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^{131}I T-3 Resin-Sponge Uptake Using Ice Water Bath Incubation— A Modified Technique[4]

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The uptake of liothyronine ^{131}I (T-3) by human red blood cells, as a method of estimating thyroid function, was described in detail by Hamolsky, Stein and Freedberg in 1957 (5). Numerous other procedures in the form of chemical protein bound iodine analysis (14), radioactive iodine uptake studies (4,6,10-12), and ^{131}PBI conversion ratio determinations (1,2,16) had been in vogue, but this method was an *in vitro* radioactivity test utilizing the tagging of red cells with a thyroxine analog (T-3). The test relied primarily on the affinity of red cells for T-3 and was based on the assumption that a number of binding sites in the blood, already filled with the patient's existing thyroxine, indicated the patient's thyroid status.

The limitations of this procedure included difficulty in uniformily washing the red blood cells and the inherent inability of duplicating the test as a routine. In 1958, Mitchell (8) modified the T-3 red cell uptake by substitution of an ion exchange resin for the red cells. It was shown that the resin had an affinity for iodides, thyroxine and analogous compounds. Thyroxine ^{131}I, (T-4), was initially used in the test and mixed with the patient's serum. With additional refinements (9,13,15), T-3 was substituted as the labelled material and more reproducible results were obtained. The T-3 uptake test was vastly improved by a resin-sponge (7), (polyurethane resin-embedded sponge), which is offered as a replacement for the red cells as well as for the loose granular resin which varies from day to day.

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The mechanism of the T-3 test is based on the relative saturation of primary thyroxine binding sites in the serum. The alpha-one and alpha-two globulins are primary binding sites. The prealbumins are secondary sites, the erythrocytes possessing only tertiary binding importance (3). In hyperthyroidism the relative number of binding sites available to any ^{131}I T-3 is reduced; therefore, the resin-sponge uptake of T-3 is increased. In hypothyroidism the relative number of binding sites is increased; therefore, the resin-sponge uptake is reduced. In restatement: The *in vitro* serum thyroxine binding capacity is inversely related to the activity of the thyroid gland.

The binding of ^{131}I labelled T-3 with serum proteins is significantly dependent upon temperature and time of incubation. So that a normal T-3 range reference may be used, corrections must be applied to normalize a T-3 uptake result as to standard room temperature and incubation time. Room temperature may vary even under ideal conditions in different parts of the laboratory. For these reasons the ^{131}I T-3 resin-sponge technique was modified to minimize the above mentioned variations and corrections. This study presents a reproducible laboratory procedure, free from temperature corrections or variations, with a close distribution and a significant separation of ranges. The routine T-3 room temperature incubation is compared statistically, by group, to the modified T-3 method, using an ice water bath incubation. Studies were repeated on pooled serum in duplicate from different resin batches for ten batches.

MATERIALS AND METHODS

The thyroid status of 147 patients was evaluated clinically and confirmed by serum protein-bound iodine, ^{131}I uptake tests, ^{131}PBI conversion ratio determinations and saliva radioactivity. The patients were then classified into euthyroid, hypothyroid and hyperthyroid groups. From each of the 147 patients, 12 ml blood samples were obtained. The blood samples were permitted to clot and 6 ml of serum were obtained. One ml duplicates of serum were incubated with ^{131}I T-3 in an ice water bath. Previous incubation studies were performed as a function of time for room temperature and for ice water bath. The values indicated that a 45 minute incubation period at room temperature and an 80 minute incubation period in ice water bath were optimum, yielding the most reproducible results for the shortest time.

A two inch by two inch NaI *(Tl)* well-type scintillation detector with a gamma spectrometer and scaler was used. The photopeak of 364 KeV for ^{131}I was set with a channel width of 80 KeV. A minimum of 10,000 counts were obtained on all samples studied. The analysis was performed in the following manner:

1. One ml of serum was pipetted into a test tube.
2. The contents, less than 0.1 μC ^{131}I T-3, of a Triomet[1] syringe was emptied into the test tube.

[1]Abbott's liothyronine ^{131} T-3 diagnostic kit.

3. Resin-sponge was added and air was expelled from the sponge by compressing it with a plastic rod.
4. Counting of the samples was performed prior to incubation.
5. The samples were then incubated, performed in duplicate, two at room temperature and two in an ice water bath.
6. Following the completion of incubation, the sponge was sucked dry, washed three times with distilled water and reconstituted to the original level.
7. The washed sample was recounted.
8. The per cent of sponge uptake was recorded as

$$= \frac{\text{Net counts of washed sponge} \times 100}{\text{Net counts of sample before incubation}}$$

RESULTS

Results of the T-3 ^{131}I resin-sponge uptake tests performed by the room temperature and ice water bath incubation methods are tabulated, Table I, according to the number in the group, mean value, standard deviation, probability (P) values and t-scores. The overall distribution of the ice water bath method compares favorably with, and is as accurate as, the room temperature method. The results are reproducible and free from temperature corrections. In the euthyroid and hyperthyroid ranges the differences are statistically significant with no overlapping of results (Table I, Graph I). The t-test of significance was based on the formula:

$$T = \frac{M_1 - M_2}{\sqrt{\frac{T_1^2}{N_1} + \frac{T_2^2}{N_2}}}$$

TABLE I

PERCENT T-3 ^{131}I RESIN-SPONGE UPTAKE

	Method	*Number in Group*	*Mean Value*	*S. D.*	*T*	*P*
Overall	Room Temperature	147	30.2	± 8.1	1.79	.04
	Ice Water Bath	147	28.5	± 8.2		
Euthyroid Group	Room Temperature	99	28.5	± 5.1	5.69	Practically Zero
	Ice Water Bath	99	25.0	± 3.4		
Hypothyroid Group	Room Temperature	24	26.4	± 3.4	8.10	Practically Zero
	Ice Water Bath	24	19.8	± 2.1		
Hyperthyroid Group	Room Temperature	24	43.4	± 9.8	0.473	.30
	Ice Water Bath	24	42.0	±11.0		

where M_1 and M_2 are the means of the two groups, T_1 and T_2 are the standard deviations of the two groups, and N_1 and N_2 are number of people in each group. This ratio compares the difference in the means obtained in the comparison study with the quantity in the denominator, standard error of means, which gives the amount by which the sample means may be expected to differ from the means of the entire population as a result of change incurred by the use of a random sample.

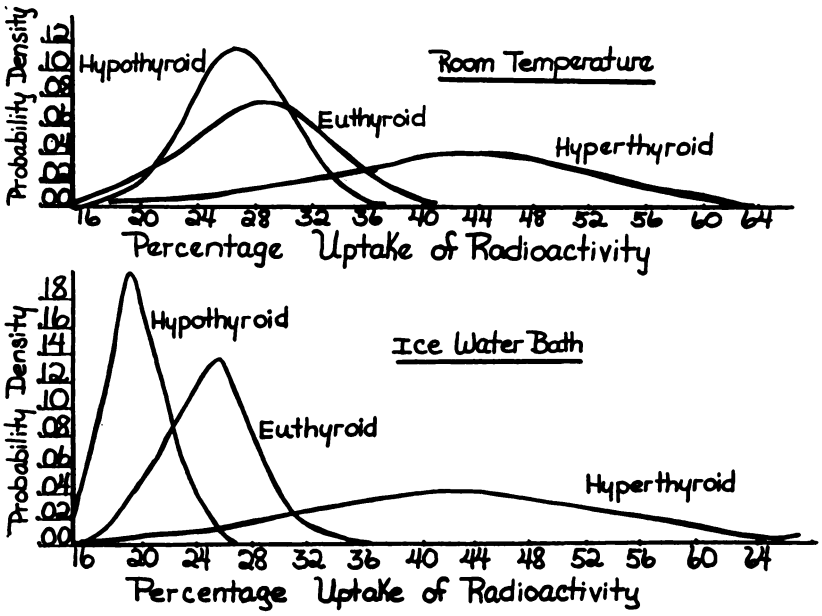
A t-score of 1.645 demonstrates that there is a five per cent probability that the difference in means occurs by change and not as a result in the difference in testing. A t-score of at least 2.327 yields, from the table of t values, indicates a probability of less than one per cent that the difference occurs by change. It is then stated that the groups differ significantly on the one per cent level instead of on the five per cent level as above.

CONCLUSION

This report describes the results of the T-3 ^{131}I resin-sponge uptake test in 147 patients calculated by the standard room temperature incubation method and by an improved technique utilizing an ice water bath incubation. The data indicate that the two methods are of equal usefulness as diagnostic procedures in the determination of thyroid activity. The ice water bath technique is free of temperature corrections and there is no overlap of results between the euthyroid and hypothyroid groups. The technique is highly reliable and easily managed. The results are reproducible and analyzed according to the t-test of significance.

GRAPH I

Comparison Percentage Uptake of Radioactivity for Room Temperature and Ice Water Bath Incubation Methods



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