pairs, and the following conclusions were obtained at the 0.05 probability level:

1. For Hyland T_3 determinations the standard deviation, 1.3, using the Res-O-Mat kit is significantly less than the equivalent, 3.1, using the Tresitope kit.
2. For Versatol T_3 determinations the difference between the standard deviations, 2.9 and 1.4, for lot c using the Tresitope and Res-O-Mat kits, respectively, is significant whereas the standard deviations, 0.8 and 0.5, for lot d are not significantly different.

Paired determinations in T_3 test. Figure 1 shows the comparison between T_3 uptake values determined with Tresitope and Res-O-Mat T_3 kits for 132 paired samples. If both techniques gave the same T_3 resin uptake values, the scatter plot would follow the 45-deg line drawn. The means of all 132 values are 33 and 29% for the Tresitope and Res-O-Mat techniques, respectively. The range of differences (Tresitope minus Res-O-Mat) is −4.8 to +23.8% with a mean difference of +3.9% and a standard deviation of 3.9%. The distribution of differences was analyzed using the Wilcoxon Test for pair differences (*10*) and gave a probability $p < 0.001$ of nonsignificance.

The clinical record of each patient included in the series of paired determinations was examined from 1 to 4 months after discharge. The T_3 results for patients who had received drugs or had conditions known to increase (salicylates, Prednisone, Heparin, Dicumarol, Dilantin, androgens, anabolic steroids, Oragraffin, pulmonary insufficiency, nephrosis, hepatitis, cirrhosis, paroxysmal atrial arrhythmia) or decrease (estrogens, antiovulatory medication, pregnancy) T_3 resin uptake were excluded from evaluation of a euthyroid range. In addition patients on treatment for hypothyroidism or hyperthyroidism or in whom a diagnosis of hypothyroidism or hyperthyroidism was made were excluded. When this was done there remained, of the 132 paired samples, 69 T_3 results for euthyroid patients which are indicated in Fig. 1. An analysis of these values is summarized in Table 3 for both T_3 techniques.

Comparison of T_3 results using both methods for patients in various categories (anticoagulant therapy, seven patients; estrogen therapy and pregnancy,

TABLE 3. THE NORMAL RANGE

Technique	Tresitope T_3 % uptake	Res-O-Mat T_3 % uptake	Tetrasorb T_4 I μg/100 ml	Res-O-Mat T_4 I μg/100 ml
Number of euthyroid patients	69	69	85	85
Range of values	24–45	19–38	2.9–9.7	3.0–12.0
Mean value	33	29	6.1	6.9
Standard deviation σ	4.2	3.1	1.5	1.8
95% euthyroid range (mean ± 2σ)	24–41	23–35	3.1–9.0	3.2–10.6

TABLE 4. DUPLICATE DETERMINATIONS OF SERUM THYROXINE

T_4 kit	Tetrasorb	Res-O-Mat
Number of duplicate samples	65	35
Range of differences between duplicates (μg% T_4I)	0–1.6	0–2.3
Mean difference (μg% T_4I)	0.3	0.7
Variances of differences σ^2	0.086	0.28
Standard deviation σ	0.3	0.5
Mean of all values	6.4	7.2
σ as a fraction of the mean	0.045	0.074

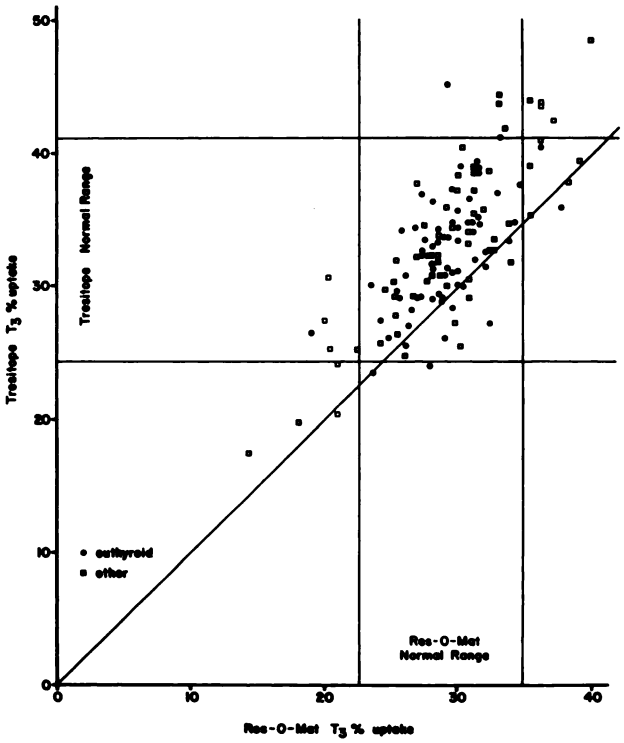

FIG. 1. Scatter plot of paired T_3 determinations using Tresitope and Res-O-Mat techniques. 95% euthyroid ranges are indicated.

TABLE 5. BETWEEN BATCH REPEAT DETERMINATIONS OF THYROXINE IN HYLAND AND VERSATOL CONTROL SERUMS USING TWO T_4 KITS

T_4 kit:	Tetrasorb			Res-O-Mat		
Control Serum:	Hyland	Versatol		Hyland	Versatol	
Lot:	b	c	d	b	c	d
Assigned Value:						
Uncorrected	4.1*	6.1†	6.4†	4.1*	6.1†	6.4†
Corrected		6.8	7.1		6.8	7.1
No. of determinations	17	11	13	12	8	5
Range (T_4I μg%)	4.2–7.1	6.2–8.5	5.5–7.7	6.5–10.8	10.3–12.4	8.4–9.9
Mean (T_4I μg%)	6.0	7.4	6.9	8.5	11.5	9.1
Standard deviation σ	0.7	0.7	0.6	1.3	0.8	0.6
σ as a fraction of the mean	0.11	0.10	0.09	0.15	0.07	0.06

* T_4I μg% (T_4 by column).
† PBI μg%, corrected assuming 90% recovery.

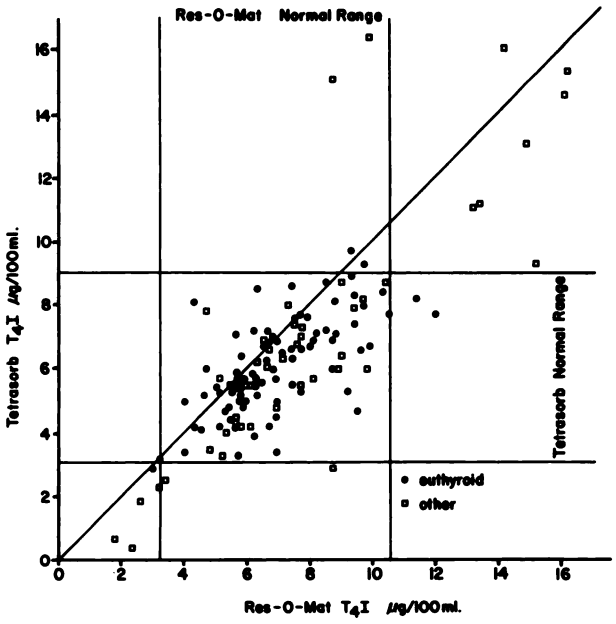

FIG. 2. Scatter plot of paired T_4I determinations using Tetrasorb and Res-O-Mat techniques. 95% euthyroid ranges are indicated.

eight patients; pulmonary insufficiency, five patients; hypothyroid, four and hyperthyroid, four patients) did not reveal significant differences when examined as groups. The numbers of patients in other categories (liver disease, etc.) were too few to draw conclusions.

Reproducibility of T_4 test. The results of duplicate determinations of thyroxine iodine are given in Table 4. The range of differences between duplicate determinations, the mean difference, and the standard deviation of the differences are greater for the Res-O-Mat kit than for the Tetrasorb kit. The Tetrasorb T_4 duplicates have a variance significantly lower ($p < 0.01$) than that of the Res-O-Mat T_4 duplicates.

Quality control of T_4 test. Table 5 gives the results of T_4I determinations for Hyland and Versatol control serums. Assigned PBI values for Versatol were corrected for 90% recovery (*11*). It is seen that T_4I values for the control serums using the Res-O-Mat technique are very high whereas Tetrasorb values are in agreement with assigned values.

The variances in Table 5 were tested in pairs, and no significant difference was found between any pair of variances at the 0.05 probability level except for the following:

1. For Hyland T_4I determinations the standard deviation, 0.7, using the Tetrasorb kit is significantly less than the equivalent, 1.3, using the Res-O-Mat kit.
2. Using the Res-O-Mat kit the standard deviation, 1.3, for Hyland T_4I determinations is significantly greater than 0.8 and 0.6 for Versatol T_4I determinations.

Paired determinations in T_4 test. Figure 2 shows the comparison between T_4I values determined with Tetrasorb and Res-O-Mat T_4 kits for 132 paired samples. If both techniques gave the same T_4I values for the paired samples, the scatter plot would follow the 45-deg line drawn. The means of all 132 values are 6.4 and 7.3 μg% for the Tetrasorb and Res-O-Mat techniques, respectively. The range of differences (Tetrasorb minus Res-O-Mat) is −5.9 to +6.5 μg% with a mean difference of −0.9 μg% and standard deviation of 1.7 μg%. The distribution of differences was analyzed using the Wilcoxon test for pair differences (*10*) and gave a probability $p < 0.001$ of nonsignificance.

The clinical record of each patient included in the series of paired determinations was examined from 1 to 4 months after discharge. The T_4 results for patients who had received drugs or had conditions known to increase (Choloxin (d-Thyroxine), estro-

gens, antiovulatory medication, pregnancy) or decrease (androgens, anabolic steroids, nephrosis, hepatitis, cirrhosis, widespread malignancy) the T_4 estimate were excluded from evaluation of a euthyroid range. In addition, patients on treatment for hypothyroidism or hyperthyroidism or in whom a diagnosis of hypothyroidism or hyperthyroidism was made were excluded. When this was done there remained, of the 132 paired samples, 85 T_4 results for euthyroid patients which are indicated in Fig. 2. An analysis of these values is summarized in Table 3 for both T_4 techniques.

Comparison of T_4 results using both methods for patients in various categories (estrogen therapy and pregnancy, eight patients; hypothyroid, four patients and hyperthyroid, five patients) did not reveal any significant differences when examined as groups. The numbers of patients in other categories, such as liver disease, were too few to draw conclusions.

Subsequent to the study of 132 paired T_4 determinations, an additional 30 euthyroid patient samples were analyzed using the Tetrasorb technique and the values were added to the original 85 to obtain a euthyroid sample size of 115 for the Tetrasorb method. This sample has a mean 6.0 μg% T_4I with a standard deviation 1.5 μg% and 95% normal range 3.1–8.9 μg%. It is seen that these additional 30 samples do not change the Tetrasorb euthyroid range.

DISCUSSION

T_3 test. The Res-O-Mat method has superior within-batch and between-batch reproducibility and a smaller euthyroid range compared with the Tresitope method. Although the total running time of the Res-O-Mat test is no shorter than that of the Tresitope test, the time spent by the technician is less. The relative diagnostic accuracy of both techniques do not appear to be significantly different. The few hypothyroid and hyperthyroid samples analyzed had resin uptakes outside both euthyroid ranges. With both methods all but three of the euthyroid samples fell within the appropriate euthyroid range.

In conclusion, we find the Res-O-Mat resin strip T_3 procedure to be satisfactory and our method of choice for determination of T_3 binding capacity.

T_4 test. The Res-O-Mat method has inferior within-batch and between-batch reproducibility, and a larger euthyroid range compared to the Tetrasorb method. We believe much of the inaccuracy in the Res-O-Mat method arises from the calibration line. It is of such shallow slope that any inaccuracy in the estimate for the bound fraction of labeled T_4 is enhanced in the T_4 estimate. Furthermore, the calibration line is drawn through only two reference points. The addition of a third standard reference point would provide a check on the validity of the calibration line.

The relative discriminatory powers of both techniques do not appear to be significantly different. None of the hypo- or hyperthyroid samples analyzed fell within the euthyroid range of either technique. With both methods all but three of the euthyroid samples fell within the appropriate euthyroid range.

Our standard deviation of 0.3 μg% for within-batch duplicate T_4I determinations using the Tetrasorb method (Table 4) compares favorably with other published values of 0.3–0.5 μg% (*12,13*). Murphy (*8*) made 12 separate T_4I determinations on pooled serum over a 6-week period and obtained a corrected mean of 6.1 μg% and standard deviation of 0.6 μg% which is 0.10 of the mean. Our values for Versatol and Hyland control serums in Table 5 compare favorably.

Subsequent to the comparative study reported here, 43 T_4 determinations (in duplicate) of Versatol standard serum were perfomed using the Tetrasorb technique over a 15-week period. The mean deviation of the measured T_4I value from the expected value was $+0.1$ μg% (2 s.d. $= 1.3$ μg%).

In conclusion, although the Res-O-Mat resin strip procedure is technically simpler and shorter, our method of choice for determination of serum thyroxine is the Tetrasorb resin sponge technique because of its superior results regarding duplicate determinations and reproduction of control serum T_4I values.

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