

Simultaneous Resin Uptake Studies of Labeled Thyroxine and Triiodothyronine in Thyroid Disease¹

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Preliminary studies reported by us (1) pointed out the relative advantages of ¹³¹I-labeled L-thyroxine (T₄-¹³¹I) over ¹³¹I-labeled L-triiodothyronine (T₃-¹³¹I) in the resin uptake of labeled iodoamino acids (T₃-RU, T₄-RU) for the diagnosis of hyperthyroidism. The present study represents further experience in the comparison of the resin uptake of these labeled thyroid hormones as measurements of thyroid function in various conditions.

MATERIAL AND METHODS

The clinical material consisted of patients on the medical services of the various hospitals of the Indiana University Medical Center and normal volunteers. The study includes 290 determinations performed on 242 subjects. In the group were 107 normal individuals, 24 hyperthyroid and 19 hypothyroid patients, 11 pregnant individuals and 24 patients with chronic debilitating illnesses. The diagnosis of thyroid disease was made on the basis of clinical findings, serum protein-bound iodine determination and thyroidal uptake of radioiodine.

Serum was stored at -20° C until used. A control serum pool, 400 ml, was prepared from samples obtained from 50 normal subjects; small aliquots were stored at -20° C for subsequent use in each run.

The procedure used was essentially that described by Mitchell, Hardin and O'Rourke (2) with certain modifications noted. The T₃-¹³¹I in Tris maleate buffer solution, 0.1 mg, pH 5.2, contained 0.10 to 0.12 μC and 0.0033 to 0.0051

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μg per ml, while the labeled thyroxine in identical buffer solution contained 0.10 to 0.13 μC and 0.0026 to 0.0064 μg per ml.¹ On arrival concentrated human serum albumin was added to the isotope solutions to prevent absorption to glassware; the final albumin concentration was 0.5 per cent. The solutions were stored at 4-10°C. No solutions were used beyond a two week period. Over this period there was no significant change in control resin uptake with any particular batch. Had dissociation of ¹³¹I occurred, an increasing trend in resin uptake would have become evident with time.

Preliminary studies indicated that equilibration was nearly complete at about 120 minutes for both T₃-RU and T₄-RU. Varying the incubation time from 30 to 120 minutes in hyperthyroid and hypothyroid sera did not alter separation from normal. Varying the temperature from 21°C to 36°C for the 120 minute incubation time showed a linear increase in resin uptake for both iodinated amino acids of about one per cent per degree. All subsequent runs were performed at room temperature, 24-28°C, corrected to 25°C. Residual sponge radioactivity was determined at the end of the incubation time with aspiration of serum only and after each of five consecutive washings. Very little decrease in sponge radioactivity occurred after the third washing; it was elected to use four washings in all subsequent runs.

The test procedure and calculations were otherwise those of Mitchell, *et al.* Control sera for T₃-RU and T₄-RU were processed simultaneously by the same method. The results were expressed as a per cent of control serum value for the particular run. The average T₃-RU and T₄-RU values on control serum for 56 runs with 22 shipments of T₃-¹³¹I and T₄-¹³¹I were 40.2 per cent (range, 33.8-45.1 per cent) and 11.7 per cent (range 8.2 - 16.0 per cent), respectively. All radioactive counting was done in duplicate in a scintillation well counter for a sufficient period of time to keep the probable error below one per cent.

Test runs were carried out in duplicate during the initial phase of the study. The mean difference between duplicate values, expressed as per cent of control, for 45 tests was 5.1 percentage points for T₃-RU and 7.7 percentage points for T₄-RU. Duplicate determinations were discontinued when it was established that the maximum possible error at the 95% confidence level² from the true value was 9.3 percentage points with single determinations and reduced only to 6.6 percentage points with duplicate determinations for the T₃-RU. Corresponding values of maximum possible errors for the T₄-RU were 14.1 percentage points with single determinations and 10.0 percentage with duplicate determinations. All statistical calculations were performed according to methods in Steel and Torrie (3).

¹The resin sponge (Triosorb) and labeled hormones were kindly supplied by Dr. Howard Glenn of Abbott Laboratories.

²A pooled estimate of standard deviation, with 30 pairs of duplicate determinations, was used in computing the confidence limits.

RESULTS

Effect of age on resin data. The effect of age on the resin uptake values in the 107 euthyroid individuals is shown in Figure 1. There was no significant trend in either resin study in this group.

Resin data in various thyroid states. The results in various thyroid states are shown in Table I and Figure 2. The data in the chronically ill group or sick patients were moderately elevated and overlapped into the hyperthyroid group in both studies. Sixty-two and a half per cent for T_3 -RU but only 25.0 per cent of the T_4 -RU data for the sick group were found to be in 95% euthyroid range (Table I). In order to overcome the diagnostic problems between the sick and hyperthyroid groups the euthyroid range was arbitrarily raised to 125% for T_3 -RU and 160% for T_4 -RU (See clear box in Fig. 2). Using these upper euthyroid limits, only two, eight per cent, of the hyperthyroid patients were found in the adjusted range for both studies. Two additional patients had only T_4 -RU data in this range while an additional hyperthyroid patient had only T_3 -RU results below 125 per cent.

Among the sick group, results in four patients, 16%, were found above the 125% limit for T_3 -RU, but no T_4 -RU data were found above the 160% level.

Correlation of resin data with PBI. The correlation of T_3 -RU and T_4 -RU data with PBI are shown in Figure 3. This group of data excludes subjects under the influence of exogenous iodide. The linear relationship is apparent except in two cases of hypoproteinemia with low PBI's and high resin uptakes.

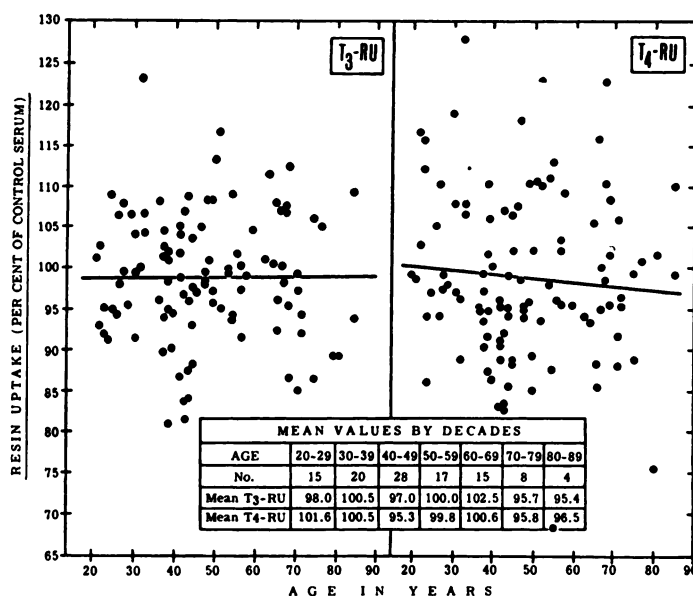


Fig. 1. Resin uptake of T_3 and T_4 in various age groups of euthyroid individuals, as per cent of control serum. The regression lines, derived by the method of least squares, are shown for both studies. The correlation coefficients for the studies with age were 0.004 and 0.07 for T_3 -RU and T_4 -RU respectively.

Studies during treatment of thyroid disease. The resin uptake tests were used in following the course of several patients being treated for thyrotoxicosis and myxedema. The results of these tests correlated well with the clinical course and serum protein-bound iodine determinations. No improvement in diagnostic discrimination over that obtained with the resin uptake was observed with the PBI. However, in the event of exogenous iodide contamination, the resin uptake provided an accurate appraisal of the clinical course in case of a grossly elevated PBI. Two such cases are graphically represented in Figures 4 and 5.

Effect of iodine and iodinated dyes. Resin uptakes and PBI determinations were performed on the sera of a number of subjects before and after the administration of both inorganic and organic iodine. It was apparent that neither inorganic nor organic iodide caused a significant change in the resin uptake results.

Miscellaneous. In a few patients receiving salicylates, ten grams every four hours, and other patients with respiratory acidosis and high arterial blood carbon dioxide tensions, moderate elevation of the T_4 -RU and less marked elevation of the T_3 -RU were observed as in the sick group. These are not included in the data because of the small number of such cases.

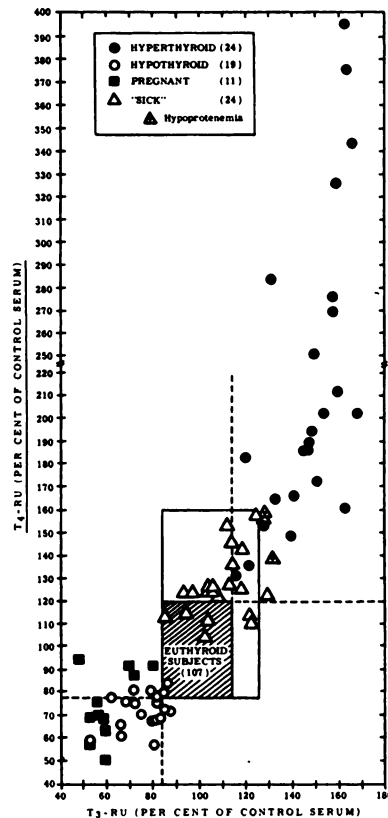


Fig. 2. Relation of T_3 -RU and T_4 -RU in the same subjects in various thyroid states.

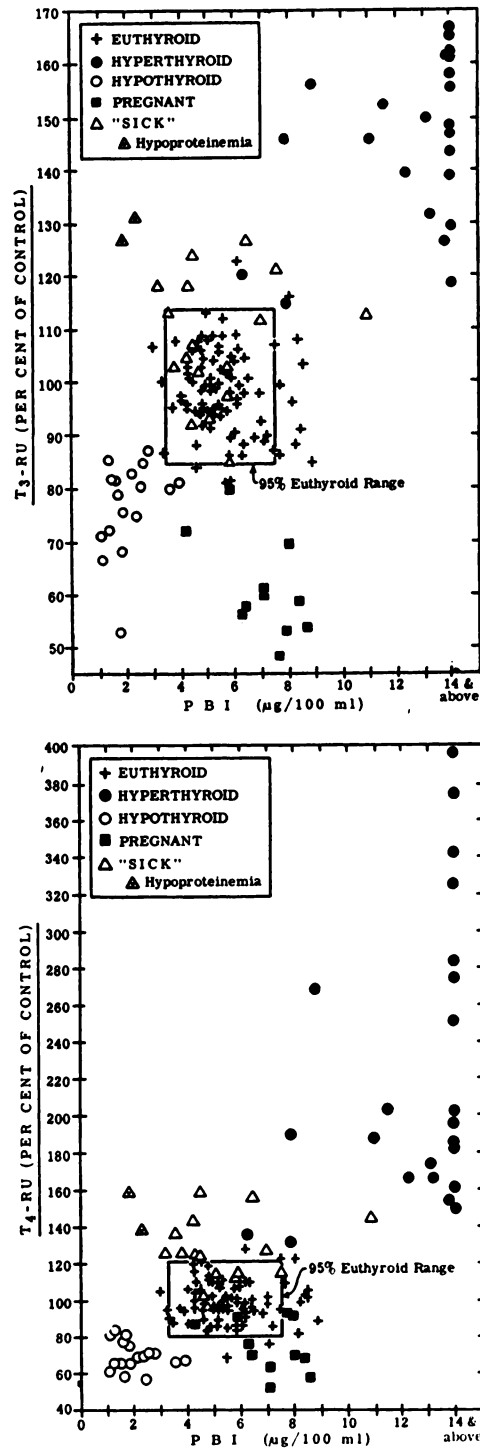


Fig. 3. Relation of T₃-RU (a) and T₄-RU (b) to PBI in various thyroid states. The PBI 95% euthyroid range for our laboratory is 3.4 - 7.6 mg/100 ml (based on 607 euthyroid individuals, using a modified chloric acid digestion technique with brucine and point stabilization) (18).

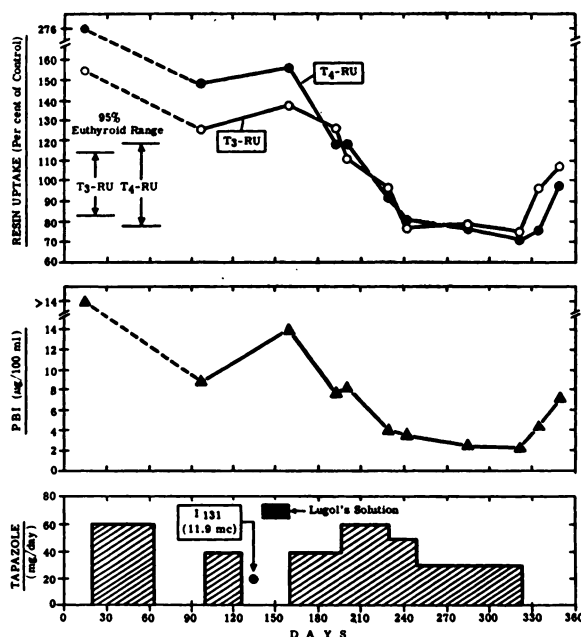


Fig. 4. Correlation of T_3 -RU and T_4 -RU with PBI in a hyperthyroid patient, C. G., following treatment.

DISCUSSION

The use of T_4 - ^{131}I in the resin uptake test for thyroid disease has received little attention. The resin uptake of ^{131}I -labeled thyroxine (T_4 -RU) was originally employed by Mitchell, *et al* on sera of pregnant and nonpregnant women (4) and later in thyroid disease (5); however, no relative differences in discrimination were noted between thyroid states from what was observed previously with the T_3 -RU (2). Brief comments on comparison of T_3 -RU and T_4 -RU were made (6, 7) with equal discriminating ability in thyroid disease being reported with the two tests. Preliminary studies reported by us (1) indicated a marked improvement in separation of normal and hyperthyroid subjects with the T_4 -RU and suggested that concomitant performance of the T_3 -RU and T_4 -RU may provide an improved diagnostic technique in this and other conditions.

The data in this study clearly illustrate that the hyperthyroid group is better separated from the euthyroid by the T_4 -RU than by the T_3 -RU, although no overlap occurred with either test. This finding should be suspected from what is known of the binding properties of L-triiodothyronine (T_3) and L-thyroxine (T_4) and of the alterations in the level of circulating thyroxine and the binding characteristics of the thyroxine-binding proteins (TBP) in hyperthyroidism.

Three separate serum proteins which bind thyroxine have been identified by paper electrophoresis in TRIS-maleate buffer using tracer amounts of ^{131}I -labeled L-thyroxine (T_4 - ^{131}I) (8). The thyroxine-binding proteins are (1) thyroxine-binding globulin (TBG) which migrates electrophoretically between alpha-1 and alpha-2 globulin; (2) thyroxine-binding pre-albumin (TBPA) which has a

slightly more anodal migration than albumin; and (3) albumin itself. L-thyroxine is bound by all three protein moieties, whereas, L-triiodothyronine is bound only by TBG and albumin (9).

Since the binding capacity of TBPA is reduced in hyperthyroidism (9, 10), the saturation of the TBP is relatively more increased for T_4 than for T_3 in this condition. Consequently, this alteration in the binding capacity of TBPA would be expected to be reflected more directly in the T_4 -RU with an increase relative to normal greater than that seen with the T_3 -RU. The somewhat higher T_4 -RU results, compared to T_3 -RU results, in the *sick* euthyroid group is due to reduced TBPA capacity in these patients (11), with a direct effect on the T_4 -RU and only an indirect or displacing effect on the T_3 -RU. This might be expected to cause less discrimination between the hyperthyroid and *sick* groups with the T_4 - ^{131}I , but adjustment of the *normal range* resulted in reduction of the overlap between hyperthyroid and euthyroid *sick* data for both tests with the T_4 -RU showing a wider separation of the two groups. Nevertheless, recognition of co-existing chronic debility or acute stress (11-13) is necessary in interpreting resin uptake values in the diagnosis of thyroid disease.

Although TBPA capacity is reduced in hypothyroidism (9, 10), the increased TBG capacity in this state (9, 10) apparently resulted in slightly better diagnostic discrimination with the T_3 -RU than with T_4 -RU. However, both studies performed concomitantly, appeared to differentiate the hypothyroid patient from the euthyroid group much better than either test alone.

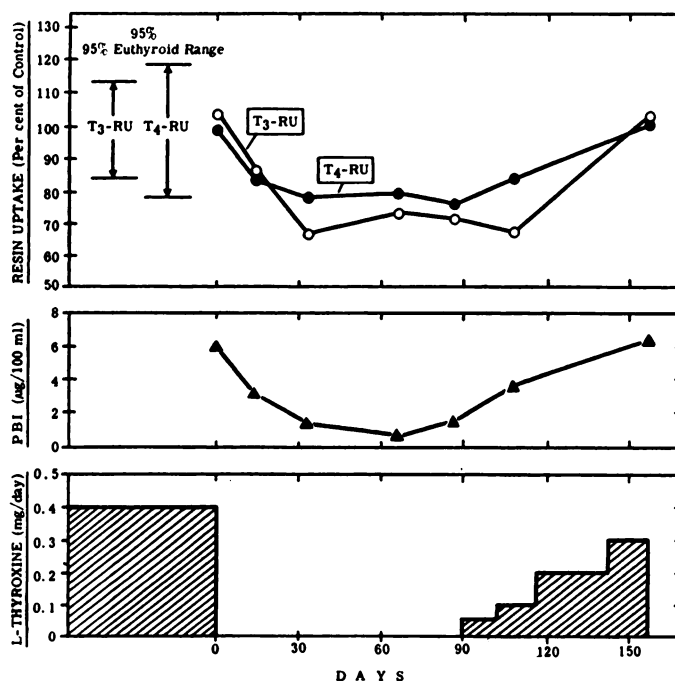


Fig. 5. Correlation of T_3 -RU and T_4 -RU with PBI in a hypothyroid subject, H. S., with and without replacement therapy.

TABLE I
SUMMARY OF RESIN UPTAKE DATA IN VARIOUS THYROIDAL AND NONTHYROIDAL CONDITIONS

Group	Resin Uptake (Per Cent of Control Serum)			Per Cent in 95% Euthyroid Range ¹	
	Test	Mean \pm S.D.	Range	T ₃ -RU (84.2-113.6)	T ₄ -RU (77.8-119.6)
Euthyroid (107)	T ₃ -RU	98.9 \pm 7.4	81.1-123.2		
	T ₄ -RU	98.7 \pm 10.6	75.6-127.7		
Hyperthyroid (24)	T ₃ -RU	146.0 \pm 15.3	115.3-167.2	0	0
	T ₄ -RU	222.1 \pm 76.2	131.9-396.0		
Hypothyroid (19)	T ₃ -RU	76.5 \pm 9.5	52.7- 87.5	15.8	31.6
	T ₄ -RU	72.6 \pm 7.8	57.6- 84.6		
Pregnancy (11)	T ₃ -RU	60.7 \pm 9.4	48.0- 79.7	0	36.4
	T ₄ -RU	74.5 \pm 15.0	50.5- 94.6		
Sick (24)	T ₃ -RU	111.2 \pm 12.5	85.1-131.1	62.5	25.0
	T ₄ -RU	129.4 \pm 16.1	104.4-159.4		

¹Calculated from Mean \mp $|2t_{.05} \times \text{S.D.} \sqrt{1 + \frac{1}{n}}|$, where t.05 is 2-tailed.

The T_3 -RU values were slightly lower than the T_4 -RU values in pregnancy. This would be expected from the known increase in TBG capacity in pregnancy (14). Not sufficient determinations were performed to correlate resin uptake values with duration of gestation.

The effect of age, studied with erythrocyte uptake of ^{131}I -labeled L-triiodothyronine, was reported to show a linear decrease of about 0.1 per cent per year, from an average of 16 per cent to 9 per cent, for normal subjects ranging from 25 to 90 years of age (15). Unpublished data (10) on normal subjects up to age 50 indicate a constant thyroxine-binding capacity of TBG, while TBPA capacity rises with age. The daily fractional turnover rate of thyroxine was reported to decrease with age up to the sixth decade (16). Since there is no trend in the protein-bound iodine determinations with age (17, 18), it would seem that the increased TBPA binding capacity would result in a declining resin uptake, and that this should be more pronounced with the T_4 -RU. Failure of our T_3 -RU and T_4 -RU values to display any trend with age would seem to indicate that these studies are not sufficiently sensitive to reflect subtle physiologic alterations. More sensitive studies, as equilibrium dialysis for free thyroxine, might shed more light on the changes with age.

In our experience the resin uptake tests have provided an accurate index of response to therapy with desiccated thyroid and L-thyroxine, antithyroid drugs, radiiodine therapy and thyroidectomy. The exogenous administration of inorganic or organic iodine, as previously noted, did not interfere with the resin uptake test in this study.

SUMMARY

The resin uptake of both ^{131}I -labeled triiodothyronine and ^{131}I -labeled thyroxine (T_3 -RU, T_4 -RU) were performed concomitantly in 107 normal subjects and patients with thyroidal and nonthyroidal disease. The results indicate definite superiority of the T_4 -RU in the diagnosis of hyperthyroidism. The delineation of the hypothyroid state is less satisfactory by either test alone although T_3 -RU results and T_4 -RU results are equally reliable. The concomitant performance of both increases the diagnostic reliability. Changes in resin uptake values with age in normal adults, as reported by others, were not seen in this group. The value of the resin uptake in following therapy of thyroid disease is particularly helpful in the event of inorganic or organic iodide contamination.

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