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THE "FREE THYROXINE INDEX"

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THE principal thyroid hormone thyroxine (T_4) is transported from the thyroid gland to the tissues in reversible combination with a group of proteins known as thyroxine binding proteins (T.B.P.) (1, 2). This association can be explained by the law of mass action which allows for the existence of a tiny fraction (0.05%) of the total thyroxine in the unbound or free state (3). This "free" fraction is dialysable, able to enter the cell and is believed to comprise the metabolically active fraction of circulating thyroxine and to represent the true determinant of thyroid status (4, 5).

Thyroid function tests such as the estimation of thyroxine iodine (T_4I), protein bound iodine (P.B.I.) and the uptake of ^{131}I labelled triiodothyronine (T_3) by red cells or resin, are usually sufficient to confirm a diagnosis in most cases of thyroid disease. These tests are found unreliable in conditions in which there is any abnormality in the protein binding of thyroxine. Deviations from normality in results obtained may occur without comparable changes in thyroid status. In such cases the serum level of free thyroxine ($F.T_4$) is the only theoretically reliable index of thyroid status. The $F.T_4$ concentration is too low to be measured directly in the laboratory so various indirect methods have been developed for its estimation. These include the use of equilibrium dialysis (6, 7, 8) and Sephadex gel filtration (9) to separate free from protein-bound thyroxine. Clark and Horn (10) combined two existing tests, the P.B.I. and resin uptake of ^{131}I T_3 to give a free thyroxine index which they considered to be proportional to the concentration of free thyroxine in the blood.

Wellby and O'Halloran (11) found a satisfactory correlation between $F.T_4$ levels measured by gel filtration (9) and by the method of Clark and Horn. The principle of this method is as follows:

$$T_4 + \text{T.B.P.} \rightleftarrows \text{T.B.P.} : T_4$$

$$K = \frac{(T_4)\,(\text{T.B.P.})}{(\text{T.B.P.} : T_4)}$$

$$1/K\,(T_4) = \frac{(\text{T.B.P.} : T_4)}{(\text{T.B.P.})}$$

T_4 = Unbound or free thyroxine.
T.B.P. = Unsaturated thyroxine binding protein.
T.B.P. : T_4 = Protein bound circulating thyroxine.
K = Equilibrium constant for the reaction.

From this model it can be seen that the free thyroxine concentration is a direct function of the total thyroxine content of serum (T.B.P. : T_4), and an inverse function of the unsaturated thyroxine binding protein (T.B.P.) (4, 9). In order to measure these two indices Clark and Horn (10) used the P.B.I. estimation as a measure of total thyroxine concentration and the resin uptake of T_3 to determine the degree of unsaturation of T.B.P.

The present authors have introduced the estimation of T_4I as a more direct measurement of total thyroxine than the concentration of all the serum protein-bound iodinated amino acids as represented by the P.B.I. This T_4I estimation has the additional advantage of being free from the effects of the usual contaminants which invalidate the P.B.I. (12, 13). We have also substituted the resin uptake of T_4 for that of T_3 as a more direct assay of the ability of the patient's serum to bind thyroxine (14).

The T_4I concentration was determined by the method of Smyth and

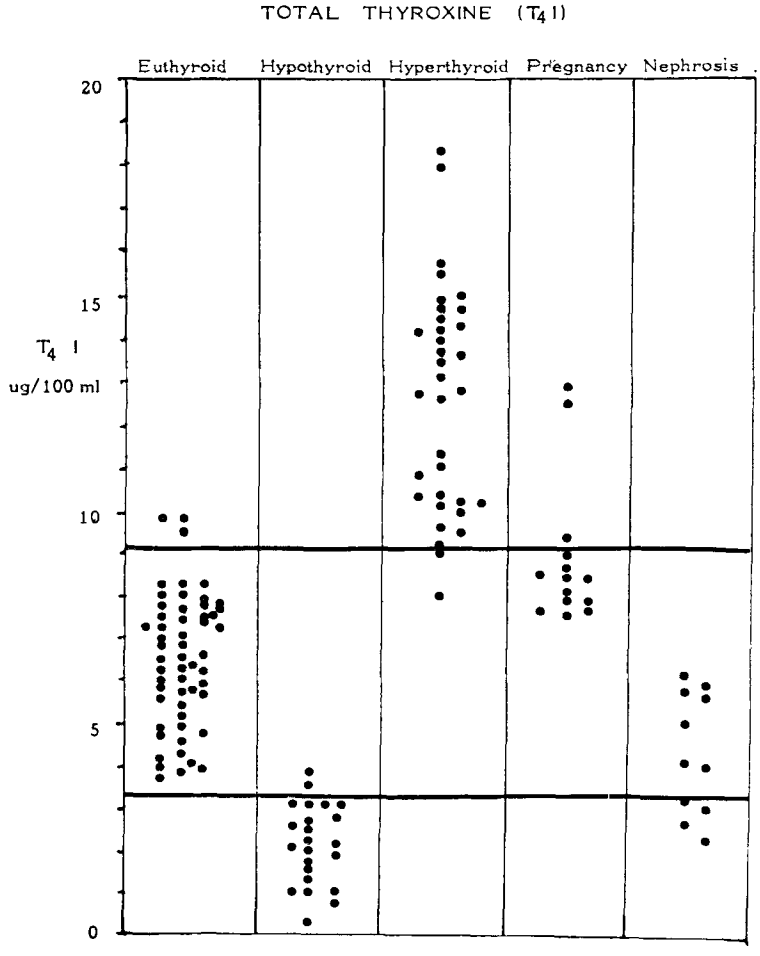

Fig. 1—Thyroxine Iodine (T_4I) levels in five groups of patients classified according to clinical status. The normal range of 3.3-9.2 ug/100 ml. T_4I is shown by the dark horizontal lines.

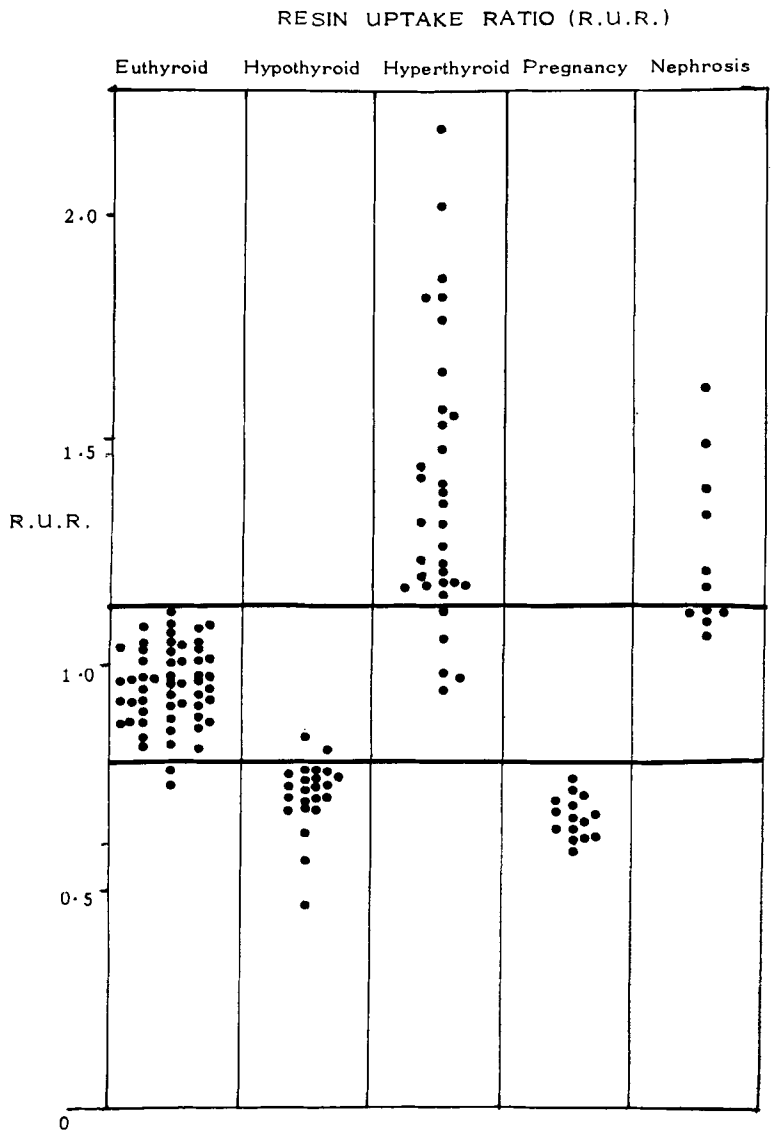

Fig. 2—Resin Uptake Ratio (R.U.R.) values. Normal range 0.79-1.15 shown by horizontal lines.

O'Donovan (1968) (13) which used the principle of competitive protein-binding analysis (C.P.B.A.) as postulated by Murphy (1964) (15). Results are expressed as ug/100 ml thyroxine iodine (T_4I).

The method used for the resin uptake of T_4 ^{125}I from serum is a modification of that proposed by Clark in 1963 (15). The estimation can be carried out on 0.5 ml. of serum with the result that both tests can usually be carried out on less than 3 ml. of serum. The resin uptake of a given serum is expressed as a ratio to a simultaneous uptake from a controlled pooled serum and reported as the resin uptake ratio (R.U.R.).

The free thyroxine index (F.T.I.) is the mathematical product of the T_4I and R.U.R. estimations expressed as an arbitrary numerical expression.

Serum total thyroxine (T_4I) and resin uptake ratio (R.U.R.) were carried out on 5 groups of patients, euthyroid, hypothyroid, hyperthyroid, pregnant and nephrotic. The latter two were chosen as conditions in which protein binding abnormalities occur. The F.T.I. was calculated and the diagnostic accuracy of the three indices of thyroid function tested for each group. Figure 1 shows T_4I levels obtained. The calculated 95% confidence limits for the normal range previously reported by the present authors were 3.3

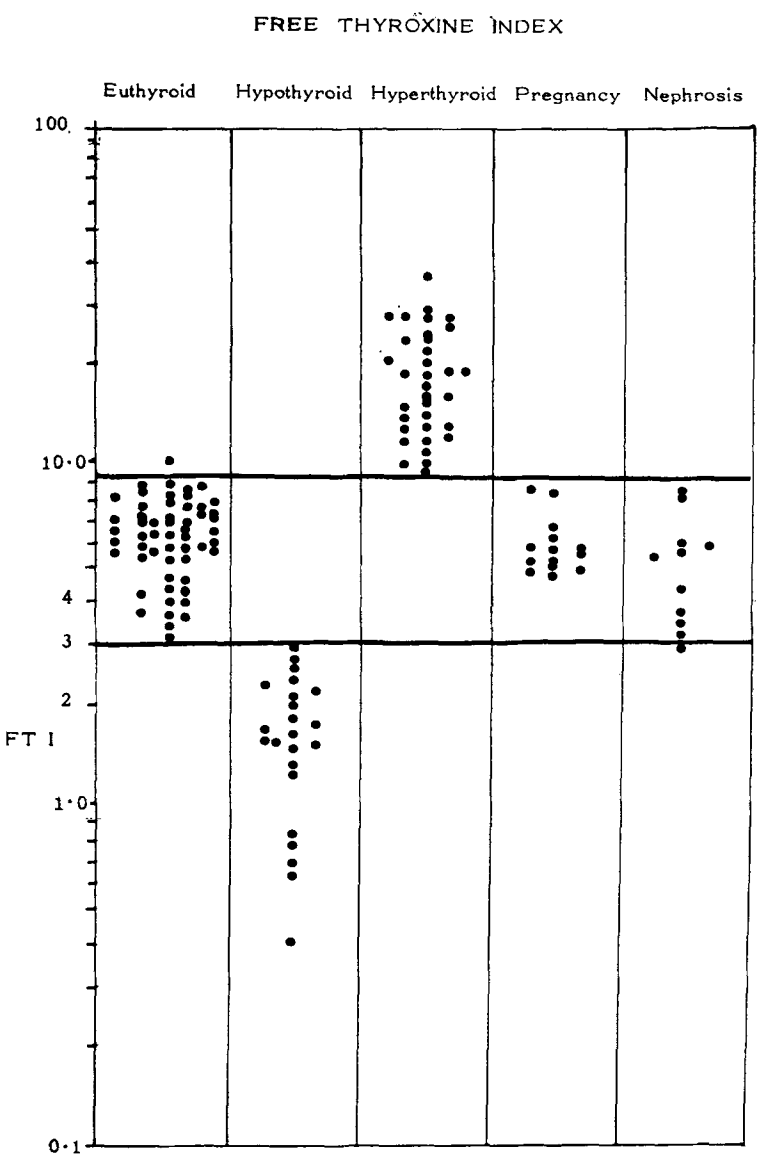

Fig. 3—Free Thyroxine Index (F.T.I.). Normal range 3.0-9.3 shown by horizontal lines.

TABLE I

GROUP	No.	T_4I	R.U.R.	F.T.I.
Euthyroid	55	94.5	96	98
Hypothyroid	23	91	91	100
Hyperthyroid	34	94	85	100
Pregnant	14	79	0	100
Nephrotic	11	64	44.5	90

The percentage agreement with clinical status of T_4I, R.U.R. and F.T.I. is shown for the five groups of patients.

to 8.6 ug/100 ml. T_4I (13). As larger numbers of results have become available these limits have been redefined as 3.3 to 9.2 ug/100 ml. T_4I. This is equivalent to a normal range of 4.0 to 11.0 ug/100 ml. T_4 uncorrected for recovery. The mean T_4I levels in the hypothyroid and hyperthyroid groups are significantly different from the euthyroid mean $p<0.001$ in each case.

In the pregnancy group, values lie in the high normal or hyperthyroid range and the mean T_4I is significantly elevated with respect to the euthyroid mean $p<0.001$.

In the group with nephrosis values lie in the low normal or hypothyroid range and the mean T_4I is significantly low with respect to the euthyroid mean $p<0.001$. All of these subjects were found to be clinically euthyroid by previously described criteria (13). The deviations from normal in the T_4I levels can be explained by abnormalities in the principal thyroxine-binding protein, thyroxine binding globulin (T.B.G.) (16, 17). In pregnancy there is an increase in the level of T.B.G. giving an elevated thyroxine-binding capacity resulting in a rise in serum T_4I. In nephrosis there is a reduction in T.B.G. giving reduced thyroxine-binding capacity and lowered T_4I.

Figure 2 shows R.U.R. values carried out on the same groups of patients. The normal range for the R.U.R. is 0.79 - 1.15 with mean 0.96 ± 0.09. The mean R.U.R. values in the hypothyroid, hyperthyroid, pregnant and nephrotic groups are significantly different with respect to the euthyroid mean $p<0.001$.

It should be noted that values for the R.U.R. and T_4I in the pregnant and nephrotic groups deviate from the normal in opposite directions. In pregnancy the T_4I is elevated, the R.U.R. reduced; in nephrosis the T_4I is reduced, the R.U.R. elevated.

Figure 3 shows the product of the T_4I and R.U.R. expressed as a free thyroxine index (F.T.I.). The normal range for the F.T.I. is 3.0 - 9.3 with a mean of 6.14 ± 1.56. The mean F.T.I. levels in the hypothroid and hyperthyroid groups are significantly different from the euthyroid, mean $p<0.001$. There is not a significant difference between the mean values of the preg-

nancy and nephrotic groups and that of the euthyroid group, mean $p<0.1$ and $p<0.2$ respectively. It will be noted that F.T.I. values were plotted on a log graph. This is a matter of convenience as the spread of values was rather large for standard graph paper.

Table 1 lists results obtained for T_4I and R.U.R. from Fig. 1 and Fig. 2 and compares the diagnostic accuracy shown in each individual test with that obtained when they are combined as a F.T.I. as shown in Fig. 3. It can be seen that the F.T.I. gives appreciably better correlation than either T_4I or R.U.R. alone.

Discussion

The estimation of total thyroxine iodine (T_4I) is usually sufficient to confirm a diagnosis in most cases of hypothyroidism or hyperthyroidism. However, in conditions where abnormal thyroxine binding proteins occur or in borderline cases where T_4I levels may be equivocal the estimation of R.U.R. and calculations of the F.T.I. can be of considerable value. The estimation of R.U.R. can be quite readily carried out in any laboratory in which the estimation of serum thyroxine is already available as no extra reagents or apparatus are required. There is no significant delay involved in this addition to diagnosis.

Summary

The direct estimation of serum thyroxine iodine (T_4I) and the uptake of ^{125}I labelled thyroxine from serum by anion exchange resin (R.U.R.) can both be used independently to assess thyroid status. The results from these two tests can be combined to give a free thyroxine index (F.T.I.) which is believed to be proportional to the concentration of free thyroxine in the serum.

A comparison has been made between the diagnostic accuracy of the T_4I, R.U.R. and F.T.I. as done by the above method, carried out on a series of 137 patients who fall into five diagnostic categories, euthyroid, hypothyroid, hyperthyroid, pregnant and nephrotic. It was found that the amended F.T.I. gives better agreement with clinical status than either the T_4I or R.U.R. alone.

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