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Red blood cell uptake of I-131 Triiodothyronine as a test of thyroid function

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THE RED BLOOD CELL UPTAKE OF I^{131} TRIIODOTHYRONINE AS A TEST OF THYROID FUNCTION

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HISTORY AND RATIONALE

Thyroid hormone substances, thyroxine and l-triiodothyronine, are known to be bound by the plasma proteins[31] and by the red blood cells.[8] It has been noted that the thyroxine-binding-globulin fraction of the plasma proteins is most active as the protein carrier.[28] The affinity of the plasma proteins for these substances is greater than that of the red blood cells, so that little is taken up by the red blood cells until the plasma proteins become relatively saturated.[6,8,40] Finally, it has been established that thyroxine is more firmly bound by either plasma proteins or red cells than is triiodothyronine.

Based on these facts, Hamolsky, Stein, and Freedberg[19] devised a test of thyroid function employing radioactive I^{131}. Essentially, the test measures in vitro the amount of l-triiodothyronine which becomes bound to the red blood cells during incubation, and this test has subsequently been adopted as a test of thyroid function.

PHYSIOLOGY

General

The radioiodinated triiodothyronine red blood cell uptake test apparently defines thyroid status in terms of serum concentration of unbound thyroidal hormone. Red blood cell uptake of labeled triiodothyronine is presently considered to be an absolute physio-chemical phenomenon consisting of a distribution of the hormonal component between binding sites on the serum proteins and erythrocytes.[4,6]

Recent studies suggest that the erythrocyte uptake of triiodothyronine is a quantitative procedure exclusively dependent upon the concentration of unbound thyroid hormone in the serum.[3] The exact physiological mechanism of the test is not clear at the present time, and further investigation is being carried out.

Physiology of Triiodothyronine

The compound 3,3,5-l-triiodothyronine was initially identified as a normal constituent of the organic iodine fraction of blood plasma, and was noted to be present both in euthyroid and hyperthyroid individuals, and then as a component of thyroid tissue.[25] Various means of evaluation revealed that it possesses up to 7 times the activity of thyroxine. Thyroxine is the major circulating thyroid hormone, and triiodothyronine is present in the blood stream only in low and variable concentrations.

Investigation has shown that triiodothyronine is primarily bound to a specific protein moiety in the globulin fraction of the blood plasma.[17] It is less firmly bound to its receptor than thyroxine and is easily displaced by thyroxine.[8,9,32,33] Triiodothyronine readily enters many of the cells and tissues of the body, while thyroxine does not. Its ability to do this may be due to its labile binding with the plasma proteins.[25]

The exact mechanism of the cellular binding of triiodothyronine is unknown at the present time. The receptors in the erythrocytes are presumably located in the stroma.[6] The present evidence in this field suggests that the uptake of thyroid hormones by red blood cells is a physio-chemical phenomenon[8,12,29] not directly dependent upon cellular respiration but rather upon the concentration of unbound hormones in the serum.[4]

Although erythrocytes *in vitro* will bind both thyroxine and triiodothyronine, studies conducted with radioactive hormones indicate the uptake or binding of triiodothyronine to be consistently greater than the uptake of thyroxine.[6,7,8,19] It has also been noted that the plasma markedly decreases erythrocyte uptake of thyroxine and, to a lesser degree, triiodothyronine, and that the plasma proteins are the main carriers of the thyroid hormones.[7]

Determinants of Triiodothyronine Red Blood Cell Uptake

At the present time, the distribution of the thyroid hormones, l-thyroxine and l-triiodothyronine, between the fluid element of

the blood and the erythrocyte appears, from experimental evidence, to be dependent upon the relative binding capacities of the plasma proteins[37,27] and some binding factor in the red blood cell stroma.[6,7,8,29] It is believed that the availability to triiodothyronine of unsaturated binding sites on the plasma proteins is the significant determinant of the concentration of unbound hormone in the blood and, consequently, of red blood cell uptake. A recent investigation suggests that this is a quantitative phenomenon.[3] In the presence of thyroid malfunction, the availability of plasma binding sites to triiodothyronine is affected by one or more factors. Quantitative differences may occur in the degree of saturation of common plasma protein binding sites by greater or lesser amounts of the more firmly bound thyroxine.[13,18] Alterations may also occur in the total amount of protein receptors present in the fluid component of the blood.[3]

The significance of the plasma as the determinant of the red blood cell uptake of triiodothyronine has been demonstrated by two series of "criss cross" studies.[13,19] In these experiments, plasma and erythrocytes taken from normal and hyperthyroid individuals were interchanged. In well over 90 percent of the cases, the red blood cell uptake was dependent upon the thyroid status of the plasma donor. Thus, the uptake by hyperthyroid erythrocytes in euthyroid plasma was in the euthyroid range,

while the uptake of euthyroid erythrocytes in hyperthyroid plasma was in the hyperthyroid range.[13,15,19]

It has been noted that as the plasma concentration decreases or is diluted there is an increase in percent red blood cell uptake, and this is presumed to be caused by a reduction in the number of available binding sites for triiodothyronine on the plasma proteins. The addition of triiodothyronine or thyroxine to plasma also increases the red blood cell uptake, evidently by saturation of the plasma protein binding sites.[13] In the case of thyrotoxicosis, both the degree of saturation and reduction in the total number of protein receptors available have been credited with the high red blood cell uptake of triiodothyronine observed.[3]

At the present time, it seems that the altered radioiodinated triiodothyronine uptakes which are diagnostic of hypothyroidism and hyperthyroidism represent quantitative differences in degree of saturation of common plasma protein binding sites[16] by greater or lesser quantities of the more firmly bound thyroxine; hence, in hyperthyroidism, more protein receptors would be occupied because of the increased thyroxine levels, and more radioiodinated triiodothyronine would be taken up by the erythrocytes. Conversely, in myxedema, with more plasma binding sites available, the concentration of unbound hormone is reduced and erythrocyte uptake is less.[3,13,18] Variations of the red cell uptake found in other disease states can generally be explained by changes in

the amount of thyroid hormones present or by changes in the amount of available plasma protein binding sites.

PROCEDURE

Blood Sample

1. Withdraw 10 ml. of venous blood into a test tube containing anticoagulant. Potassium oxalate (0.1 ml. of a 40% solution), heparin, or ACD solution may be used. In bloods with hematocrit values outside the range of 30-55, one should correct values within this range by the removal or addition of appropriate aliquots of the donor's plasma prior to adding the radioactive triiodothyronine. In handling blood samples with very rapid sedimentation rates, special care should be exercised to adequately mix the samples immediately before each pipetting step.

Addition of I^{131} l-triiodothyronine

1. Radioactive l-triiodothyronine is diluted with saline or distilled water to a concentration of 0.01 to 0.12 micrograms per milliliter.
2. Add 0.1 ml. of the dilute radioactive triiodothyronine to 3 ml. aliquots of whole blood in stoppered 10 ml. Erlenmeyer flasks. Flasks must be well stoppered, since uptake is strikingly decreased when incubation is carried out in unstoppered flasks, with resultant alterations in carbon dioxide concentration and a change in final values.[42]
3. The flasks are shaken at 37 degrees C. for 2 hours.
4. Two 1.0 ml. aliquots of whole blood are removed from each flask.

The radioactivity of each is determined with a well-type scintillation counter at counting rates sufficient to permit $\pm$ 2 percent accuracy.

5. Each sample is then centrifuged for 5 minutes at 3000 r.p.m., the supernatant plasma is removed, and the erythrocytes are washed 5 times with 10 volumes of isotonic solution. Volume and temperature of the saline washes as well as time between washes must be constant for reproducible results.

6. The radioactivity remaining in the erythrocyte fraction of each aliquot is then determined as above.

7. The percent radioiodinated triiodothyronine red blood cell uptake is then calculated:

$$\% \text{ uptake} = \frac{\text{net counts in erythrocytes}}{\text{net counts in whole blood}} \times 100$$

8. The uptake is expressed as an average of the four determinations, and corrected to a hematocrit of 100.

9. If more than 3 percent difference occurs in duplicate samples, the results should be considered inconclusive and the tests repeated.[35] The above procedure is basically that of Hamolsky and associates.[19]

VARIATIONS OF THE MAJOR TEST

It has been noted that modifications of the major test may be advantageous. One group of investigators[8] using a larger volume of a more dilute solution performs the original count on the dilute radioactive l-triiodothyronine first; then, after incubating the blood in the solution, washes the cells and makes the second count. Since the radioactive triiodothyronine is absorbed onto glass, the count of the sample and the addition of whole blood must be accomplished almost immediately to avoid the loss of significant radioactivity.

Recently a modification of the basic test has been developed using an ion-exchange instead of red blood cells to measure the uptake of radioactive triiodothyronine. These investigators noted that the red blood cells played a relatively passive role, their uptake being largely determined by the amount of triiodothyronine left free in the plasma, and noted that the resin seemed to work in place of red blood cells. The technique is similar to the procedure described above, and the results of their test were comparable to those obtained by RCU (red cell uptake) tests. The advantage of the resin test are its simplicity and its independence of hematocrit values and of red blood cell anomalies.[28]

VARIANTS AFFECTING THE TEST PROCEDURE

Hematocrit

It has been noted that variation in hematocrit can cause variation in the amount of triiodothyronine uptake by the red cells, and this is especially noticeable with hematocrits above 55 and below 30. Hamolsky and associates noted that consistent results were obtained after correction to 100 hematocrit with bloods over a hematocrit range from 30 to 55. Variable results were sometimes found using bloods with hematocrit values outside this range; such bloods were, therefore, corrected before final determination of uptake to hematocrit values within this range by removal or addition, respectively, of the appropriate aliquots of the donor's plasma prior to the addition of I^{131} l-triiodothyronine and incubation.[18]

Adams and associates[1] discovered in 1960 that red cell labeling seemed to obey a simple equation derived from the law of mass action. The studies showed the labeled triiodothyronine taken up by the red cells was proportional to the product of the red cell mass and the concentration of labeled triiodothyronine remaining in the plasma after incubation. They worked out a constant of proportionality, or binding coefficient, that was supposed to be characteristic of a given plasma sample and was essentially independent of the hematocrit over a wide range. At the present time, they are using this instead of the hematocrit

and believe it is a more reliable index of thyroid function than the percentage red cell uptake which depends on the hematocrit reading.

In 1962, Parrow[30] reviewed the concept of binding coefficient; and, in his estimation, it is invalid and should not be used in determinations. At the present time, it seems that the original method outlined by Hamolsky is probably the most reliable method to be used.

CO_2

High concentrations of carbon dioxide increase the red cell binding. This effect of CO_2 provides a possible explanation of the increased triiodothyronine red cell uptake observed in patients with pulmonary ventilatory disease. At low CO_2 values, red cell binding was markedly reduced below normal values. These results support the concept that it is mandatory to keep the blood in stoppered containers to prevent diffusion of carbon dioxide from the system prior to and during the test procedure.[42]

Incubation Time

Experiments were carried out and the incubation period was varied from 5 minutes to 2 hours and showed only a slight increase in the uptake of I^{131} triiodothyronine by red cells after the first half hour. In a series of 120 cases, there was no significant difference in the values at 30 minutes and 1 hour (Figure 1).[14]

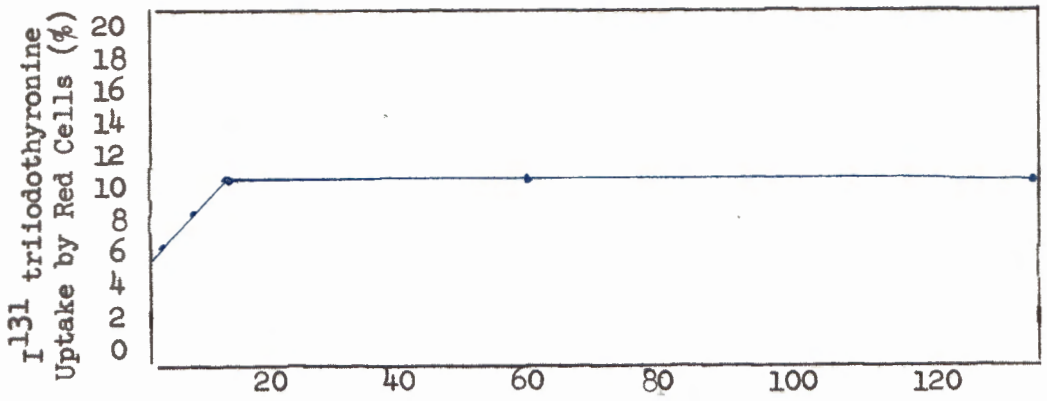

Fig. 1-Relation between the uptake of I^{131} triiodothyronine by red cells, and the duration of incubation.

In another study, significant incorporation of I^{131} triiodothyronine by erythrocytes has been noted, and the longer the cells are incubated, the more triiodothyronine is taken up (Table 1).[18] These studies give varying results; however, generally it can be stated that, the longer the incubation time, the higher the red blood cell uptake will be.

Table 1-The in vitro Red Blood Cell Uptake Of I^{131} l-triiodothyronine At Varying Periods Of Incubation Of This Compound In Whole Blood From Euthyroid And Hyperthyroid Subjects

Incubation Period	Uptake of I^{131} T_3 (%/100 Ht.)	
	Euthyroid	Hyperthyroid
¼	10.3	17.4
½	11.8	20.2
1	12.6	21.9
2	13.3	23.9
3	15.4	26.8
4	15.5	28.0
5	16.8	28.8

Number of Washes

The relationship between the number of washes and the uptake of I^{131} triiodothyronine by the red cells is shown in Fig. 2.

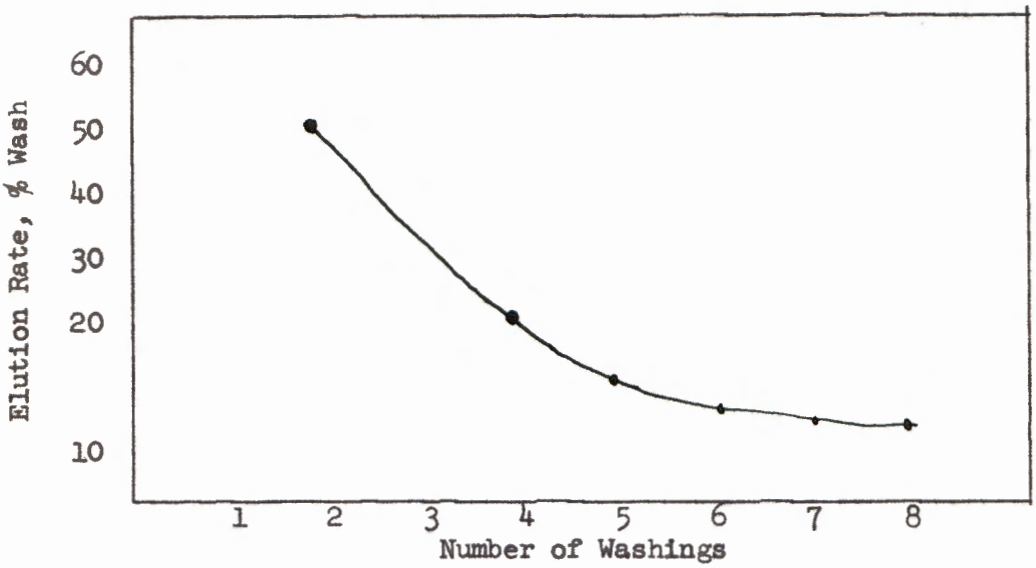

Fig. 2-Relationship between the number of washes and the uptake of I^{131} triiodothyronine by red cells.

The curve is made up of two components: A rapid initial fall in radioactivity, which represents removal of plasma from cells and is followed by a slower exponential phase due to elution of triiodothyronine from cells. The authors feel that 4-5 washings are sufficient and further washing will slowly decrease the triiodothyronine uptake by red blood cells because it is washed away.[14,36]

Temperature

A group of tests were conducted with saline solution wash and centrifuge temperatures at 4, 24, and 37 degrees C., as illustrated in Fig.3. The change brought about by variation of

temperature was striking. It was observed to be a major cause of the lack of reproducibility of the test results. The investigators point out that the temperature of the centrifuge should be watched throughout the day, as they tend to build up heat as they are used.[38]

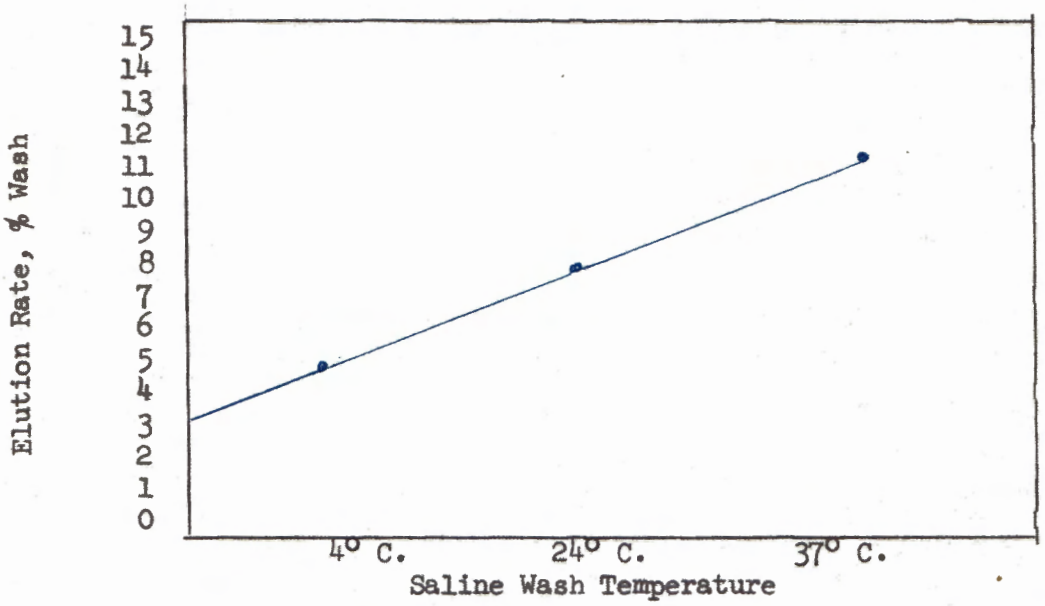

Fig. 3-Arithmetic plot indicating effect of temperature of saline solution wash on the elution rate of I^{131} triiodothyronine from erythrocytes. The rate increases approximately 1 percent per wash with each 5° C. elevation in temperature.

Concentration of Saline Washes

The amount of saline solution used has no effect on washing the radioactivity off the surface of the red cells; however, it did have some effect on washing the radioactivity out of the cells, and it is recommended that a standard solution be used in addition to a specified number of washes to keep the results constant.[38]

Volume of Saline Wash

Five washes of 5 and 10 ml. were carried out on paired duplicates. The average result obtained on the blood of six patients is shown in Table 2. In all cases studied, the difference between results with the 5 ml. and the 10 ml. wash was relatively constant and showed that volume affects the final results.[24]

Table 2-I^{131} l-triiodothyronine Activity Remaining In Erythrocytes As Affected By Volume Of Wash

Volume of Saline Wash	Erythrocyte I^{131} T_3 Activity After 5 Washes	
	%	%/100 Hematocrit
5 ml.	6.86	13.8
10 ml.	5.62	11.6

From the above studies, it can be seen that the results of the RCU test will be more reproducible and uniform in a laboratory if the above variables are rigidly controlled.[44]

CLINICAL STUDIES

General

Hamolsky, Stein, and Freedberg[19] evaluated the test using data from 305 subjects. Hamolsky, Golodetz, and Freedberg[18] later summarized the data from more than 2,900 determinations. They found that the test:

1. Was simple and accurate.
2. Yielded results of diagnostic accuracy comparable to those of standard methods.
3. Permitted thyroid function assessment under conditions precluding other methods.
4. Was useful in assessing therapy in thyroid patients.
5. Might become useful in the study of thyroid physiology.

From the larger experience, reviewed in Table 4, euthyroid limits are established ranging from 11.0 to 17.0 percent for females and from 11.8 to 19.0 percent for males, when corrected to a hematocrit of 100.[18] The statistical validity of the suggestively higher values in males may be due to a higher hematocrit found in most males.

Euthyroid

Of the total of 1,943 euthyroid individuals tested, 76, or 3.9 percent, presented uptake values falling outside normal limits. The euthyroid clinical group was comprised of individuals presenting no known abnormality, as well as persons showing such

thyroid and non-thyroid pathology as nontoxic nodular goiter, colloid goiter, thyroid carcinoma, diabetes mellitus, Addisons disease, hypertension, congestive heart failure, severe anxiety states, polycythemia vera, and severe malnutrition.

Hyperthyroidism

In hyperthyroidism, uptake limits are placed between 17.0 and 35.0 percent for females and between 19.5 and 37.9 percent for males, when corrected to a hematocrit of 100. In a series of 279 hyperthyroid patients subjected to examination, 4, or 1.4 percent, had uptake values below the euthyroid level.[18] A distinct relationship between the severity of thyrotoxicosis and the extent of red blood cell uptake of radioiodinated triiodothyronine has also been reported.[12]

Hypothyroidism

Uptake limits in hypothyroidism ranged from 6.1 to 11.0 percent for females and from 5.5 to 11.6 percent in males, when corrected to a hematocrit of 100. Only 1, or 0.5 percent, of the 195 hypothyroid patients tested had an uptake level above the lower euthyroid level. In diagnostic cases, low values were obtained in all cases of spontaneous myxedema, panhypopituitarism, and cretinism, and, ultimately, in hypothyroidism developing after treatment of hyperthyroidism by surgery, propylthiouracil, or radioactive iodine.

Summary

Results of the *in vitro* radioactive triiodothyronine red

cell uptake test are also reproducible. In a series of 300 consecutive uptake procedures, the difference between duplicate determinations ranged from 0.0 to 1.6 percent, averaging 0.6 percent with a standard deviation of ± 0.5.

Table 3-Differential in vitro Red Blood Cell Uptake Of Radioiodinated Triiodothyronine Consistent With Thyroid Status

Thyroid Status	No. of Observations	(%/100 Hematocrit)
Euthyroid Female	1301	11.0-17.0 (Av. 13.9)
	3 (0.2%)	11.0
	49 (3.6%)	17.0
Euthyroid Male	566	11.8-19.0 (Av. 15.2)
	24 (4.1%)	19.0
Hyperthyroid Female	211	17.0-35.0 (Av. 22.5)
	2 (0.9%)	17.0
Hyperthyroid Male	64	19.5-37.9 (Av. 23.7)
	2 (3.0%)	19.5
Hypothyroid Female	129	6.1-11.0 (Av. 23.7)
	1 (0.7%)	11.1
Hypothyroid Male	65	5.5-11.6 (Av. 6.7)

The results of other laboratory tests were diagnostically distinctive, in varying degrees, in the three major categories of thyroid function. However, there was no apparent correlation

within any single category between the absolute RBC I^{131} T_3 uptake values for given patients and their BMR, serum PBI level, or 24-hour I^{131} uptake.

INFLUENCE OF THYROID FACTORS ON TEST VALUES

Influence of Therapy for Hyperthyroidism

Repeated observations were carried out on 76 hyperthyroid patients, during and after therapy with stable iodides, propylthiouracil, surgery, and radioactive iodine. In four instances, the red blood cell uptake of I^{131} T_3 persisted in the hyperthyroid range for as long as one year after attainment of the euthyroid (3 pts.) and hypothyroid (1 pt.) states. Subsequently, the red blood cell uptakes were consistent with the attained clinical state in three of these subjects; the fourth was lost to follow-up. In all other instances, correlation of red blood cell uptake and thyroid status was excellent. The administration of propylthiouracil often caused a decreased red blood cell uptake as early as twenty-four to forty-eight hours after institution of therapy, before there was any apparent clinical improvement. Failure of red blood cell uptake to decrease within one week presaged failure of clinical response, necessitating increasing dosage. Similarly, failure of an increased red blood cell uptake to reach normal levels within two or three months following surgery or administration of I^{131} was correlated in every instance with the ultimate diagnosis of persistent hyperthyroidism. Decrease of uptake to hypothyroid levels was an early and reliable index of overtreatment.

The in vitro addition to hyperthyroid bloods of large amounts of stable iodides or propylthiouracil failed to lower the subsequent red blood cell uptake. As in the untreated states, there was no apparent direct correlation between red blood cell uptakes of I^{131} T_3 and other parameters of thyroid function. Although results obtained by these methods were usually in general agreement, in several instances the red blood cell uptake reached a level consistent with the evaluated thyroid status in advance of the other parameters.

Influence of Therapy for Hypothyroidism

Observations were carried out during treatment of 41 adult hypothyroid patients with desiccated thyroid, l-thyroxine, l-triiodothyronine, or various combinations thereof. The overall correlation was excellent. In all instances, ultimately, adequate therapy resulted in a progressive increase of red blood cell uptake of I^{131} T_3 to the normal range. Failure of attainment of normal levels reliably indicated undertreatment. If the levels became elevated, they were generally accompanied by clinical signs of hyperthyroidism. The red blood cell uptake is uniquely useful in following therapy in those cases in which l-triiodothyronine has been selected as the therapeutic agent, since in these patients the serum PBI level cannot be used.

Experience with more than 40 cretins for periods up to two years has revealed the radioiodinated triiodothyronine red blood

cell test to be a highly satisfactory check on therapeutic course in this condition. With proper therapy, uptake levels within the normal range are achieved and maintained.

Influence of Exogenous Iodine

Various investigators have carried out red cell uptake tests in patients who received exogenous iodine. In these cases the PBI and the 24-hour thyroid I^{131} uptake values were markedly affected, the red cell uptake of I^{131} T_3 was slightly elevated but was not enough higher to prevent the test from being useful in euthyroid patients.

The administration of iodides to hyperthyroid patients caused a progressive decrease in the red blood cell uptake of I^{131} triiodothyronine.

Patients who had recently received radiographic contrast media for thyroid or kidney studies showed no alteration in the T_3 test, while the PBI and I^{131} tests were distorted.[12,42]

Iodine and Thyroid Administration in Euthyroid Patients

In 10 of 12 euthyroid subjects receiving varying doses of desiccated thyroid, l-thyronine, or l-triiodothyronine, erythrocyte uptakes remained in the euthyroid range. In one subject, the daily administration of 30 drops of compound solution of iodine (Lugols) had no effect for ten days; then there was a decrease to low borderline euthyroid values.[19]

Nontoxic Nodular Goiter

Six patients were studied. These patients were within the euthyroid range when tested with the erythrocyte uptakes ranging from 13.6 to 16.8 percent. These patients were also euthyroid when tested by other procedures.[42]

INFLUENCE OF UNRELATED NON-THYROID FACTORS ON TEST VALUES

Pregnancy

In a study conducted by McGoogan and associates,[23] it was noted that the I^{131} T_3 uptake tests in the first trimester of pregnancy of 98 normal pregnancies that went to term showed an almost continuous decrease from the fifth to thirteenth week after the last menses, decreasing from 16.0 to 10.9 percent. This decrease remained in all cases until delivery, and then the test results returned to a euthyroid, or occasionally a hyperthyroid, level postpartum.

There appears to be some relationship between the red blood cell uptake of triiodothyronine and abortions. In a series of six pregnancies followed by Hamolsky and associates,[18] the red blood cell uptake was found to be in the normal, euthyroid range beyond the eighth week of gestation. Each of these pregnancies subsequently terminated in abortion. Similar results were obtained in a study conducted by Mitchell and associates using resin uptakes.[26]

Investigative work by Dowling and associates[10,11] and others has shown that thyroxine is avidly bound by thorixine binding globulin, and that the peripheral utilization of this hormone is in part conditioned by this protein binding. They have shown that large doses of estrogen increase the amount of thyroid binding globulin and in these patients there is a marked retardation

of the peripheral rate of turnover of thyroxine. If there is an increase in thyroid binding globulin, which is known to be caused by increased estrogens, and pregnancy, these additional globulins would be in direct competition for any free hormone circulating and would, because of their preferential absorptive powers, decrease the amount of free hormone available for bondage with erythrocytes and other peripheral tissues. This may be what causes the decreased values noted in the triiodothyronine test during pregnancy.

Children

In several studies carried out on newborn infants and children, it has been noted that the range of 20 to 26 percent uptake is present in newborn infants. This value decreases as the children become older. This range is distinctly higher than that of normal adults and is a finding that is compatible with that of the serum protein-bound iodine and I^{131} uptake, which are also generally higher in the newborn infant than in the older child and the adult.[20] Crigler found the levels in children to be lower than the above studies and stated that they were only slightly higher than the normal adult levels, especially in older children.[5] Kunstadter made a rather exhaustive study of the test in all age groups from newborn to adulthood, and the results show that the values in newborns are greatly elevated as compared to those of euthyroid adults, with progressive decreases up to the

age of 2 years; thereafter the mean levels are essentially the same until adulthood, but still above euthyroid levels.[21]

Erythrocyte Abnormalities

Barrett and associates[2] studied various erythrocyte abnormalities to see what effect, if any, these abnormalities would have on the red blood cell uptake of I^{131} l-triiodothyronine. In 3 cases of sickle-cell trait, normal values were observed. Normal uptakes were also recorded in 1 case of sickle-cell anemia, 1 of sickle-cell thalassemia, and 3 of thalassemia trait. Varying results were obtained in 9 cases of hemolytic anemia; in 2 cases associated with acute leukemia the uptakes were elevated despite low hematocrits; in 3 cases associated with Hodgkins disease, normal values were noted in 2 and an increased uptake in 1; values were normal in a case of congenital spherocytic hemolytic anemia, and elevated in a case of congenital hemolytic anemia with unusual inclusion bodies. Uptakes were elevated in 6 of 8 cases of polycythemia vera. Criss cross experiments in many of the above cases showed that the abnormal results were generally caused by the plasma and not the red cells. These investigators believe that whenever there is a change in the hematocrit, there is also a variation in the amount of plasma thyroxine-binding protein which is probably responsible for the variation in the results of the red blood cell T_3 uptake test.

Estrogens

On repeated observations, red blood cell uptake was increased following administration of estrogens to male or female subjects. This can probably be explained by the same mechanism as is present in pregnant women, as is discussed under "Pregnancy."[18,40]

Anticoagulants

Studies have revealed that there is an increase in red blood cell uptake of I^{131} triiodothyronine after administration of Dicumarol, in about 81 percent of the cases. Elevated values were observed as early as twenty-four to forty-eight hours after administration. Once increased, the levels seemed to remain essentially the same through the period of treatment. After stoppage of therapy, return to normal levels was noted within 14-18 days. Comparable increased uptakes have also been observed with Heparin. At the present time, the cause of this increase in uptake is unknown, and further study is needed in this area.[18,19,42]

Miscellaneous Disease Processes

The red blood cell uptake of I^{131} l-triiodothyronine has also been noted to be consistently increased in nephrosis, severe hepatic disease, extensive metastatic malignancy, paroxysmal atrial arrhythmias, uremia, and chronic pulmonary disease. A low level was noted in menstruation.

The exact mechanism determining these increased red blood cell uptakes is not clear at the present time and will require

further investigation. It has been postulated that the red cell uptake depends on two basic mechanisms, these being (1) increase or decrease in thyroxine and (2) change in the number of thyroid hormone binding sites in the plasma proteins. If this is true, the above disease processes which show an increase in red cell uptake may do so because of a decrease in serum protein and, hence, a decrease in available binding sites. One exception to this theory is paroxysmal atrial arrhythmias, in which the increased uptake is probably due to a hyperthyroid condition that has not been recognized clinically.[10,12,18, 19,22,35,39,42,43]

In summary of the previous two sections, Table 4 is presented, which outlines the various factors that affect the red blood cell uptake of I^{131} triiodothyronine from whole blood.

Table 4-Summary Of The Effect Of Various Factors On The Two-Hour in vitro Red Blood Cell Uptake Of I^{131} Triiodothyronine From Whole Blood

I. Decrease

A. Pregnancy
B. Estrogen
C. Propylthiouracil in Hyperthyroidism
D. Iodide in Hyperthyroidism
E. Menstruation

II. No (or Slight) Effect

A. Thyroid Enlargement--Colloid Goiter, Nontoxic Nodular Goiter, Thyroid Carcinoma
B. Exogenous Iodine
C. Anxiety
D. Congestive Heart Failure
E. Hypertension
F. Administration of Mercury
G. Other Endocrinopathies--Diabetes Mellitus, Addisons Disease

III. Increase

A. Anticoagulants--Dicumarol, Heparin
B. Nephrosis
C. Severe Liver Disease
D. Severe Metastatic Malignancy
E. Severe Pulmonary Insufficiency
F. Paroxysmal Atrial Arrhythmias
G. Uremia

COMPARISON WITH OTHER THYROID TESTS

General

All tests of thyroid function developed to date suffer from shortcomings. Determination of the protein-bound iodine in the serum is a difficult and time-consuming test and subject to many variables in previous treatment and technique. It is highly susceptible to the exogenous administration of iodine and mercury. Iodized radiographic media may increase the values for months or even years after their administration.

In considering the I^{131} uptake it is easier to perform and requires less time; however, it is also susceptible to exogenous iodine intakes, and is not suitable for following the patients during treatment with desiccated thyroid and anti-thyroid agents. Lastly, the patients are exposed to some, though slight, radioactive irradiation.[12]

The I^{131} l-triiodothyronine test has distinct advantages over the above tests because exogenous iodine or thyroid medications do not impair the accuracy of the test, the results of thyroid and anti-thyroid therapy can be assessed, it avoids the administration of radioactive material to the patient, and it is a simple, rapid test.

Availability

All three procedures are readily available in larger communities. In rural areas, it is often simpler to send a blood

sample to the city, and this can be done in the case of PBI and RCU tests.

Cost to Patient

The costs of these tests vary from location to location. Well-run PBI's are from $10 to $15. The thyroid uptake of radioactive iodine, as usually performed, includes both 6 and 24-hour readings with a definite increase in information for $20 to $25. A single uptake is generally cheaper. The cost of a red blood cell uptake test generally runs from $20 to $25.

Patient Time and Convenience

The PBI and RCU tests require only the time necessary to draw blood samples and answer a few questions. The uptake of I^{131} requires about the same initial amount of time, and two return visits of 5 minutes each.

Technician Time

The technician time in performing a PBI and RCU varies, of course, with the number of tests done daily, the procedure used, cleaning of equipment, and so forth. On the average, each PBI represents an investment of 30-45 minutes of trained personnel time, while a RCU requires about 1½-2 hours. An I^{131} uptake test, including both 6- and 24-hour readings, entails only 12-15 minutes, including standardization.

Cost to Laboratory

The PBI test, necessitating more time, more expensive materials,

higher-paid personnel, the need for redoing contaminated runs, and a special work area, costs more per determination. The higher initial cost of isotope equipment partly offsets this temporarily; but, in the long run, the I^{131} uptake and the RCU tests are less costly. The isotope equipment is useful in performing both isotope tests and other tests, while the PBI equipment is useful for nothing else.

Accuracy

If done properly, the three tests should be about equally accurate, but there are a greater number of pitfalls with the PBI in inexperienced or careless hands.

Clinical Studies

Ureles and Murray[40] have studied the correlation of the red blood cell uptake of $I^{131}T_3$ with clinical diagnosis, PBI, and 24-hour I^{131} uptake in 81 patients.

Two of four patients with hypothyroidism had studies in complete agreement. In the third, only the RCU was confirmatory. The PBI and 24-hour I^{131} uptake were disturbed by organic iodide and a hyperplastic gland. The fourth patient with chronic potassium thiocyanate poisoning, and an elevated RCU and 24-hour uptake in contrast to a low PBI, which was consistent with the clinical state of myxedema.

In 34 of 57 euthyroid subjects, the RCU, PBI, and 24-hour I^{131} uptake agreed with the clinical diagnosis. In 12 patients,

the RCU was disturbed by liver disease, hypoalbuminemia, and renal disease.

20 patients were studied in the hyperthyroid range, and 13 had RCU's, PBI's, and 24-hour I^{131} uptakes that agreed.

Thus, in 81 patients in whom all determinations were made, there was a positive correlation of the three laboratory procedures with the clinical state in 49 cases (60.5%). It should be noted that, in the cases that did not correlate, some exogenous factor or other disease process was involved.

ADVANTAGES AND DISADVANTAGES

Advantages

The red cell uptake test does not supersede any of the standard laboratory diagnostic tests but does have the following advantages:

1. A simple, rapid, and accurate determination of thyroid function.
2. Useful parameter for screening larger groups of patients.
3. Diagnostically significant in the presence of unrelated non-thyroidal factors which complicate interpretation of other test findings; examples are anxiety, hypertension, congestive heart failure, polycythemia, ingestion of mercury, and particularly the administration of exogenous iodine.
4. In cases presenting borderline, unusual, or bizarre features or where treatment for hyperthyroidism has been instituted, results are more closely correlated with the ultimate diagnosis of thyroid function than those obtained by means of other examinations.
5. A valuable index of the adequacy of treatment of hyper- and hypothyroidism, and a suitable method for measuring and following the course of any therapeutic program using thyroid hormones.
6. Avoids the administration of radioactive material to the

patient; hence, it can be used in children, pregnant women, or in adults who fear ingestion of even tracer doses of I^{131}.

Disadvantages

1. Laboratory procedure and technique must be controlled and watched closely.
2. Various disease processes which either increase or decrease the red cell uptake in euthyroid patients.
3. It is unknown what is actually being measured by the test.
4. Erythrocyte fragility and various erythrocyte abnormalities.

SUMMARY

History

Hamolsky and associates began working on the thyroid hormone complex in 1955 and developed the red cell uptake test in 1957 which measures thyroid function.

Physiology

Exhaustive studies concerning the thyroid hormones revealed that thyroxine and l-triiodothyronine are bound by plasma proteins and by red blood cells. It has been established that thyroxine is more firmly bound by either plasma proteins or red cells than is triiodothyronine. It has also been noted that the affinity of the plasma proteins for these substances is greater than that of the red blood cells, so that little is taken up by the red blood cells until the plasma proteins are saturated. The amount of red cell uptake is determined by the plasma of the donor and not his red blood cells, and it has been established that two mechanisms are probably responsible for the amount of red cell uptake, these being the amount of thyroxine and the number of thyroid binding sites on the plasma proteins.

Laboratory Factors

It should be emphasized that carbon dioxide concentration, incubation time, number of washes, temperature, concentration of saline washes, and volume of saline washes should all be rigidly controlled to obtain consistently reproducible results.

Cost

The cost runs from $20 to $25 and is comparable to the cost of an I^{131} uptake.

Clinical Studies and Use

The results of many clinical studies indicate that this is a simple and rapid test which avoids the administration of radioactivity to the patient and possesses a diagnostic accuracy comparable to other standard methods. It permits the assessment of thyroid function under circumstances in which other standard methods may not be applicable, particularly after the administration of organic iodine-containing compounds. It is useful in following the course of therapy for hyper- and hypothyroidism. The uptake is decreased in normal pregnancy, after estrogen administration, and in propylthiouracil treatment and iodine treatment in hyperthyroidism. The uptake is increased in nephrosis, in certain cases of liver disease and metastatic malignancy, in pulmonary insufficiency with carbon dioxide retention, in paroxysmal atrial arrhythmias, and following administration of anticoagulants.

CONCLUSIONS

1. It appears that the triiodothyronine red blood cell uptake test is a practical and useful additional tool for the diagnosis, therapy, and further investigation of thyroid physiology and disease.
2. It is a simple and accurate test.
3. It yields results of diagnostic accuracy comparable to those of standard methods.
4. It permits thyroid function assessment under conditions precluding other methods.
5. It is useful in assessing therapy in thyroid patients.
6. As with other thyroid function tests, its limitations must be further explored and understood in order to place its value in proper perspective.
7. Moreover, a more definite understanding of the exact meaning of the test is necessary in the future in order to be certain that it truly measures thyroid function and the exact way in which this is done.

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