AN ASSESSMENT OF THE RED-CELL UPTAKE OF ^{131}I-LABELLED TRIIODOTHYRONINE IN THE DIAGNOSIS OF THYROID DISORDERS*

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In 1957 Hamolsky *et al.*[1] described the red-cell uptake of ^{131}I-labelled triiodothyronine as an accurate test of thyroid function, and in 1959 they reviewed 2,900 determinations.[2] From their reports and those of others,[3-9] it appears that this test has advantages over standard tests of thyroid function in that it is not affected by the previous intake of substances containing iodine and does not involve the administration of radioactive material to the patient. The red-cell uptake (RCU) of triiodothyronine (T3) is believed to be a measure of the degree of saturation of the available thyroxine- and T3-binding sites on the serum proteins. The greater the binding capacity of the serum proteins, the lower will be the RCU of T3. It has been shown by Albright *et al.*[10] that the thyroxine-binding capacity of the plasma is increased in myxoedema and decreased in hyperthyroidism. Hamolsky *et al.*[1] showed that red blood cells from euthyroid persons, incubated in hyperthyroid serum, have a raised uptake and that cells from a hyperthyroid patient, incubated with normal serum, have a normal uptake. No metabolic activity on the part of the red cells is required.[11] Thus it would seem that the RCU is the result of competition between the red cells and the plasma protein-binding sites for the T3. This relationship can be disturbed by oestrogens, pregnancy, dicoumarol, abnormality of serum proteins, liver disease, CO_2-retention and polycythaemia.[3]

Similar tests have been devised, using a resin sponge[12] or resin,[13,14] which compete for T3 added to the plasma in a manner similar to the red cells.

In the present study, the results obtained with the RCU test over a period of a year were correlated with the level of protein-bound iodine (PBI) and with the 24-hour thyroidal ^{131}I uptake.

METHOD AND MATERIAL

Method

The method of Hamolsky *et al.*[1] was used, the only difference being the use of 5 ml. glass tubes in the place of Erlenmeyer flasks. The method consists of adding 0·1 ml. of a standard solution of ^{131}I-labelled T3† to 3 ml. of heparinized blood in a small glass tube. The tube is then rotated in a constant temperature incubator at 37°C. for 2 hours. Duplicate 1 ml. samples of the blood are then pipetted into test-tubes and the radioactivity in each tube is measured in a well-type scintillation counter. The red cells in each tube are separated by centrifugation and are then washed 5 times with 10 ml. of isotonic saline. The cells are left suspended in 1 ml. of saline and the residual radioactivity is counted. The ratio of the residual to the initial count is expressed as a percentage and corrected to a theoretical haematocrit of 100%.

Clinical Material

Altogether, 262 tests have been carried out. Of these, 154 were performed on 133 patients attending the radiotherapy department for thyroid investigation, 42 on 36 normal students and technicians, and 24 on normal pregnant women and newborn infants.**

Fifty-eight patients were euthyroid (mainly patients with nodular, colloid or simple goitre), 20 were euthyroid following treatment for hyperthyroidism or myxoedema, 46 were hyperthyroid and 9 were hypothyroid. Thyroid status was determined in all cases by clinical assessment, correlated with results of estimations of serum-PBI levels, 24-hour thyroidal ^{131}I uptake, blood-cholesterol levels and, in some cases, the 24-hour conversion ratio and 48-hour PB^{131}I levels.

RESULTS

Red-cell Uptake

The RCU of T3 in euthyroid, hyperthyroid and hypothyroid subjects is tabulated in Table I. Of the 46 hyperthyroid patients, 8 had RCUs within the normal range. This group consisted of 7 patients with autonomous hyperactive nodules, and 1 patient having treatment with 'tapazole' who, on other criteria, was felt to be still hyperthyroid.

TABLE I. RCU OF T3 IN VARIOUS THYROID STATES

Thyroid status	*Sex*	*No. of cases*	*RCU of T 3 %*
Euthyroid (including normal subjects) 36 ..	F	92	13·9 (range 10·4–18·5)
	M	22	16·0 (range 13·0–20·0)
Hyperthyroid	F	41	23·2 (range 13·8–33·0)
	M	5	27·3 (range 23·3–30·9)
Hypothyroid	F	6	11·2 (range 9·6–12·8)
	M	3	11·0 (range 9·2–12·5)

The Effect of Therapy on the RCU of T3 (Table II)

Twelve patients were followed-up for a period of 3 - 9 months after ^{131}I therapy for hyperthyroidism. Two remained hyperthyroid and two became hypothyroid. The remainder became euthyroid. The RCU closely paralleled the PBI levels and the clinical state of the patient. It is apparent from Fig. 1 that the RCU was perhaps even more reliable than serial PBI levels in this respect. The patients who failed to respond to treatment were more clearly identified by their RCU than by their PBI levels.

Thirteen hyperthyroid patients taking tapazole and 1 having potassium perchlorate were tested; 3 of them serially. As in the previous group, the results of the RCU

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†Obtained from Abbott Laboratories, Oak Ridge, Tennessee.

**Forty-two of the 262 tests have been discarded from this analysis because of the totally bizarre results obtained. There were T3 possible reasons for this high degree of unreliability. Firstly, it has been shown by van Zyl[15] that, during storage, radio-chemical decomposition of ^{131}I-labelled T3 may occur from self-radiation. The use of old material, i.e. ^{131}I-T3 kept for a period of more than 2 weeks, often resulted in high RCUs. Secondly, the use of concentrations of ^{131}I-T3 greater than the recommended 0·006 - 0·012 μg. per 0·1 ml., in an effort to obtain higher degrees of radioactivity and thus prolong the 'life' of a particular batch, yielded higher uptake results. Thirdly, one batch of radioactive T3 produced extremely low results on all patients. Recently Nelson (quoted by Sterling and Tabachnick[16]) commented on the low results yielded by some shipments of ^{131}I-T3 in a related resin-uptake test and suggested the use of normal standard to overcome this. Meade[14] has drawn attention to other possible sources of error, all of which can be overcome by careful attention to the technique of the test.

TABLE II. THE EFFECT OF ^{131}I THERAPY FOR HYPERTHYROIDISM ON THE RCU AND ON THE SERUM PBI LEVEL

	Initial level		*3 months*		*6 months*		*9 months*		
Patient	*RCU* %	*PBI (μg./100 ml.)*	*RCU* %	*PBI (μg./100 ml.)*	*RCU* %	*PBI (μg./100 ml.)*	*RCU* %	*PBI (μg./100 ml.)*	*Thyroid status after treatment*
1	25·8	14·0	25·0	—	20·8	10·5	16·5	8·0	Slow response
2	22·7	9·0	12·7	7·0	—	—	—	—	Hypothyroid
3	27·0	18·0	25·0	12·0	25·0	10·0	26·0	9·0	Hyperthyroid
4	28·4	10·0	25·6	8·8	—	—	26·0	9·8	Hyperthyroid
5	28·6	11·0	19·2	7·0	13·5	7·0	12·3	—	Euthyroid
6	18·1	12·5	16·4	13·4	12·6	8·5	—	—	Euthyroid
7	29·4	13·0	—	3·5	12·5	1·5	—	—	Hypothyroid
8	33·0	11·3	16·2	6·0	—	—	—	—	Euthyroid
9	20·8	8·2	16·3	6·8	—	—	—	—	Euthyroid
10	20·0	12·8	13·4	10·5	10·8	12·0	—	—	Euthyroid
11	16·6	8·5	12·6	7·0	—	—	—	—	Euthyroid
12	26·1	13·0	—	—	16·7	7·2	—	—	Euthyroid

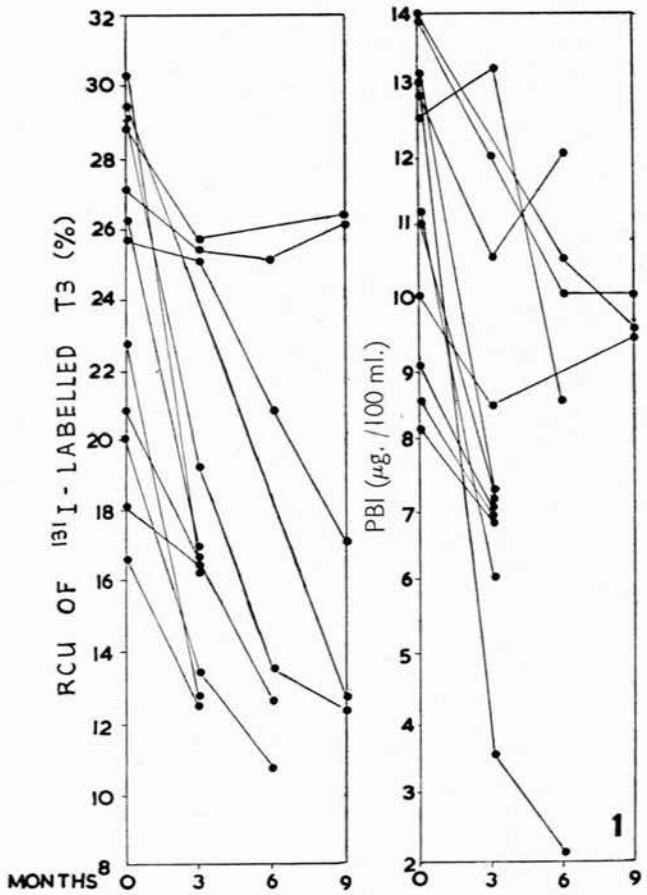

Fig. 1. The effect of ^{131}I therapy for hyperthyroidism on the RCU of T3 and the serum-PBI level.

correlated well with the clinical impression and the PBI levels. Several hypothyroid patients were tested while taking thyroid extract or thyroxine. The RCU was in keeping with the clinical state and correlated well with the PBI level.

The Effect of Exogenous Iodine (Table III)

There were 11 euthyroid patients and 1 hyperthyroid patient (No. 6, Table III) who had raised PBI levels owing to administration of iodine either orally or parenterally. Five of these patients had depressed 24-hour thyroidal ^{131}I uptakes for the same reason. All 11 euthyroid patients had normal RCUs.

Patient 6 (Table III) was a woman of 60 years who had been taking a mixture containing potassium iodide for bronchial asthma. She complained of weight loss, fatigue and nervousness and had a firm, nodular goitre. Her serum-PBI level was over 25 μg. per 100 ml., her 24-hour thyroidal ^{131}I uptake was 2%, and her RCU was 21·0%. Three weeks after stopping the medicine, the serum-PBI level was 12 μg. per 100 ml. and the RCU 21·3%. After a further 3 weeks the serum-PBI level was 9 μg. per 100 ml. and the RCU 24·3%. The 24-hour thyroidal uptake of ^{131}I was 80%, and following the oral administration of 80 μg. of T3 daily for a week, it remained 70%, indicating a hyperthyroid state. The RCU had all along suggested hyperthyroidism and the rise in the RCU has been mentioned by Hamolsky *et al.*[2] as occurring in hyperthyroid patients following withdrawal of exogenous iodine.

TABLE III. EFFECT OF IODINE

Patient	*RCU* %	*PBI (μg./100 ml.)*	*24-hour thyroidal ^{131}I uptake* %	*Source of iodine*
1	13·4	10·6	31	Pot. iod.
2	15·8	11·8	25	?
3	11·3	9·3	15	Pot. iod.
4	16·9	11·0	14	?
5	15·2	25+	—	Cholecystogram
6	21·0	25+	2	Pot. iod.
7	16·5	25+	—	?
8	17·7	7·8	14	Pot. iod.
9	13·3	9·8	34	Pot. iod.
10	17·9	25+	—	Cholecystogram
11	15·0	25+	—	Cholecystogram
12	20·0	9·8	5	Lugol's iodine

Factors Influencing Interpretation of RCU Tests

Pregnancy (Table IVA). Twenty normal pregnant women were tested. The RCUs were well below normal in 12. Four newborn infants were tested, and their RCUs were 10·2, 10·9, 9·1 and 15·7% respectively.

TABLE IVA. EFFECT OF PREGNANCY ON RCU OF T3

	Number	*RCU* %	
Pregnant patients ..	20	10·7	(range 7·4–13·6)
Newborn infants ..	4	11·5	(range 9·1–15·7)

TABLE IVB. EFFECT OF MENSTRUATION ON RCU OF T3

Patient	Day of cycle 1st %	15th %	25th %
1	11·3	16·5	16·0
2	13·9	14·1	15·8
3	15·5	15·8	—
4	10·8	—	13·5
5	11·1	—	—
6	10·9	—	—

Menstruation (Table IVB). Six women were tested during or just after menstruation and 5 showed low RCUs. Three of the 4 who had repeat tests showed a rise in the RCU later in the cycle.

Cardiac arrhythmias. A patient with paroxysmal nodal tachycardia had an RCU of 27·1% and a patient with auricular fibrillation and heart failure an RCU of 37·7%. In both patients, all other tests of thyroid function were normal.

Serum-protein abnormality. Three patients with grossly disturbed serum-protein patterns were tested. In 2 of them who had low albumin levels, the RCU was raised. In the third patient, who had only slight reduction in the albumin content and a raised globulin level, the RCU was low.

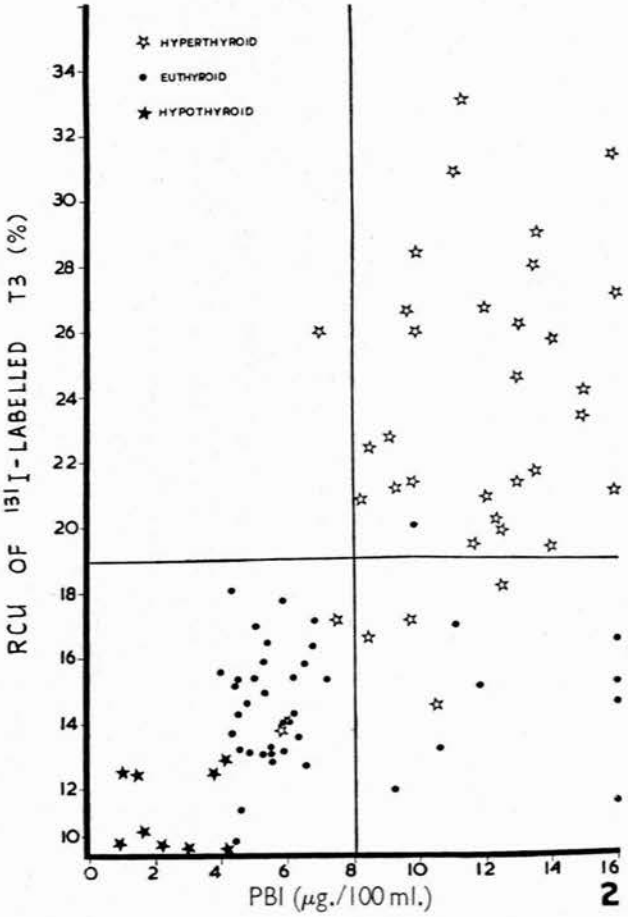

Fig. 2. The correlation between the RCU and the serum-PBI level in hyperthyroid, euthyroid and hypothyroid patients. The lines indicate the upper limit of normal for each test.

Correlation of the RCU with Other Tests

1. *Serum PBI (Fig. 2).* Thirty-six patients with hyperthyroidism had both their RCU and their serum-PBI level estimated. In 2 cases both tests were within normal limits. In 4 cases the RCU was normal and the PBI level raised, whereas in 1 case the opposite was found. Of 42 euthyroid patients, 11 had raised serum-PBI values resulting from exogenous iodine. Nine hypothyroid patients were similarly tested. In 6 of these both tests were below normal. However, in most the PBI level was unequivocally low whereas the RCU in hypothyroidism, with only 2 exceptions, was at, or only just below, the lower limit of normal.

2. *24-Hour thyroidal uptake of ^{131}I (Fig. 3).* There were 43 hyperthyroid patients on whom both this test and the RCU were performed. In 11 patients the 24-hour thyroidal ^{131}I uptake was normal while the RCU was raised. Five of these patients had single hyperactive thyroid nodules, 2 were taking tapazole, and 2 were being treated with

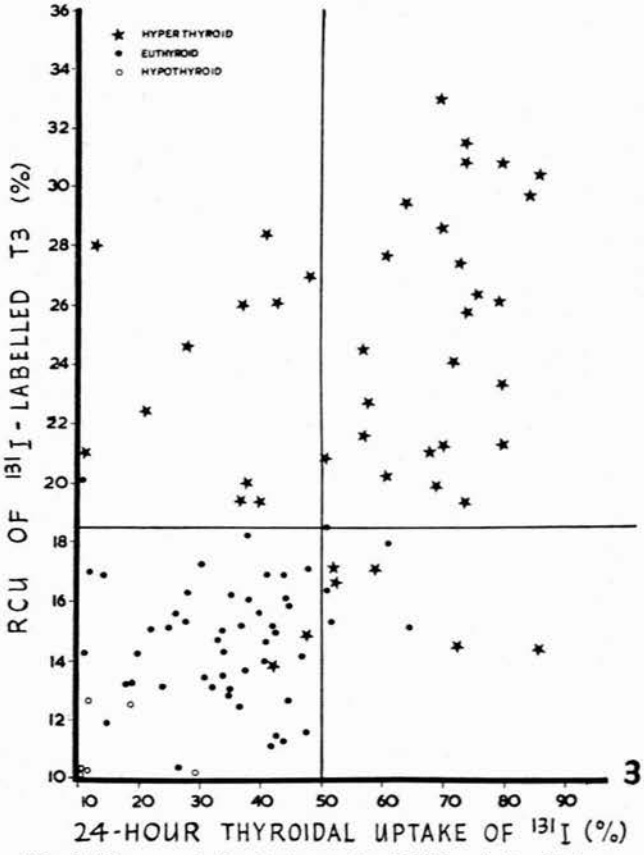

Fig. 3. The correlation between the RCU and the 24-hour uptake of ^{131}I in hyperthyroid, euthyroid and hypothyroid patients. The lines indicate the upper limit of normal for each test.

iodine. In 5 cases the RCU was normal and the 24-hour thyroidal ^{131}I uptake was raised, and in 2 cases both the tests gave normal results. These last 7 patients all had hyperactive nodules and have been discussed above. Of 60 euthyroid patients similarly tested, 7 had low 24-hour thyroidal ^{131}I uptakes associated with a normal RCU. Four

of these had had excessive iodine administration resulting in a depression of thyroid function. Five patients had high levels of 24-hour thyroidal ^{131}I uptake and a normal RCU, this combination of results possibly being due to a degree of iodine deficiency.

3. *T3-suppression test.* It was considered that, following the administration of 80 μg. of T3 daily for 7 days, a 24-hour thyroidal uptake of ^{131}I of less than 20% of the administered dose indicated a euthyroid state.[17] Thirty-two patients were tested, most of whom had presented considerable difficulty in diagnosis. Of 15 euthyroid patients, 3 showed inadequate suppression, but normal RCUs. All of these had 'hot nodules'. All 17 hyperthyroid patients undergoing this test showed incomplete suppression. These included 7 of the 8 patients with normal RCUs.

DISCUSSION

The values for the RCU in euthyroid, hyperthyroid and hypothyroid patients tended to be slightly higher in the present study than in other reported series[1-6] (Table V). The RCU is increased by high haematocrit values,[2,18,19] and it is possible that the altitude in Johannesburg, resulting in a higher average packed-cell volume, produced this effect. Adams *et al.*[19] suggested that the differences noted in all series between males and females may be explained by the higher haematocrit values found in males.

TABLE V. COMPARISON OF THE RCU OF T3 WITH OTHER SERIES

	Euthyroid		*Hyperthyroid*		*Hypothyroid*	
	Mean %	*Range* %	*Mean* %	*Range* %	*Mean* %	*Range* %
Hamolsky, *et al.*[1,2]	13·9	11·0–17·0	22·5	17·0–35·0	9·3	6·1–11·0
Ureles and Murray[4]	14·0	11·5–18·5	22·7	18·5–32·0	9·9	7·5–11·0
Robbins and Murphy[3]	15·4	10·1–23·0	21·3	17·1–26·4	12·1	6·4–16·2
Crigler *et al.*[5]	—	10·7–17·0	—	15·8–27·0	—	6·0– 9·8
Sterling[6]	13·1	—	17·3	—	8·6	—
Present series	15·0	10·4–20·0	23·2	13·8–33·0	11·2	9·6–12·6

The finding of 8 out of 46 hyperthyroid patients with normal RCUs differs from the experience of Hamolsky *et al.*[2] and Ureles and Murray,[4] both groups finding a smaller proportion. These 8 patients were all mildly hyperthyroid and presented some difficulty in diagnosis. It is felt that the mild degree of the hyperthyroidism rather than the fact that 7 of them had autonomous nodules was the reason for the normal RCU. It is, however, conceivable that in hyperthyroidism caused by 'hot nodules', there may be a difference in the type of thyroid hormone produced and possibly a difference in the binding sites the hormones occupy on the plasma proteins.[20] In any event, it would seem that the RCU, like most other tests of thyroid function, will not on its own prove of value in borderline cases of hyperthyroidism.

There were too few patients with hypothyroidism for any firm conclusions to be drawn, but the impression was gained that the serum-PBI level is more reliable than either the 24-hour thyroidal uptake of ^{131}I or the RCU in making this diagnosis.

Hamolsky *et al.*[2] tested 42 patients before and after the administration of organic iodine compounds when both the serum-PBI level and the 24-hour thyroidal ^{131}I uptake became spuriously abnormal. Slight increases in the RCU were noted in 4 cases, while in the remaining 38 it was unchanged. This failure of exogenous iodine to affect the RCU significantly has been confirmed by Robbins and Murphy[3] and by Ureles and Murray,[4] and is apparent in the 12 cases described in this series.

Apart from basal metabolic rate and cholesterol studies which are not specifically related to thyroid function, the RCU and similar resin tests[12-14] are the only tests of value in the presence of iodine contamination.

An increase in the binding capacity of the serum proteins for thyroxine has been shown during pregnancy[21] and is associated with a rise in the PBI level.[22] In Hamolsky's series[2] a low RCU was found from the third week of gestation onwards, the average uptake being 7·4%. Where a normal RCU persisted beyond 8 weeks in 6 pregnancies, each pregnancy terminated in miscarriage. The relationship of spontaneous abortion to normal PBI levels during pregnancy has been noted previously.[23] The low RCU in pregnancy has been confirmed by Robbins and Murphy[3] and by Ureles and Murray,[4] and is also apparent in this series. Ureles and Murray drew attention to the effect of menstruation in producing a reduction of the RCU of the same order as that found in this series. The reason for these changes in pregnancy and menstruation is not known. A similar rise in the PBI level[24] and fall in the RCU can be produced by oestrogen administration.[2]

Kurland *et al.*[25] have observed the presence of a high RCU in patients with paroxysmal atrial fibrillation and tachycardia in whom the PBI level, 24-hour thyroidal ^{131}I uptake, and basal metabolic rate were within normal limits. They found an increased rate of turnover of labelled thyroxine in such patients. Five out of 39 were treated with potassium iodide, even though they were considered to be euthyroid, and improvement was noted in each case. The first of the 2 such patients tested in this series was treated with tapazole, although euthyroid, and for the first time in over 6 months of observation obtained relief from daily attacks of nodal tachycardia.

Low levels of PBI have been described in association with severe hypoalbuminaemia.[26] Hamolsky *et al.*[2] and Ureles and Murray[4] have reported on the rise in the RCU in the same circumstances, presumably owing to a decrease in the T3-binding capacity of the plasma proteins. Hamolsky *et al.*[2] noted the effect of dicoumarol, liver disease and CO_2-retention in causing a rise in the RCU. These effects have been confirmed[3,4] and have not been further investigated in this series. In the routine work of the radiotherapy department which forms the basis of this

study, these biological abnormalities have not occasioned much difficulty in the interpretation of the results of RCU estimations.

CONCLUSIONS AND SUMMARY

The red-cell uptake of ^{131}I-labelled triiodothyronine is an *in vitro* test of thyroid function, which avoids the necessity for the administration of radioactive material to the patient. In the present series it proved particularly valuable in the follow-up of patients with hyperthyroidism treated with ^{131}I or with drugs, and in the diagnosis of patients in whom previous administration of iodine had invalidated the results of thyroidal ^{131}I uptake and serum-PBI studies. In following the progress of hypothyroid patients treated with thyroid hormones, the RCU has also proved useful. It may be of special value in patients treated with T3 where the serum-PBI level fails to mirror clinical improvement.[3,6]

Correlation with the PBI levels and 24-hour thyroidal ^{131}I-uptake studies, showed that the RCU as a single test of thyroid function was on a par with both these tests in diagnostic accuracy. The results in this series did, however, confirm strikingly the great value of 2 or more tests in combination as opposed to reliance on any one test alone in the diagnosis of thyroid disorders. There are several biological factors that may interfere with the test, but these occasioned little practical difficulty. The short useful 'life' of the radioactive T3 is a distinct disadvantage when using this test in South Africa. Variations in the quality of the T3 make the use of a normal standard advisable. In addition, the test requires careful attention to detail in its performance.

With better understanding of the mechanisms involved in the protein binding of thyroid hormones and perhaps the substitution of other indicators for the red cells, there would seem to be a definite place for this type of test in the clinical investigation of thyroid function.

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