EVALUATION OF A SIMPLIFIED METHOD FOR DETERMINING SERUM THYROXINE BY COMPETITIVE PROTEIN BINDING ANALYSIS

By

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ABSTRACT

Serum thyroxine concentrations were determined in 66 euthyroid, 30 hyperthyroid and 13 hypothyroid patients using both the established Murphy method and a simplified method of competitive protein binding analysis. A diagnosis compatibility of 96% was found with both methods indicating that the simplified method has comparable clinical application as an initial screen of thyroid status.

Since Ekins (1960) first applied the principle of competitive protein binding analysis (CPBA) to estimate serum thyroxine, considerable evidence has accumulated to indicate that this parameter of thyroid function is a valuable screen of thyroid status (Murphy et al. 1966; Kennedy & Abelson 1967; Sparagana et al. 1969).

Recently we have employed a simplified method of estimating serum thyroxine by CPBA. The present study purports to show that this method of analysis compares favourably with the established Murphy procedure (Murphy 1965) and has comparable accuracy in defining thyroid status.

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MATERIALS AND METHOD

Standard aqueous ethanol solutions of crystalline L-thyroxine sodium pentahydrate were prepared to contain 0, 6, 12 and 18 ng thyroxine/0.3 ml of solution. The radioactive reagent (Mallinckrodt/Nuclear) was prepared at two monthly intervals to consist of 0.5 ml pooled normal serum, 2.5 μCi ¹²⁵I-thyroxine (specific activities approximating 100 mCi/mg) and sufficient 0.1 m barbital buffer - pH 8.6 to make 100 ml of solution. To irreversibly bind free ¹²⁵I-thyroxine, fibre strips impregnated with a quaternary ammonia base ion exchange resin (Mallinckrodt Res-O-Mat strips) were used. When not in use, standard solutions and radioactive reagent were refrigerated at 5°C.

Preparatory to the test procedure, standard solutions and radioactive reagent were brought to room temperature. To test tubes containing 2 ml of 95%/ ethanol was added dropwise 1 ml of the serum to be tested. After a 15 second mix with a Vortex mixer, the tubes were centrifuged at 2000 rpm for 4 minutes. From each tube, 0.3 ml of the ethanol supernatant was transferred to vials containing 4 ml of radioactive reagent. The vials were inverted several times, and the mixtures allowed to equilibrate 10 minutes. After the addition of a resin strip to each vial, the vials were placed on a rotatory mixer revolving at 12 rpm for 1 hour. At the end of this time, the resin strips were removed and discarded.

For each set of 8 to 12 serum specimens tested, a standard curve was derived. To 4 vials each containing 4 ml of radioactive reagent was added 0.3 ml of each standard solution. These were processed in exactly the same manner as were the test specimens after addition of the ethanol extract to the radioactive reagent.

Following resin equilibration, the concentration of radioactivity remaining in each standard and test vial was determined as time/10,000 counts. A pre-count determination was made at the same time on a vial containing only 4 ml of radioactive reagent. By plotting the pre-count/post-count ratio against the concentration of thyroxine in the standards (equivalent to 0, 6, 12 and 18 μg thyroxine/100 ml) a standard curve was derived as shown in Fig. 1. The pre-count/post-count ratios of the test specimens were similarly determined and their equivalent concentrations of thyroxine read from the standard curve. These values were multiplied by a factor of 1.32 to correct for a predetermined ethanol extraction efficiency of 76%.

Serum thyroxine concentrations were determined by both the Murphy and resin strip methods in serum specimens drawn from 109 patients who presented to the Department of Medicine at Vancouver General Hospital for assessment of thyroid function. Thyroid status of each patient was determined from history and physical examination by one of 4 physicians regularly assigned to the thyroid clinic and confirmed by a battery of laboratory procedures which, with few exceptions, included determination of serum PBI, T₃ uptake, serum thyroxine (Murphy) and 4 hour and 24 hour ¹³¹I uptakes. Of the 66 patients categorized as being euthyroid, 13 were women who were taking a contraceptive (oestrogen) medication. Of the remaining 43 patients, 30 were diagnosed as being hyperthyroid and 13 as being hypothyroid.

RESULTS

Almost invariably a straight line standard curve was obtained as shown in Fig. 1. On occasion, a slight plateauing-off of the curve was noted with the
higher standard concentrations which was included in the calculation of a least squares line. Mean in-run replicability of 0.81 ± sd 0.54 μg/100 ml was found between 22 duplicate estimations of normal pooled serum. For this same pool of serum, 43 estimations of thyroxine concentration determined over the course of 14 days showed a mean concentration of 10.1 ± sd 1.1 μg/100 ml.

Fig. 2 compares the concentrations of serum thyroxine determined by both the Murphy and resin strip methods for each of the 4 categories of patients studied. By the resin strip method, a mean euthyroid concentration of 8.5 (2 sd limits 5.4–11.6) μg/100 ml was found compared to a Murphy euthyroid mean of 9.1 (2 sd limits 4.9–13.3) μg/100 ml. Resin strip concentrations for hyperthyroid and hypothyroid patients (11.4 to 29.6 and 0.8 to 5.3 μg/100 ml, respectively) were comparable to those found by the Murphy method. For the group of euthyroid, oestrogen treated females, a mean resin strip concentration of 10.6 (2 sd limits 6.9–14.3) μg/100 ml was found compared to a Murphy mean of 11.6 (2 sd limits 8.5–14.7) μg/100 ml. Of the hyperthyroid and hypo-thyroid patients, 4 showed resin strip and Murphy thyroxine concentrations that fell into the actual range found for the euthyroid group (diagnosis compatibility for both methods – 96 %).

In Fig. 3, serum thyroxine concentrations determined by the Murphy method have been plotted against those determined by the resin strip method. A linear relationship throughout the entire range of thyroxine concentrations

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**Fig. 1.**
A typical standard curve calculated by the least squares method.
Fig. 2.
Frequency distribution of serum thyroxine concentrations determined by both the Murphy and resin strip methods in normal, hyperthyroid, hypothyroid and oestrogen treated patients. Resin strip values are to the right and Murphy values to the left of each y axis. Mean and 2 sd confidence limits are indicated for euthyroid and oestrogen treated patients.

Fig. 3.
The relationship between serum thyroxine concentrations determined by the Murphy and resin strip methods on 109 patients.

is apparent \((r = 0.957)\). By \(t\) test there was no significant difference between the two sets of thyroxine concentrations \((t\) probability \(= 0.432)\).

PBI estimations were determined on 98 of the 109 patients studied. In Fig. 4,
these have been plotted (as thyroxine equivalents) against the concentrations of serum thyroxine found for each patient using the Murphy method. Below serum thyroxine concentrations (and PBI equivalent concentrations) of 20 μg/100 ml, the correlation is linear. Above concentrations of 20 μg/100 ml, there appears to be a plateauing-off of the serum thyroxine concentrations. This same upper range non-linearity is suggested in Fig. 5, where the same PBI values have been plotted against serum thyroxine concentrations determined by the resin strip method. In both Fig. 4 and Fig. 5, lines representing the linear relationships for concentrations below 20 μg/100 ml are indicated. Also indicated are the 2 sd limits of the estimates which are 2.46 and 3.44, respectively.

**DISCUSSION**

The resin strip method of estimating serum thyroxine is a much simpler test to perform than is the Murphy method. Noteworthy is the omission of the evaporation procedure during which ethanol extracts of thyroxine from both

![Fig. 4.](image)

The relationship between PBI concentrations (expressed as thyroxine equivalents) and serum thyroxine concentrations determined by the Murphy method in 98 patients. Open circles indicate concentrations in patients whose serum PBI was spuriously elevated by iodide. The solid and dotted lines indicate respectively the linear relationship calculated in the 2 sd limits of the estimate.
The relationship between PBI concentrations (expressed as thyroxine equivalents) and serum thyroxine concentrations determined by the resin strip method in 98 patients. Open circles indicate concentrations in patients whose serum PBI was spuriously elevated by iodide. The solid and dotted lines indicate respectively the linear relationship calculated and the 2 sd limits of the estimate.

standard solutions and test sera are reduced to a dry residue. With the Murphy method, precise timing, constant shaking and low temperature control during resin equilibration are necessary. Small variations in the timing of resin equilibration particularly influence the accuracy of the procedure. With the resin strip method a much longer period of resin equilibration is required at room temperature, which means that small variations in timing and temperature are relatively without effect.

Although in-run and day to day variability of the resin strip method was found to be greater than with the Murphy method, it appears that this difference is not significant and is lost within the wider limits of biologic variation. This is attested to by the narrower range of euthyroid concentrations found with the resin strip method (Fig. 2) and the absence of a statistical difference between concentrations found by the two methods on 109 patient estimations. Further, serum PBI concentrations correlated more closely with resin strip thyroxine concentrations than with Murphy thyroxine concentrations (Figs. 4 and 5). When abnormalities of binding protein concentration, namely the elevations of TBG concentration attending oestrogen administration, were taken into account, a diagnosis compatibility of 96% was found with both the resin strip and Murphy method indicating that they have comparable clinical application as an initial screen of thyroid status.
REFERENCES


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