THYROID FUNCTION MEASURED BY IN VITRO ERYTHROCYTE UPTAKE (RED-CELL UPTAKE) OF I^{131}-LABELED *l*-TRIIODOTHYRONINE*

Results of 133 Determinations

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SINCE it is now known[1-3] that there are differences in the amount of thyroxin-binding protein in the blood, in the various grades of thyroid activity, the work of Hamolsky and associates,[4,5] and Crispell, Kahana, and Hyer[6] suggested that one might estimate the amount of thyroxin-binding protein, and study thyroid function clinically, by the in vitro erythrocyte uptake of I^{131}-labeled *l*-3, 5, 3 prime-triiodothyronine (TRI^{131} red-cell uptake, hereinafter called RCU). Others have shown that there are many factors that altered the RCU of *l*-triiodothyronine other than the grade of thyroid function. It is decreased in pregnancy,[4-8] during estrogen therapy, and increased with Dicumarol† therapy, nephrosis, hepatic disease, emphysema, and severe illness[9] of any cause. Since the evidence to date indicates that this parameter of thyroid function is rarely affected by exogenous iodine medication, there are many[4-8] who believe it probably will have considerable clinical usefulness. This report is a summary of our experience with 133 determinations in patients who had various thyroid disorders.

Method

As previously described by Hamolsky and associates,[4,5] and by Crispell, Kahana, and Hyer[6] the method consists principally of adding a standard solution of TRI^{131} to 5 ml. of oxalated, citrated, or heparinized blood. In our initial experiment, oxalated blood was used, but now EDTA‡ is used as an anticoagulant. The blood was collected in a commercially prepared vacuum collection tube, and it was found that some coagulation took place when the TRI^{131} was added to the oxalated blood unless the amount of oxalate in the tube was doubled, and unless oxalate was added to the saline solution used for washing the cells. EDTA prevented this coagulation and in addition decreased the hemolysis during the washing procedure.

The TRI^{131} solution was added to the specimen in the collecting tube so that

**The radioactive material (Radio-L-Triiodothyronine Sterile Solution) used in this investigation was supplied by Abbott Laboratories on the authorization of the Isotopes Division, United States Atomic Energy Commission, Oak Ridge, Tennessee.*

†Dicumarol, Abbott Laboratories.

‡EDTA is dipotassium-ethylenediaminetetraacetate.

when 1 ml. of the TRI^{131}-blood mixture was tested in a scintillation (well) counter,* about 8,000 to 10,000 counts per minute were registered. This was determined by experience to be the optimum amount of radioactive material to give the most consistent and reproducible results. After adding the TRI^{131}, the blood was incubated on a vertically rotating mixing wheel at 37 C. for three hours. At the end of the incubation time, three aliquots of 1 ml. each were taken for analysis. The first aliquot was used as the whole blood control, and was pipetted directly into a 12 mm. by 100 mm. test tube and held until the other aliquots had been processed. The other two aliquots were duplicate specimens used to measure the residual radioactivity present after washing the cells four times with physiologic saline solution. These specimens were pipetted directly into polystyrene, round-bottom centrifuge tubes, 15 mm. by 100 mm. This plastic is believed to cause less hemolysis than does glass; the tube can be freed from radioactivity faster than can glass after it has been used, and, moreover, plastic eliminates breakage. After the cells were washed, they were lysed and were transferred quantitatively to the test tubes. The three tubes were then made up to equal volumes with distilled water, which also lysed the cells. Radioactivity of the material in the tubes was determined with a scintillation (well) counter. Three one-minute counts were taken for each specimen. The RCU was calculated by dividing the counts of the washed cells by the counts of the whole blood, multiplying the result by 100, and correcting for cell volume. The results were then reported as per cent per 100 per cent hematocrit reading. The formula is:

$$\text{RCU} = \frac{\text{Counts of washed cells}}{\text{Counts of control tube}} \times \frac{100}{\text{Hematocrit reading, ml./100 ml.}}$$

One hundred thirty-three determinations were done on hospital or clinic patients who also underwent the standard tests for thyroid function; these included: protein-bound iodine, basal metabolic rate, and radioiodine uptake. The final diagnosis was then compared with results of the RCU.

Results *(Fig. 1)*

Euthyroidism. Seventy-nine euthyroid patients were studied in the manner described, and the results were tabulated. The range of the RCU was from 12 per cent to 26.5 per cent, or an average of 17.8 per cent. Seventy-one patients (90 per cent) had values over 14 per cent; 74 patients (94 per cent) had values under 22.5 per cent; and 77 patients (97 per cent) had values under 24.0 per cent. It should be noted that 97 per cent of patients (77) had values between 12.0 and 24.0 per cent; whereas, 81 per cent of patients (64) had values between 14.0 and 22.5 per cent.

Hyperthyroidism. The 40 patients with active hyperthyroidism (Graves' disease) had an average RCU of 29.5 per cent in a range of 10.8 to 89.1 per cent. Twenty-six

*The determinations were made with the technical assistance of Miss Doris Reep, under the guidance and with the co-operation of Dr. Otto Glasser and Mr. Bernard Tautkins, Department of Biophysics.

(65 per cent) hyperthyroid patients had values above 26.5 per cent, which was the highest value obtained in euthyroid patients. Thirty-six (90 per cent) hyperthyroid patients had values higher than 22.5 per cent *(Table 1),* and 38 patients (95 per cent) had values higher than 21.0 per cent.

Hypothyroidism. The 14 patients with myxedema had an average RCU of 11.1 per cent. All of these patients had values below 14.5 per cent; 12 patients (86 per cent) had values below 14.0 per cent *(Table 1);* and 9 (65 per cent) had values under 12.0 per cent.

Continuing Studies on the Same Patients

Table 2 lists the RCU values for eight hyperthyroid patients before and during therapy. There is good correlation between the patient's clinical response and the

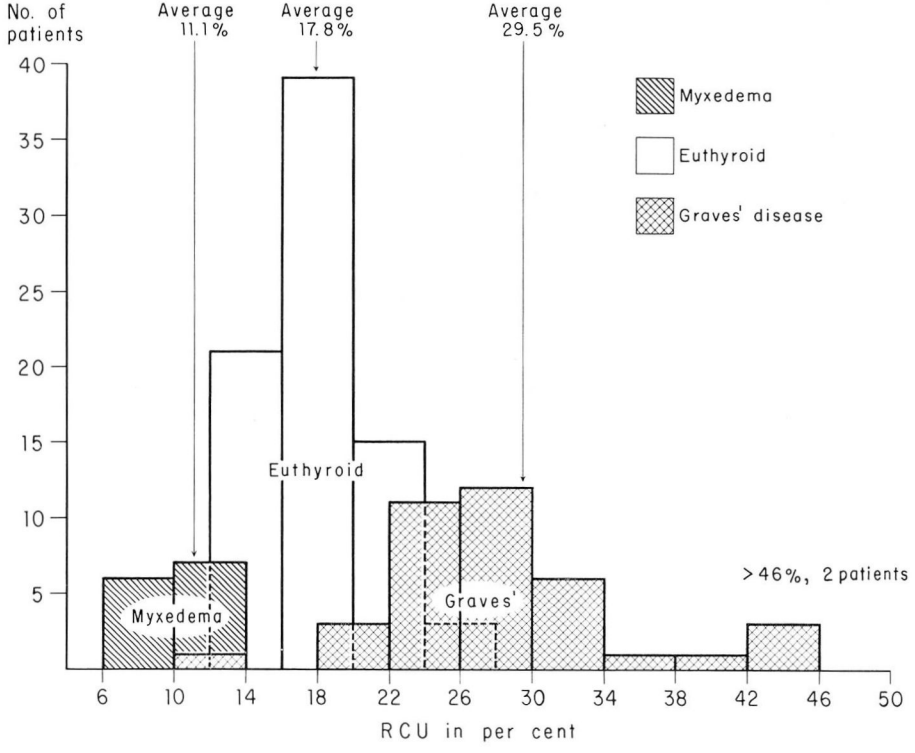

Fig. 1. Graph showing the results of the RCU in 14 patients with myxedema, 79 euthyroid patients, and 40 hyperthyroid patients. The number of patients with values of the RCU in intervals of 4 per cent is plotted vertically. There were no patients with myxedema with values above 14 per cent (actual range 6.5 to 14.5 per cent). The euthyroid patients had values between 12 and 28 per cent (actual range 12 to 26.5 per cent). The hyperthyroid patients had values between 10 and 86 per cent (actual range 10.8 to 89.1 per cent).

change in the RCU. The usefulness of the radioiodine tracer in diagnosing thyroid overactivity becomes impaired[10] after radioiodine therapy or after surgery. Perhaps the RCU will be particularly useful in treated patients.

Table 1.—*Summary of 133 determinations of RCU*

	Thyroid status	
	Myxedema	Euthyroid
Average RCU	11.1% *Under 14.0% (86%)*	17.8% *Over 14.0% (90%)*
	Hyperthyroid	
Average RCU	29.5% *Over 22.5% (90%)*	17.8% *Under 22.5% (94%)*

Table 2.—*Comparison of the RCU in eight hyperthyroid patients before and during treatment*

	% RCU, phase			
Patient no.	Toxic	Incomplete treatment	Euthyroid	Myxedema
1	61.7	23.5	12.5	—
2	21.1	—	14.3	—
3	43.9	28.0 (Lugol's solution)	—	—
4	30.2	—	15.6	—
5	32.0	—	16.0	—
6	26.3	—	20.0	12.9
7	22.7	—	—	7.7
8	—	—	17.24	10.8

Factors That Affect the RCU

Since we have not had the opportunity to make a systematic study of the factors that alter the RCU, we are unable to draw any conclusions in this regard, although we have studied seven pregnant, healthy patients who had low normal values, as has been previously[5] described, and have observed unusually high values

in an occasional patient taking Dicumarol. Other cases in which there may have been factors influencing the RCU are summarized in *Table 3.*

Table 3.—*Alterations in the RCU in eight patients*

Patient no.	Summary of data
1	Myxedema; PBI, 8.3 μg./100 ml.; B.M.R. (−29%); RCU, 16.4%; gallbladder roentgenograms 2 months before studies.
2	Active Graves' disease; PBI, 8.1 μg./100 ml.; B.M.R. (+20%); RCU, 21.1%; Lugol's solution until 1 week before studies.
3	Emphysema; euthyroid; RCU, 34.9%.
4	Euthyroid; bromism (serum bromide, 170 mg./100 ml.); RCU, 27.6%.
5	Myxedema with postnecrotic cirrhosis; RCU, 19.6%.
6	Euthyroid with severe intercapillary glomerulosclerosis; RCU, 26%.
7	Severe Graves' disease with severe secondary hepatic disease; Bromosulphalein, 28%; RCU, 6.0% with active hyperthyroidism; RCU, 20.3% when euthyroid.
8	Graves' disease; B.M.R. (+20%); PBI, 8.0 μg./100 ml.; RCU, 21.8%; Lugol's solution until 3 weeks before studies.

We have observed *(Table 2)* that there is an excellent correlation between the change in the RCU and the patient's clinical response, particularly in the treatment of hyperthyroidism. Because of its effect on thyroid function, iodine may indirectly influence the RCU. One patient (case 3, *Table 2),* in whom the RCU was greatly changed by Lugol's solution also had an excellent clinical response to 10 days of this therapy. Two patients (cases 2 and 9, *Table 3)* also had RCU values considerably lower than the average value obtained in hyperthyroidism (29.5 per cent). Both of the patients had had excellent clinical responses to Lugol's solution.

Discussion

As has been previously shown with paper electrophoresis,[1-3] small amounts of I^{131}-labeled thyroxin or *l*-triiodothyronine are localized in the inter-alpha-globulin zone at pH 7.4 and 8.6. Further studies disclosed that in blood from patients with various thyroid disorders, the amounts of added I^{131}-labeled thyroxin or *l*-tri-

iodothyronine bound by the thyroid-binding globulin varied inversely with the thyroid function, indicating that in hyperthyroidism, the thyroid-binding globulin is relatively saturated as compared with the thyroid-binding globulin in hypothyroidism. This saturation can be measured indirectly by the RCU.

Hamolsky, Stein, and Freedberg[4] observed that the red-cell uptake in the euthyroid range was principally between 10.3 and 17.0 per cent. In the series reported by Ureles and Murray[8] the range was between 11.5 and 18.5 per cent. Our values are considerably higher than those (14.0 to 22.5 per cent); the reason for this difference is not obvious, although we used plastic tubes instead of glass flasks for the washing. Ureles and Murray[8] stated that determinations carried out in open flasks were significantly depressed and led to false values, but this was not a factor here, since the tubes were all stoppered. We have not as yet studied enough patients with various conditions that alter the thyroxin-binding protein, to draw any firm conclusions as to what other factors may alter the results of this test.

Summary and Conclusions

Patients with various grades of thyroid function were studied with the in vitro test of erythrocyte or red-cell uptake of I^{131}-labeled *l*-triiodothyronine (RCU). The average value for patients with myxedema was 11.1 per cent, for euthyroid patients was 17.8 per cent, and for hyperthyroid patients with hyperthyroidism due to Graves' disease was 29.5 per cent. Twelve (86 per cent) of 14 patients with myxedema had values under 14.0 per cent. Of 79 euthyroid patients, 71 (90 per cent) had values higher than 14.0 per cent. Of 40 patients with hyperthyroidism secondary to Graves' disease, 36 patients (90 per cent) had values more than 22.5 per cent. In comparison, 74 patients (94 per cent) of the euthyroid group had values less than 22.5 per cent. It is obvious that some values obtained in euthyroid patients overlap some of those obtained in hyperthyroid and hypothyroid patients.

The measure of the clinical usefulness of the RCU, as with all thyroid-function tests, will depend on further understanding of those pathologic states that affect the RCU, and which are not related to the patient's thyroid function. The test has the advantage that no radioactive substance is administered to the patient, and it is relatively easy to do in comparison with the test for protein-bound iodine. The results are also unaffected by the previous administration of iodine.

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