

Radioassay

A New Economical Method for Determination of Triiodothyronine (T_3) Uptake Using Talc Tablets

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The determination of T_3 uptake is necessary to find the free thyroxine index, increasingly used as the simplest accurate measure of thyroid dysfunction. A T_3 uptake determination requires a reproducible, readily automated, and cost-effective method of adsorbent separation. Five adsorbent separating reagents were studied: hemoglobin-coated charcoal, dextran-coated charcoal, magnesium carbonate, anion-exchange resin, and talc tablets. Of these, binding efficiency was best with talc tablets. Talc binding was insensitive to incubation time, eliminating problems associated with other reagents. To maintain the conventional normal range for T_3 uptake (25–35%), the quantities of talc, tracer, and serum used required adjustment. Results obtained with talc were compared to those obtained with a commercial kit, using paired sera from 897 patients. The correlation coefficient was 0.95 ($P < 0.0005$). The coefficients of variation (CV) for 36 runs were 4.6%, 2.3%, and 2.1% inter-assay, and 3.3%, 1.7%, and 1.04% intra-assay for hypothyroid, euthyroid, and hyperthyroid controls, respectively.

When first introduced, the in vitro T_3 uptake test for estimation of unsaturated thyroxine binding globulin capacity was tediously performed, with human erythrocytes used as the adsorbent separator (1). When anion-exchange resins (2), coated charcoal (3), or magnesium carbonate (4) were substituted as the adsorbent separator, the test could be performed in fewer steps, but the time-sensitive incubation required for resin or coated charcoal presented a significant drawback when large numbers of tests were performed. The low binding efficiency of magnesium carbonate and the need to scoop magnesium carbonate powder tended to decrease the reproducibility of this assay. We compared five separating reagents and selected talc tablets because of their high binding efficiency, time independence, and excellent reproducibility of results. The talc method was evaluated and

the validity of these results were demonstrated by comparison with those obtained using a Res-o-Mat kit (Mallinckrodt, Inc., St. Louis, MO). Clinical correlations performed on 158 patients showed that the normal range, defined by nonparametric methods, was 24–35%.

Method

Barbital buffer (pH 8.6, ionic strength 0.075) was made by dissolving sodium barbital (291.06 g), barbital (51.16 g), and sodium azide (9.45 g) in 5 gal of deionized water in an appropriate container. This solution is stable for 2 months at room temperature.

Talc tablets, 100 mg, (Pan-Ray Div. Ormont Drug and Chemical Co., Inc., Inglewood, NJ) were used. Hormone I (DADE Div., American Hospital Supply Co., Miami, FL) was used as standard serum. After reconstitution of lyophilized standard and control sera, the daily assay aliquot was pipetted and stored in the freezer at -10°C .

Then [^{125}I] triiodothyronine was obtained (Abbott Laboratories, North Chicago, IL) as Triomet-125, Catalogue No. 6769. A stock solution of 100 ng/ml [^{125}I] T_3 (specific activity, 50 $\mu\text{Ci}/\text{ng}$) was prepared weekly with the use of barbital buffer containing 350 mg/dl of human serum albumin. Working tracer solution was prepared daily by diluting 2 ml of stock solution with 500 ml of barbital buffer (1 ng [^{125}I] T_3 /2.5 ml buffer).

Procedure

A 0.4-ml aliquot of serum or standard was added to 2.5 ml of working tracer solution in a test tube. One 100-mg talc tablet was added to each tube and the entire rack of tubes placed on a rotator, which provided end-over-end mixing action for 15 min. The tubes were then centrifuged for 10 min at 2,200 rpm. The supernatant was discarded, and the tubes drained by touching the lip of each tube to absorbent paper. The precipitate was counted in a gamma well counter for 1 min. The thyroxine binding ca-

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capacity index for each serum sample was calculated by the following formula

$$\frac{\text{net patient count}}{\text{net standard count}} \times \text{normalizing factor} = \text{TBC index.}$$

The TBC index was converted to percent uptake by the conversion table generated by the equation:

$$T_3 \text{ uptake} = \frac{\text{TBC index} - A}{M}$$

When TBC index > 1, A (intercept) = 0.004, and M (slope) = 0.0332. When TBC index < 1, A = 0.25; M = 0.025. The calculations were programmed into a mini-computer or programmable calculator and the percent uptake printed out.

The hemoglobin-coated charcoal method was performed according to Herbert (3). The dextran-coated charcoal method was a modification of Herbert's procedure, in which dextran-80 was substituted for hemoglobin. The magnesium carbonate method used was that developed by Deutsch (4). Anion-exchange resin was prepared according to the thyroxine determination method of Murphy and Pattee (5).

TABLE 1. Binding Efficiency of Five Separating Reagents

Reagent	Binding efficiency (%)
Hemoglobin-coated charcoal	91
Dextran-coated charcoal	92
Magnesium carbonate	70
Anion-exchange resin	92
Talc tablets (100 mg)	95

Results

The time dependence of the five separating reagents over 36 min of incubation time was calculated (Fig. 1). After addition of tracer, serum, and separating reagent, the reaction was terminated at various time intervals and the real count divided by the total count, to obtain the percent of total count. Binding efficiency was determined by substituting buffer for serum in the assay tube. The percentages of tracer bound to the separating reagents in the absence of serum were then calculated as binding efficiency (Table 1). Each measurement was done in duplicate. While both magnesium carbonate and talc tablets satisfied the requirement for a short incubation period that is insensitive to time variations, the binding efficiency study clearly showed the superiority of talc tablets.

In an effort to maintain the conventional normal range (25–35%), the quantities of talc, tracer, and serum used in the assay were adjusted. A series of known percent uptake control sera was studied by the talc method and TBC indices obtained. For this study of approximate normal ranges, the biphasic linear equation for TBC percent uptake for the three typical proportions of serum, tracer, buffer, and talc was approximated by the best fitting line (Fig. 2). Since this method is calibrated by commercial kits with 25–35% normal range, a

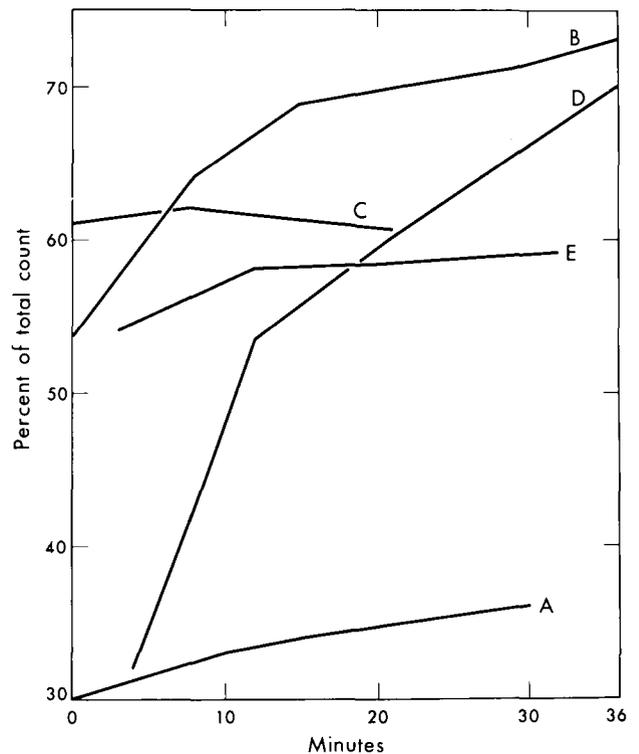


FIG. 1. The time-dependence of five separating reagents: A=hemoglobin-coated charcoal; B=dextran-coated charcoal; C=magnesium carbonate; D=anion-exchange resin; and E=talc tablets.

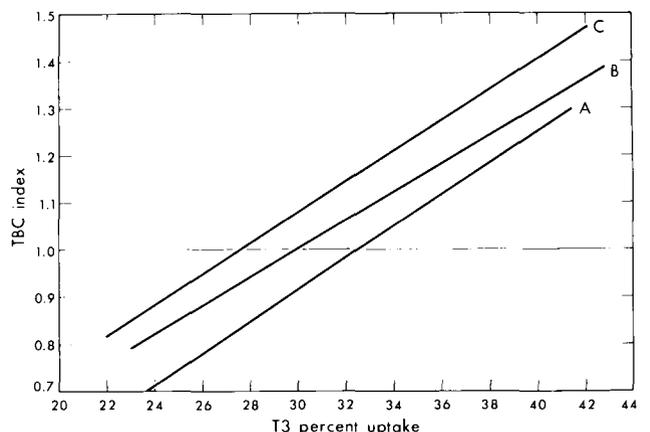


FIG. 2. Best fitting lines for three different sets of serum, tracer, buffer, and talc.

rough estimation of normal range was done by taking $\pm 5\%$ of the intercept of 1.0 TBC index from the best fitting line. The proportions finally selected were 400 μ l serum, 1.0 ng tracer, 2.5 ml buffer, and 100 mg talc (Table 2).

The correlation between results from the talc method and those from one commercial kit in sera from 897 patients is shown in Figure 3. A correlation coefficient of 0.95, $P < 0.0005$, was obtained. Interassay and intra-assay CV values, calculated according to the method of Rodbard (6), are shown in Figure 4; CV values for 36 runs were 4.6%, 2.3%, 2.1% (interassay) and 3.3%, 1.7%, and 1.04% (intra-assay) for hypothyroid, euthyroid, and hyperthyroid controls, respectively. On the basis of findings in sera from the same 897 patients, a table was generated for conversion of the TBC index to percent uptake. In our laboratory, this conversion is currently made by preprogrammed minicomputer.

TABLE 2. Calculated Approximate Normal Ranges for Various Proportions of Serum, Tracer, Buffer, and Talc

Serum (μ l)	Tracer (ng)	Buffer (ml)	Talc (mg)	Intercept	Approx-
				for TBC index=1 (%)	imate normal range (%)
A	60	0.75	1.0	25	32.5 27.5-37.5
B	400	1.0	2.5	100	30.0 25.0-35.0
C	400	0.75	2.0	100	27.7 22.7-32.7

Discussion

A normalizing factor must be derived to relate different lot numbers of Hormone I as they are received. A year's quantity of Hormone I may be ordered to eliminate the necessity for frequent calibration.

The normal range for our laboratory—24–35%—was determined by clinical correlation using a nonparametric method (7) on sera from 158 patients. Women receiving estrogens have lower values and their sera were excluded from this calculation. The free thyroxine index computed as the product of the T_3 uptake and the total serum thyroxine concentration is proportional to the concentration of free thyroxine in the serum, which is the variable that determines physiologic status (8). As this fact becomes more widely appreciated, the demand for T_3 uptake determination will increase markedly, making highly desirable a rapid, reproducible, and readily automated method such as we report. In our laboratory, a maximum of 154 sera can be incorporated into one run. With the use of a Micromedic (Horsham, PA) pipetting station and entire-rack centrifugation and decanting, a run of this quantity can be accomplished within 90 min.

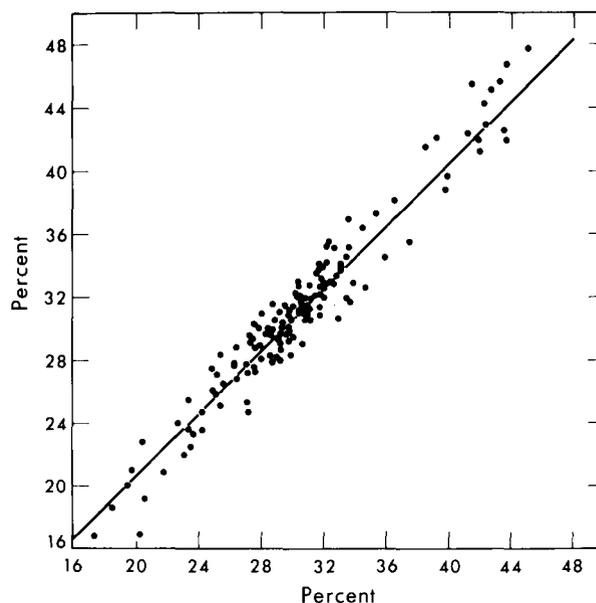


FIG. 3. T_3 correlation between talc method and commercial kit; $N=897$; $R=0.95$; and $P < 0.0005$. Abscissa = % uptake, commercial kit; ordinate = % uptake, talc tablets.

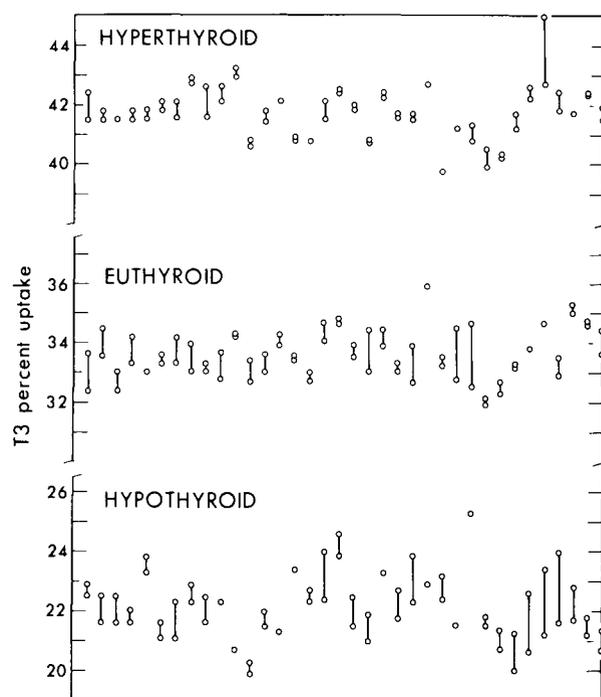


FIG. 4. Top: T_3 hyperthyroid control for 36 runs: $41.7\% \pm 0.86\%$ (1 s.d.). CV of interassay = 2.1%; CV of intra-assay = 1.04%. Center: T_3 euthyroid control for 36 runs: $33.6\% \pm 0.78\%$ (1 s.d.). CV of interassay = 2.3%; CV of intra-assay = 1.7%. Bottom: T_3 hypothyroid control for 36 runs: $22.2\% \pm 1.02\%$ (1 s.d.). CV of interassay = 4.6%; CV of intra-assay = 3.3%.

The cost is minimal for all materials, including tubes, tracer, talc, buffer, standard and control sera.

Acknowledgment

This work was supported in part by a grant from the Community Service Program of Kaiser Foundation Hospitals.

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