A NEW PARAMETER OF THYROID FUNCTION— THE EFFECTIVE THYROXINE RATIO

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The two generally available blood parameters of thyroid function—estimation of serum T_4 concentration (or PBI) and T_3 uptake test—are often deviated from normal in the presence of an abnormal concentration of T_4-binding serum proteins. Frequently encountered are the elevated T_4 concentrations (*1*) and decreased T_3 uptakes (*2,3*) associated with "hyperTBGemia" of pregnancy and the taking of estrogen (contraceptive) medication. Although estimation of serum-free T_4 has been shown to define thyroid status in the presence of binding protein abnormality, this procedure is yet too complex to be useful as a routine clinical test (*4*). Likewise, the calculation of a "free-thyroxine index" usually calculated as the product of T_3 uptake $\times$ serum T_4 (or PBI) (*5*), although correlating well with thyroid status in the presence of binding protein abnormalities, leaves much to be desired in that two separate procedures involving many steps are required.

This study purports to show that when serum T_4 concentration in test serum is determined in the presence of test serum itself and expressed as a fraction of serum T_4 concentration similarly determined in normal serum, the ratio so obtained ("effective thyroxine ratio, ETR*") provides a precise measure of thyroid status irrespective of serum binding protein concentration.

MATERIALS AND METHOD

Radioactive reagent (Mallinckrodt/Nuclear) was prepared to consist of 0.5 ml pooled normal serum, 2.5 μCi ^{125}I-T_4 with specific activities approximating 100 mCi/mg and sufficient 0.1 *M* barbital buffer, pH 8.6 to make 100 ml of solution. To bind displaced ^{125}I-T_4, fibre strips impregnated with a quaternary ammonia base ion exchange resin (Mallinckrodt ResOMat strips) were used. Reconstituted freeze dried normal serum (Monitrol I—Lot No. LTD-107CD, Dade Div., American Hospital Supply Corp.) was used as normal control serum.

All of the following was completed at room temperature. Ethanol extraction of T_4 from both test and control serum was carried out by the addition of 2 ml of 95% ethanol to test tubes containing 1 ml of serum followed by Vortex mixing and centrifugation at 500 g for 5 min. From each tube, 0.3 ml of supernate was transferred to stoppered vials containing 4 ml of radioactive reagent. After inverting the vials several times, 0.005 ml of the original serum was added to each vial. The vials were again inverted several times, and the mixtures were allowed to equilibrate 10–15 min. Following insertion of a resin strip into each vial, the vials were placed on a rotary mixer (12 rpm) for 1 hr. At the end of this time the resin strips were removed and discarded. Residual radioactivity in test and control vials was then determined simultaneously by counting the vials in a well counter to 10,000. From these counts, ETR was calculated from the formula

$$\text{ETR} = \frac{\text{cpm control vial}}{\text{cpm test vial}}.$$

Duplicate estimations of ETR were determined in serum from 178 euthyroid adult subjects of both sexes, 51 pregnant euthyroid women, 1 pregnant hyperthyroid woman, 36 euthyroid women taking estrogen (contraceptive) medication, 18 hypothyroid patients, 37 hyperthyroid patients, 36 euthyroid patients critically ill from nonthyroidal disease, and three euthyroid patients demonstrating significant deficiency of TBG. The 178 euthyroid subjects studied consisted of 26 healthy laboratory and hospital personnel and 152 ambulatory outpatients presenting for assessment of thyroid function or thyroid gland

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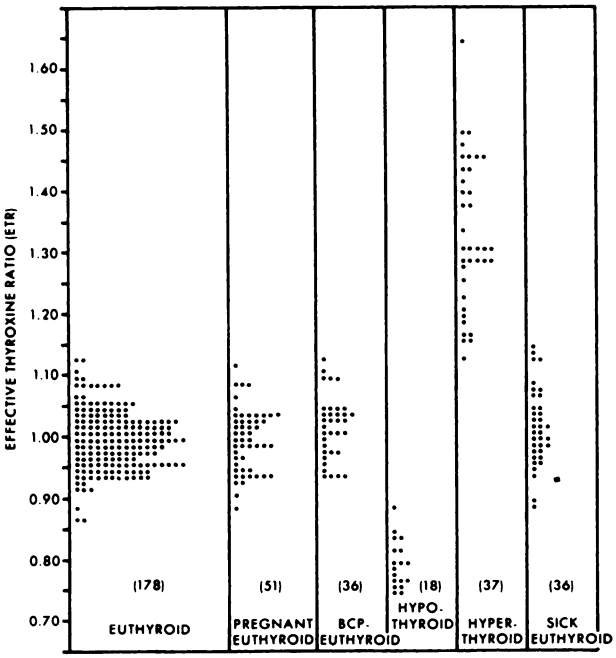

FIG. 1. ETR values determined in different patient categories.

abnormality. Serum T_4 concentration (*6*) and T_3 uptake (Mallinckrodt ResOMat T_3 test) was determined in all patient sera studied to confirm thyroid status. All hyperthyroid patients were shown to have abnormally elevated thyroidal uptakes of ^{131}I or demonstrated failure of thyroidal ^{131}I uptake suppression with T_3.

Estimation of serum-free T_4 concentration was determined by ultrafiltration (*7*) in serum from 24 of the patients studied and correlated with ETR.

RESULTS

A mean difference of 0.026 ± 0.020 (s.d.) was found between duplicate ETR estimations determined for all patients studied. A mean ETR value of 0.997 ± 0.024 was found for reconstituted freeze-dried normal serum when this was determined on a daily basis over 31 days.

Figure 1 shows the average ETR value determined for each patient studied. The 178 euthyroid patients showed values ranging from 0.87 to 1.12 (95% confidence range = 0.91–1.09). Euthyroid pregnant women, euthyroid women taking contraceptive medication, and all but two of the euthyroid critically ill patients showed ETR values that were within the range of the euthyroid group. In contrast, all but one each of the hyperthyroid and hypothyroid patients showed ETR values that were respectively above and below the euthyroid range. The one pregnant hyperthyroid patient studied showed an ETR of 1.28.

In Table 1 are shown ETR values, serum T_4 concentrations, T_3 uptake values, and TBG binding

TABLE 1. SERUM T_4, T_3 UPTAKE, TBG BINDING CAPACITY, AND ETR VALUE IN SERA FROM THREE TBG-DEFICIENT PATIENTS

Patient	Serum T_4 (μg%)	T_3 uptake (%)	TBG binding capacity (μg/ml)*	ETR
C	3.7	40.4	0.005	1.07
K	3.0	45.0	0.060	1.06
P	2.4	40.4	0.060	0.97

* Countercurrent paper electrophoresis, Trismaleate buffer, pH 8.6. Normal range, 0.15–0.25 μg/ml.

capacities determined in serum from three additional adult, euthyroid males who demonstrated significant TBG deficiency. Although serum T_4 concentrations and T_3 uptakes were reciprocally deviated from normal, ETR values confirmed their euthyroid status.

Figure 2 shows the relationship of ETR and serum-free T_4 concentration determined in serum from 24 patients randomly selected from the study group. The data indicate a linear relationship ($r = 0.98$) between the log values of ETR and serum-free T_4 concentration.

Figure 3 shows initial and subsequent ETR values, serum T_4 concentrations, and T_3 uptake values determined in serum from three hyperthyroid patients who became hypothyroid with antithyroid drug therapy (CS and SJ) and following a therapeutic dose of ^{131}I (CB). In every instance the close correlation of ETR with other blood parameters and thyroid status was apparent.

DISCUSSION

Preliminary experiments showed that when increasing volumes of pooled serum from euthyroid pregnant women were added with 0.3 ml ethanol extract of the same pool to the presently described displacement system and the concentration of T_4 expressed as a fraction (ETR) of that similarly determined for pooled normal serum, there was a progressive reduction of ETR from values significantly above 1.00 (when no serum was added) to ultimate values significantly below 1.00. When 0.005 ml of serum was added, an ETR value approximating 1.00 was found. The present study indicates that euthyroid patients having abnormally high serum concentrations of TBG (pregnancy and estrogen medication, Fig. 1), patients having abnormally low serum concentrations of TBG (Table 1), and patients having low serum concentrations of TBPA (sick euthyroid, Fig 1) (*8*) essentially all show ETR values that are within the range of normal when 0.005 ml of serum is similarly added to the system. These findings are explainable on the basis that it is the concentration of free T_4 that determines thyroid status and that the

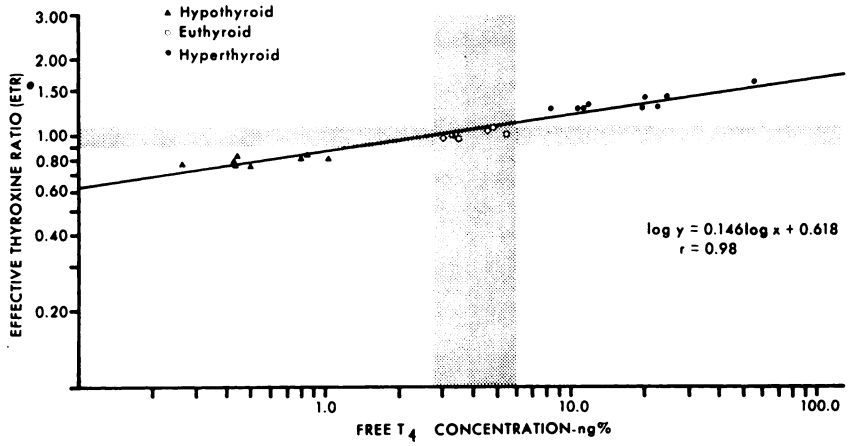

FIG. 2. Relationship of ETR to serum-free T_4 concentration. Shaded areas indicate 95% confidence limits for euthyroid patients.

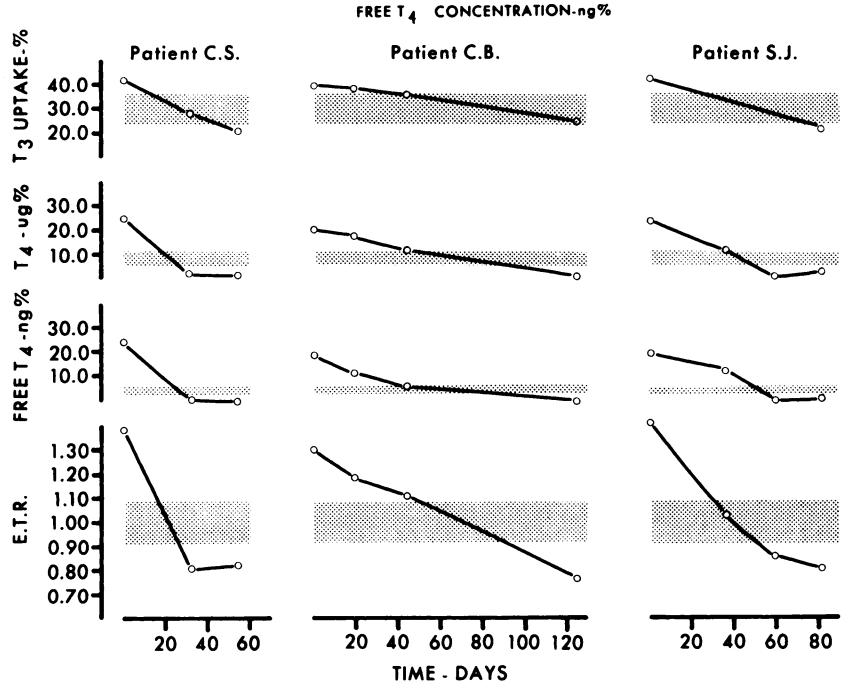

FIG. 3. Correlation of ETR with serum-free T_4, serum T_4 and T_3 uptake in three hyperthyroid patients before and following therapy with ^{131}I or antithyroid drugs. Shaded areas indicate 95% confidence limits for euthyroid patients.

ratio of total serum T_4 to total T_4-binding protein concentration (TBP) is constant in euthyroid patients according to the mass action equation (*9*)

$$[T_4] = \frac{K\,[T_4\,TBP]}{[TBP]}.$$

On the same basis, ETR values of hyperthyroid and hypothyroid patients were found to be respectively greater and less than 1.00.

The clinical application of the ETR as an easily determined index of thyroid status is apparent from the data presented. Of the 55 patients studied who were either hyperthyroid or hypothyroid, only two (3.6%) showed values that were within the range determined for euthyroid patients. The precise correlation of ETR with serum-free T_4 concentration shown in Fig. 2 and also apparent from inspection of Fig. 3 further indicates the value of ETR as a free thyroxine index. Of the 126 patients known or presumed to have a binding protein abnormality, only two of the sick patients showed ETR values that were slightly elevated above the euthyroid range. The one pregnant hyperthyroid patient studied showed an ETR of 1.28 indicating further the applicability of the test in the presence of a binding protein abnormality. Of the total 360 patients studied, only four showed ETR values that were not compatible with the final diagnosis of thyroid status (a diagnosis compatibility of 98.8%).

SUMMARY

Estimation of serum T_4 concentration in test serum was determined in the presence of test serum itself and expressed as a fraction (effective thyroxine ratio, ETR) of serum T_4 concentration similarly determined in normal serum. Studies of serum from hyperthyroid and hypothyroid patients and euthyroid patients with and without abnormalities of serum T_4-binding proteins indicate a high order of speci-

ficity of the ETR in defining abnormal thyroid status irrespective of serum binding protein abnormality.

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