J. clin. Path., 1974, 27, 372-376

An evaluation of absorbent granule kits for determining serum thyroxine concentration and free thyroxine index in the diagnosis of thyroid function

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SYNOPSIS Serum thyroxine concentrations were measured in five groups of subjects using Thyopac-4 kits containing absorbent granules. A free thyroxine index was determined from these estimations in association with triiodothyronine uptake ratios using Thyopac-3 kits. The values obtained were compared with thyroxine concentrations and T-7 values using kits containing resin sponges. Measurements with the absorbent granule kits and in particular the calculated free thyroxine index have proved very valuable in distinguishing between euthyroid, hyperthyroid, and hypothyroid subjects. The advantages of these kits over resin sponge kits are discussed.

The clinical assessment of thyroid function has been greatly assisted by the recent development of sensitive *in-vitro* methods using tracer amounts of radioactive labelled compounds for measuring thyroid hormone concentrations in blood. Techniques include anion exchange resin (Murphy and Jachan, 1965), resin sponges (Godwin and Swoope, 1968), or strips containing resin embedded in a fibre matrix (Thorson, Tsujikawa, Brown, Morrison, and McIntosh, 1970) for measuring the total serum thyroxine concentration which is bound mostly to proteins. These materials act as secondary binders of thyroid hormones after their release from proteins by a suitable extraction solution.

Thyroid function, however, appears to be more specifically influenced by unbound thyroxine concentrations in blood and methods for determining this free thyroxine fraction include the dialysis and magnesium-precipitate technique of Sterling and Brenner (1966). Harvey (1971) reported that the assessment of the free thyroxine concentration provides a better diagnostic confirmation of thyrotoxicosis than seven other indices of thyroid function, including total thyroxine concentrations, ^{131}I neck uptakes, 48-hour protein-bound ^{131}I, and the Wayne diagnostic index.

Attempts have been made to simplify the assessment of the free thyroxine concentration by call culating a free thyroxine index which is considered to be proportional to the concentration of free thyroxine in serum. The calculation uses the total serum thyroxine concentration or chemical protein-bound iodine concentration (PBI) in conjunction with a ratio of radioactive triiodothyronine or radioactive thyroxine taken up by one of the secondary binding materials in the presence of the serum under test.

Thyopac-3 kits alone, obtainable from the Radiochemical Centre Amersham, which utilize absorbent granules to give ^{125}I-triiodothyronine uptake ratios, have been explored by Clark and Brown (1970a). They have also investigated a free thyroxine index calculated from the PBI and Thyopac-3 measurements (Clark and Brown, 1970b). Chan, McAlister, and Landon (1972) have compared the results from kits of Thyopac-3 and Triosorb[1] and investigated the free thyroxine index also calculated from the PBI values.

Recently the Radiochemical Centre, Amersham, developed a new Thyopac-4 kit for determining the total thyroxine concentration in serum. This preparation also incorporates absorbent granules in place of resins and utilizes the principle of competitive protein binding of thyroxine. Preliminary results on the value of this new kit as a routine test

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Received for publication 3 January 1974.

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for determining the quantity of thyroxine (T4) in sera from five groups of subjects are reported here. A Thyopac free thyroxine index (TFTI) has been calculated using these thyroxine concentrations and the triiodothyronine (T3) uptake ratio on the same sera using Thyopac-3 kits. The results obtained have been compared with estimates of total serum thyroxine concentrations and a free thyroxine index in the form of a T-7 value (Hamberger, 1970) using the Triosorb and Tetrasorb kits incorporating resin sponges.

Method

Five to ten millilitres of blood was taken from all subjects. After clotting the samples were centrifuged and the serum removed and stored at −18°C. These samples were slowly thawed to room temperature before measuring the T3 uptake ratio and total serum thyroxine levels with the different kits.

THYOPAC-4 KIT

This consists of extraction tubes and two samples of desiccated standard reference serum one of which when reconstituted with distilled water has a value of zero and the other a known amount of total thyroxine of the order of 18 μg/100 ml respectively. In addition there are 14 identical Thyopac-4 vials each containing absorbent granules, buffered thyroxine-binding globulin, and ^{125}I-thyroxine solution.

MEASUREMENT OF TOTAL SERUM THYROXINE

The thyroid hormones in the patient's serum are released from the proteins by adding 1·0 ml ethanol to 0·5 ml serum in an extraction tube, mixing thoroughly for two to four minutes and centrifuging for five minutes at about 2000 rpm. An accurate volume of 0·5 ml of clear supernatant liquid is transferred to a Thyopac-4 vial using an Oxford or Eppendorf pipette and after recapping the total contents of this vial are mixed on a rotator at room temperature for at least 30 minutes. The absorbing granules are allowed to settle for two minutes and 1·0 ml of the supernatant liquid is transferred to a counting tube and the radioactivity is determined using a well type scintillation counter set to detect ^{125}I. Generally less than two minutes are required to obtain more than 10 000 counts.

A volume of 0·5 ml of each reconstituted standard reference serum is processed in a similar manner to the test sera using two other Thyopac-4 vials. The reciprocal of the net radioactive counts from the standard reference sera, ie, background subtracted, are plotted on the y axis of linear graph paper against thyroxine concentration on the x axis and the two points joined by a straight line since the relationship is claimed by the manufacturer to be linear over the range of 0 to 20 μg T4/100 ml. Investigations of this linear relationship are summarized below.

The thyroxine concentration of the test serum is determined directly from this line using the reciprocal of the net radioactive counts in the 1·0 ml supernatant. The method requires no correction for extraction efficiency provided the same ethanol solution is used in each batch of determinations.

Total serum thyroxine concentrations were also determined in the manner recommended by the manufacturers using Abbott's Tetrasorb kits incorporating resin sponges and ^{125}I-thyroxine binding globulin solution.

TRIIODOTHYRONINE UPTAKE RATIO

Estimates of this ratio were carried out using Thyopac-3 kits and Abbott's Triosorb kits containing ^{125}I-triiodothyronine in accordance with the manufacturers' instructions.

FREE THYROXINE INDEX

With the Thyopac-3 test the uptake by the serum is measured instead of the uptake by the secondary binder, as in most other techniques, consequently the TFTI was calculated using Thyopac values in the formula.

$$\text{TFTI} = \frac{\text{Thyopac-4}}{\text{Thyopac-3}} \times \frac{100}{1}.$$

A free thyroxine index using the resin sponge kits was determined as a T-7 value obtained by multiplying the Triosorb and Tetrasorb results for each serum.

Patients

The subjects studied included 41 euthyroid, 33 hyperthyroid, 16 hypothyroid, 44 pregnant women, and 71 medical students. The thyroid status of patients was established using radioiodine neck uptake, PB^{131}I, estimations of thyroid-stimulating hormone (TSH), PBI serum concentrations, and clinical assessment. None of the patients in the hyperthyroid, hypothyroid, and pregnant groups were receiving treatment for thyroid abnormalities. All patients studied were in the age range 18 to 64 years while the medical students had an age range of 20 to 23 years.

Results

The serum thyroxine concentrations obtained using the Thyopac-4 kits are displayed in fig 1 and the Thyopac free thyroxine index values are shown in

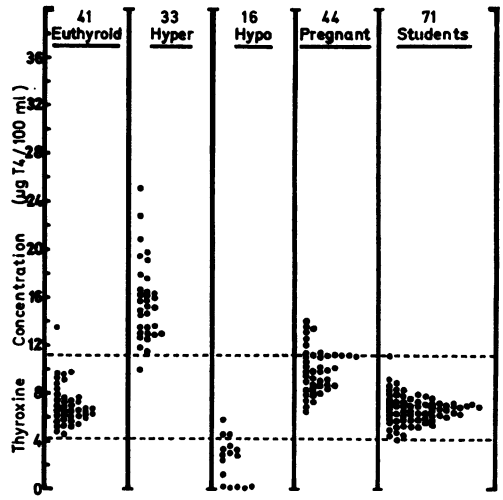

Fig 1 *Serum thyroxine concentrations using Thyopac-4 kits in the euthyroid, hyperthyroid, hypothyroid, pregnant women, and medical student subjects.*
The normal range lies between the dotted lines.

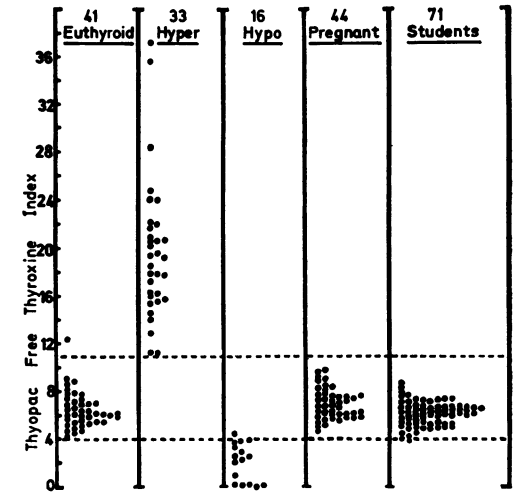

Fig 2 *Thyopac free thyroxine index (TFTI) for the five groups of subjects studied.*
The normal range lies between the dotted lines.

figure 2. A summary of all the results obtained using the absorbent granule and resin sponge kits is given in table I.

GRANULAR KITS

A normal range by inspection containing over 95% of the results from the euthyroid subjects was found to be 4·2-11·2 μg T4/100 ml serum using the Thyopac-4 kit. The normal range for the calculated Thyopac free thyroxine index was 4·0 to 11·0.

Thyopac-4

One out of 33 hyperthyroid results, three out of 16 hypothyroid, 70 out of 71 medical student values, and 36 out of 44 results from the pregnant women fell inside the normal range for Thyopac-4. One euthyroid patient had a Thyopac-4 value in the hyperthyroid range (13·5 μg T4/100 ml).

Thyopac free thyroxine index

None of the 33 hyperthyroid results, one of the 16 hypothyroid, and 70 out of the 71 medical student results fell within the normal range for TFTI. All the results from the pregnant subjects fell inside this normal range. The euthyroid patient with the abnormally high Thyopac-4 value gave an abnormally high TFTI value of 12·4.

RESIN SPONGE KITS

Over 95% of the results from the euthyroid subjects using Tetrasorb kits gave a normal range of 6·2 to 12·0 μg T4/100 ml serum. The calculated T-7 values gave a normal range, 1·4-4·4.

Tetrasorb

None of the 33 hyperthyroid patients, four of the 16 hypothyroid, and 70 of the results from the 71 medical students were within the normal range for this kit. Only 17 of the 44 results from pregnant women fell inside this normal range.

T-7 value

Two of the 33 hyperthyroid, all of the 71 results from the medical students, and all of the values obtained for the 44 pregnant women fell within the normal range obtained for the T-7 value. The results for hypothyroid subjects were not recorded as it was not possible to estimate the Triosorb values in the same sequential manner as was carried out for all the other groups.

REPRODUCIBILITY

Investigation of the reproducibility of the Thyopac-3 and Thyopac-4 and the Triosorb and Tetrasorb methods within the same batch was carried out using 20 aliquots for each test from a pool of serum from normal persons. Thyopac-3 and Thyopac-4 kits yielded coefficients of variation of 0·9 and 7·4% while Triosorb and Tetrasorb kits gave coefficients of 5·2 and 7·8% respectively. Measurements of the concentration of T4 in a pool of sera were investigated on different days using the Thyopac-4 kits and yielded a coefficient of variation of 10·3%.

Group	*No. of Subjects*	*Thyopac-3 T_3 Uptake (%)*	*Thyopac-4 T4 (μg/100 ml)*	*Thyopac Free Thyroxine Index*	*Triosorb T_3 Uptake (%)*	*Tetrasorb T4 (μg/100 ml)*	*T7 Value*
Euthyroid	41	107·9 ± 7·2	7·0 ± 1·7	6·5 ± 1·6	30·3 ± 4·9	8·9 ± 1·7	2·7 ± 0·7
Hyperthyroid	33	81·4 ± 9·3	15·7 ± 3·3	19·7 ± 5·8	39·7 ± 6·0	19·2 ± 3·6	7·6 ± 1·9
Hypothyroid	16	122·6 ± 12·5	2·4 ± 1·9	2·0 ± 1·6	—	4·6 ± 2·3	—
Pregnant women	44	142·7 ± 5·0	9·9 ± 1·9	7·0 ± 1·3	18·1 ± 3·3	12·9 ± 1·7	2·3 ± 0·5
Students	71	109·0 ± 7·9	6·7 ± 1·2	6·1 ± 1·0	31·0 ± 3·3	8·8 ± 1·4	2·7 ± 0·4

Table I *Summary of results for the five groups of patients using the absorbent granule and resin sponge kits (mean ± standard deviation)*

LINEAR RELATIONSHIP

The relationship between the reciprocal of the net radioactive counts and the concentration of thyroxine in serum plotted on graph paper was investigated using added known amounts of T4 to pools of sera prepared from the zero standard reference sera supplied by the Radiochemical Centre and patients' sera. In addition, estimations were made on dilutions of the high standard reference sera supplied by the Radiochemical Centre using appropriate amounts of the zero reference sera.

In all three cases the linearity of the slope of the graph for thyroxine concentrations between zero and 20 μg T4/100 ml was confirmed to within 10%. At concentrations greater than 28 μg T4/100 ml the graph became non-linear and indicated decreased readings of more than 16%.

Discussion

The results obtained in this series indicate that estimations of thyroxine concentrations in serum using the Thyopac-4 kits provide a useful means of selecting patients with abnormal thyroid function. The mean value for pregnant women at 9·9 ± 1·9 μg T4/100 ml was significantly different ($P < 0·001$) from the euthyroid mean of 7·0 ± 1·7 μg T4/100 ml indicating a slightly raised normal range for thyroxine concentrations for this group. We are, however, unable to explain the reason for the abnormally high Thyopac-4 and TFTI for the euthyroid subject whose results fell in the hyperthyroid ranges. She was an obese euthyroid adolescent admitted to the metabolic unit for weight reduction.

Thyroxine concentrations obtained using the Tetrasorb kits showed a similar discrimination between hyperthyroid and hypothyroid subjects but yielded a significantly higher range of normal values, 6·2 to 12·0 μg T4/100 ml ($P < 0·001$), compared with the normal range obtained with the Thyopac-4 kits. Watson and Lees (1973) also found that Tetrasorb kits gave higher T4 concentrations than Thyopac-4 kits and suggested that results obtained with the latter should have a correction applied to bring them into line with the resin sponge values. The thyroxine results obtained here with Thyopac-4 for euthyroid subjects are close to those obtained by Howorth and Maclagan (1969) who found values 4·0 to 11·0 μg T4/100 ml using resin particles, and a correction has not been considered necessary.

The Thyopac-3 results obtained here gave a normal range of 95 to 122% which is similar to the 97 to 122% range found by Chan *et al* (1972). When the Thyopac-4 results are used with the Thyopac-3 results to give values for TFTI, discrimination between different thyroid conditions is better than using the Thyopac-3 or Thyopac-4 kits alone. Only 6% of the hypothyroid results fell in the normal range of 4·0 to 11·0 for the TFTI of euthyroid subjects while all hyperthyroid subjects were outside this range. Compared to the Thyopac-4 results alone all calculated Thyopac free thyroxine index results from the pregnant subjects fell inside this normal TFTI range.

A normal range of 1·4 to 4·4 was obtained for the T-7 value and this included 6% of the hyperthyroid, all of the pregnant women, and all of the T-7 results for the group of medical students.

Overall the values obtained for T4 serum concentration using Thyopac-4 kits, and particularly the Thyopac free thyroxine index calculated from Thyopac-3 values, provide a useful means of determining abnormal thyroid function and a reliability similar to that obtained from Tetrasorb kits and the T-7 values calculated from the Triosorb estimations.

The Thyopac free thyroxine index is easier and cheaper to obtain than the T-7 value. Thyopac-3 kits have the advantage that the results are not time dependent compared with those of Triosorb kits (Chan *et al*, 1972). Watson and Lees (1973) have discussed the limitations of PBI estimations, including iodine contamination, compared with serum T4 estimations and have concluded that the serum PBI test is obsolete as a test of thyroid function. Thyopac-4 kits have distinct advantages over the Tetrasorb kits in the estimation of thyroxine concentrations in serum. No washing or evaporation procedures are required, and, because the estimations are not dependent on time and temperature corrections, calculation of the results is easier.

100 *Test Vials*	*Cost*
Thyopac-3 (large pack)	£50.00
Thyopac-4 (large pack)	£57.00
Triosorb-125 T3 diagnostic kit	£55.10
Tetrasorb-125 T4 diagnostic kit	£80.41

Table II *Comparative costs of Thyopac-3, Thyopac-4, Triosorb, and Tetrasorb kits excluding value added tax*

Table II shows that the costs of Thyopac-3 and Thyopac-4 kits per 100 test vials, excluding carriage, are less than the costs for Triosorb and Tetrasorb kits.

We wish to thank members of the medical staff of this hospital and the Royal Maternity Hospital for permission to investigate their patients, and Sisters M. Murphy and E. Good for their help. We are also grateful to Dr J. S. Glover and Dr D. Huckle, Radiochemical Centre, Amersham, for the supply of Thyopac kits used in this evaluation and Mrs Linda Ingles and Mr J. McClintock for technical assistance.

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