NORMALIZED RESIN UPTAKE OF DISPLACED $^{125}$I-T$_4$

USING DENATURED SERUM

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A procedure has been devised that exploits the ability of heat-denatured serum to displace $^{125}$I-T$_4$, from a source of labeled thyroxine-binding protein onto a resin sponge. Heat-treated sera from pregnant women and women on oral contraceptives result in resin uptake values of displaced $^{125}$I-T$_4$, that are the same as those obtained with heat-treated sera from normal controls. However, resin uptake values in hypothyroidism are significantly lower and in hyperthyroidism significantly higher than in the normal or pregnant groups. This procedure has “normalized” the behavior of sera with elevated thyroxine-binding capacities, thereby eliminating a major source of confusion seen with measurements of total thyroxine and the resin T$_4$ uptake.

Introduction of the principle of differential adsorption of hormones by resin in 1959 (1) subsequently resulted in the development and application of resin uptake techniques for the assessment of thyroid function (2). Since then, the resin test has proved to be a reasonably reliable aid in the diagnosis of thyroid disorders except when the thyroxine-binding capacity of the serum is abnormal. Most troublesome have been the inordinately low resin uptake values found in pregnancy and in women receiving oral contraceptives, a phenomenon caused by an enhanced capacity of serum proteins to bind thyroxine (3). Because the levels of thyroxine are usually elevated in these situations, errors in diagnosis can be kept to a minimum if the serum total thyroxine is also determined.

Because of the frequency with which alterations in thyroxine-binding capacity are encountered in nonthyroidal conditions, the two complementary in vitro tests of thyroid function are almost mandatory if mistakes in diagnosis are to be avoided. To perform two laboratory procedures routinely on every person suspected of thyroid disease, however, is impractical; therefore, it seemed worthwhile to try developing a single system that would “normalize” the behavior of serum with excessive protein binding by adopting key features of both the resin uptake and tracer displacement techniques (4). After a period of trial and error, a procedure was devised based on the ability of thermally denatured serum to displace $^{125}$I-T$_4$, from an exogenous source of labeled thyroxine-binding protein onto a resin sponge that self-corrected for an increase in thyroxine-binding capacity. This procedure, now referred to as the normalized resin uptake of displaced thyroxine (NRUD T$_4$), yields values that are essentially the same for pregnant and nonpregnant normals but are significantly different among normal, hyperthyroid, and hypothyroid groups. The procedure is rapid, easy to perform, uses small serum samples (0.1 ml), and does not require a standard curve.

Previous unpublished observations in this laboratory had shown that small amounts of whole serum, when added to an exogenous source of $^{125}$I-T$_4$, labeled thyroxine-binding protein, were capable of displacing the radiothyroxine onto a resin sponge. Sera from normal, hypothyroid, and pregnant individuals produced low resin uptakes of approximately the same magnitude whereas values of sera from hyperthyroid patients were significantly higher. Inferred from these observations was that the reduced binding capacity of serum in hyperthyroidism promoted an increased displacement of the $^{125}$I-T$_4$, because of the large fraction of unbound endogenous thyroxine (5,6). Therefore, it followed, that by lowering the binding capacity of almost any serum,

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more $^{125}\text{I}-\text{T}_4$ could be displaced as a consequence of the liberation of nonprotein-bound endogenous thyroxine. Moreover, the use of such sera should make it possible to separate normal and pregnant groups from hyperthyroid and hypothyroid groups on the basis of their differences in the resin uptake of the displaced $^{125}\text{I}-\text{T}_4$. According to this line of reasoning, inactivation of a constant fraction of thyroxine-binding protein would lead to the release of varying amounts of endogenous thyroxine, which in hyperthyroidism would be greater than, and in hypothyroidism less than, in normal. However, in pregnancy the greater residual binding capacity of the serum should be counterbalanced by the larger fraction of liberated endogenous thyroxine resulting in displaced $^{125}\text{I}-\text{T}_4$ resin uptake values similar to those of the nonpregnant normals.

In order to test the validity of the foregoing hypothesis, some means of destroying or inactivating a portion of thyroxine-binding protein in a reproducible fashion was essential. After a variety of chemical procedures were tried without success, it was found that the controlled heating of serum produced the desired degree of protein denaturation.

**MATERIALS AND METHODS**

**Thermal denaturation of thyroxine-binding protein.** Samples of sera that had been stored in the freezer or collected freshly and ranging in volume between 0.5 ml and 4.0 ml were allowed to reach room temperature (23.5°C). The sera, in stoppered test tubes, were placed in a constant temperature water bath (±0.5°C) for 30 min at 60°C. (In subsequent discussion it will be shown that this interval of time and elevation of temperature proved to be optimal for the degree of inactivation of thyroxine binding protein.) Neither minor deviations in temperature (59°C–61°C) nor differences in time (26 min–34 min), which occurred sporadically during the course of repeated experiments, seemed to influence the results. At the end of the period of incubation, the tubes were removed and promptly placed in a refrigerator at 4°C until the sera were ready to be analyzed.

**Procedure for resin sponge uptake of displaced $^{125}\text{I}-\text{T}_4$.** Resin sponges and $^{125}\text{I}-\text{T}_4$, thyroxine-binding protein were removed from the Abbott Tetrasorb-125 T-4 Diagnostic Kit* and used without additional modification. One-ml samples of $^{125}\text{I}-\text{T}_4$, thyroxine-binding protein ($^{125}\text{I}-\text{T}_4$-TBP) were transferred from the Abbott solution to thin-walled glass test tubes (13 mm × 100 mm). Next, 0.1-ml aliquots of the heat denatured sera were added in duplicate to the tubes that were shaken briefly by hand in order to mix serum and $^{125}\text{I}-\text{T}_4$-TBP. The tubes were placed in an automatic gamma detector and the total radioactivity in each tube recorded. Following this, resin sponges were placed at the bottom of each tube and squeezed several times with applicator sticks in order to express the air and absorb the solution. The rack of test tubes was placed in a plastic bag and set aside at room temperature (23.5°C) for 60 min to allow time for sponge and reactants to interact. The sponges were then compressed against the bottom of the tubes with a plastic aspirator connected to a vacuum line that forcefully removed the liquid; distilled water was run into each tube and the sponges aspirated a second time. Then the radioactivity remaining on the sponges was measured in the counter and the fraction of $^{125}\text{I}-\text{T}_4$, taken up by the sponge was calculated by dividing the residual radioactivity by the initial radioactivity and multiplying by 100.

**Clinical application.** Sera were obtained from 22 hyperthyroid and 10 hypothyroid patients who had unequivocal clinical and laboratory evidence of the disorder and 20 women who were either pregnant or receiving oral contraceptives. The control group consisted of 57 men and women selected from the house staff and from outpatients who had been referred for conditions unrelated to endocrine or metabolic diseases.

Measurements of total serum thyroxine were done using the Abbott Tetrasorb-125 Kit.

**RESULTS**

**Incubation time.** In order to assess the effect of time on thermal denaturation of serum proteins, resin uptakes of displaced $^{125}\text{I}-\text{T}_4$, were done using samples of the same serum heated for 30 and 60 min at 60°C. The results (Fig. 1) indicated that incubation for 30 min provided excellent discrimination among normal, hyperthyroid, and hypothyroid groups and that at the same time brought sera from the pregnant group into the range of normal. Sera incubated for 60 min, on the other hand, proved to be unsatisfactory because some normal and most pregnancy resin values were similar in magnitude to those of the hyperthyroid population. The reason for this similarity became evident when the denatured sera, with $^{125}\text{I}-\text{T}_4$, added, were subjected to agar gel electrophoresis (7). Examination of the stained protein patterns and their autoradiographs (Fig. 2) revealed that serum incubated for 30 min, despite moderate distortion of the protein bands, still retained some ability to bind radiothyroxine as shown by the faint zones of radioactivity in both the interalpha and

the effect of variations in equilibration temperature was studied on the magnitude of uptake of displaced $^{125}$I-T$_4$. The results (Fig. 3) indicated that within a relatively broad range, an elevation in the temperature of equilibration between resin sponge and reactants produced an increase in uptake values (Fig. 3). Since variations in resin uptake values can be related to changes in temperature, the ambient temperature should be constant and a quality control serum should be included in each set of determinations.

**Group values.** The values of normalized resin sponge uptake of displaced $^{125}$I-T$_4$, obtained for different groups have been depicted in Fig. 4 and can be summarized as follows: Normals, mean 40.7% ± 0.4 s.e. (range 34–46%); Pregnant or Pill, mean 39.3% ± 0.6 s.e. (range 33–45%); Hyperthyroid, mean 51.8% ± 0.8 s.e. (range 46–60%); Hypothyroid, mean 28.2% ± 0.8 s.e. (range 23–31%). It is evident from these results that the separation among the groups is excellent and in contrast to the conventional resin T$_3$ or T$_4$ uptake; there was no overlapping of normal and pregnant group values with those of the hypothyroid group.

Replicate samples obtained from pooled serum, when analyzed repeatedly during a 5-week period, yielded values that ranged between 38.5% and

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**FIG. 1.** Normalized resin sponge uptake values of displaced $^{125}$I-T$_4$ using either unheated sera or samples of some sera incubated for 30 and 60 min at 60°C. It is evident that 30-min period of incubation results in values that are similar for both the normal and pregnancy groups but distinctly different in hypothyroidism and hyperthyroidism.

**FIG. 2.** Agar gel electrophoresis on samples of some sera either unheated or heated at 60°C before $^{125}$I-thyroxine was added. It is clear from both stained gels and their autoradiographs that 60-min period of incubation destroyed completely normal configuration of proteins and their thyroxine binding patterns. Apparent band of radioactivity in prealbumin zone in F. is artifact that occurred during processing of film.

prealbumin regions. However, sera heated for 60 min suffered complete loss of normal configuration and the ability to bind any thyroxine as manifested by the absence of radioactivity in the interalpha and prealbumin zones.

**Equilibration temperature.** Because the resin uptake of labeled thyroxine and triiodothyronine had been shown to be influenced by temperature (2),...
thyroxine, the normalized resin uptake of displaced $^{125}$I-T$_4$ can be substituted for the two separate techniques. The normalized resin uptake procedure can be viewed as a self-regulating system that depends on the balance between the amount of thyroxine-binding protein destroyed and the fraction of endogenous thyroxine liberated from the denatured proteins. If, for example, too little binding protein were destroyed, the influence of the intact endogenous binding protein when coupled with the lack of sufficient free thyroxine would result in minimal displacement of $^{125}$I-T$_4$ from the labeled thyroxine-binding protein.

On the other hand, excessive denaturation of binding protein, especially if in sera from pregnant women, could result in the liberation of abnormally large concentrations of endogenous thyroxine that, in the absence of any binding protein, would produce the opposite effects. As a consequence, the high normalized resin $^{125}$I-T$_4$ values would be indistinguishable from those found in hyperthyroidism, thus presenting the same problem in diagnosis found so frequently with the measurement of serum total thyroxine. It should be noted that total thyroxine can be estimated utilizing the normalized resin uptake procedure if the serum sample is heated at 60°C for 1 hr and the value extrapolated from a standard curve obtained from pooled sera, incubated under identical conditions, containing known concentrations of thyroxine.

Serum incubated for 30 min retained some ability to bind thyroxine whereas 60 min of heat destroyed both interalpha and prealbumin binding. However, others using a system of paper electrophoresis have

41.0% at equilibration temperatures between 23.0°C and 23.5°C.

Correlation with total thyroxine. Normalized sponge uptake values of the control, hyperthyroid, and hypothyroid groups were plotted against concentrations of total thyroxine analyzed on the same samples of sera. Examination of Fig. 5 reveals extremely close agreement of both sets of measurements at both high and low values (coefficient of correlation is 0.9). This is in marked contrast to the lack of correlation between the normalized uptake values of the pregnant group and serum total thyroxine that was caused by abnormally high levels of serum thyroxine and normal resin uptake values.

**DISCUSSION**

Measurement of total thyroxine in serum by saturation analysis has become a popular and useful test of thyroid function, largely because of its specificity and lack of reaction to organic and inorganic iodine. One feature that has proved to be troublesome, however, has been the inability to discriminate between the high thyroxine values of hyperthyroidism and those resulting from an enhanced binding of thyroxine by serum proteins in nonthyroid-related conditions, e.g., pregnancy, hepatitis, and the pill. Because the resin uptake is invariably low in these conditions (2), the depressed resin values, when considered in conjunction with the elevated serum thyroxine levels, have been invaluable in helping to assess accurately the condition of the patient. By exploiting the most favorable elements of both the resin uptake and saturation analysis systems and combining them into a single procedure that automatically compensates for any enhanced binding of

![FIG. 4. Normalized resin sponge uptake of displaced $^{125}$I-T$_4$, from thyroxine-binding protein using thermally denatured sera. Each dot represents uptake value of single person with mean value and standard error for group displayed next to cluster of dots.](image)

![FIG. 5. Correlation of normalized resin sponge uptake values with corresponding serum total thyroxine concentrations for normal, pregnant, hypothyroid, and hyperthyroid groups. Pregnant women or women on pill, indicated by x, were not included in calculation of coefficient of correlation because of obvious absence of any relationship between their high serum thyroxine content and their normal uptake values.](image)
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reported that even though a 30-min period of incubation at 60°C completely inactivated the interalpha binding protein, 60 min had no demonstrable effect on the prealbumin binding of thyroxine (8). It is difficult to reconcile such discrepancies except to point out that perhaps a system using paper electrophoresis is not capable of detecting subtle changes in protein-binding behavior.

Although the normalized resin uptake procedure has proved to be successful in its application to serum with increased thyroxine binding, it has not yet been possible to extend these observations to include other kinds of binding abnormalities. Whether this procedure will also be capable of normalizing values obtained in cases of congenitally low thyroxine-binding protein, or when thyroxine binding has been decreased by drugs or hormones, remains to be determined.

It is of interest that during the preparation of this manuscript another system was described that in effect also normalizes values from sera with increased thyroxine-binding protein (9). However, the application of that procedure is complicated by a series of steps that include alcohol extraction, centrifugation, evaporation, and reintroduction of the serum before the resin sponge is added.

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REFERENCES

4. ELKINS, R: Radioaktive Isotope in Klinik und Forschung, Munich, Urban and Schwarzenberg, 1963