142 Thyroid Function Tests

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Definition

Thyroid function tests are designed to distinguish hyperthyroidism and hypothyroidism from the euthyroid state. To accomplish this task, direct measurements of the serum concentration of the two thyroid hormones—triiodothyronine (T3) and tetraiodothyronine (T4)—more commonly known as thyroxine, are extensively employed. The T3 resin uptake (T3RU) is used as an indirect measure of serum thyroid hormone binding capacity, and the Free T4 index (FT4I), derived from the T4 and T3RU, corrects estimates of T4 for serum binding abnormalities.

Direct measurement of serum concentration of thyroid stimulating hormone (TSH) has been used extensively to establish the presence of primary hypothyroidism and more recently to identify hyperthyroid states. In selected clinical settings measurement of the serum concentration of free T4 by dialysis may be used to clarify thyroid status, and measurement of serum thyroid binding globulin (TBG) to identify hormone binding disorders. Production rates of T4 and T3 can be estimated, but the techniques are time-consuming and expensive, and would be indicated only infrequently even if readily available. Similarly, a free T3 has been used on a limited basis, but it too is not generally available.

Estimates of fractional thyroid iodide uptake have been employed for decades using ^{131}I as a marker. Because of the relative low cost and accuracy of the direct measurements of serum hormone concentration, the ^{131}I uptake has fallen into disuse.

In addition to the measurement of basal hormone concentrations, a number of techniques have been devised to determine whether the normal homeostatic feedback mechanisms are operative and whether normal functional reserve is present. The most useful of these is the TSH releasing hormone (TRH) challenge wherein TSH is measured before and after TRH infusion. The characteristic response patterns in hyperthyroidism and in various types of hypothyroidism are diagnostic when taken in conjunction with the rest of the clinical picture.

There currently is no generally available test that specifically measures the end-organ response to thyroid hormone. Consequently, the diagnosis of thyrotoxicosis rests on the assumption that appropriate signs and symptoms accompanied by "confirmatory" lab results are sufficient evidence to warrant this conclusion. In the majority of instances this assumption is correct; however, there are exceptions. A sensitive and specific test of the impact of thyroid hormone on peripheral tissues would reduce the chance of error.

Technique

The frequency of thyroid dysfunction in our population clearly dictates the need for every practicing physician to be skilled in the diagnosis of thyroid disease. Thyroid function studies must be selected and interpreted with a specific question in mind. This requires a grasp of thyroid physiology, a knowledge of the limitations of the tests in question, and a thorough understanding of the patient.

Given these prerequisites, the outcome will often be helpful, but a number of common clinical circumstances cause changes in the tests that can mislead the unknowing. In this section, the generally available tests of thyroid function and their limitations are described. Their appropriate use and interpretation should allow an accurate assessment of thyroid status in almost all cases.

Thyroxine

Serum total T4 concentration is measured by radioimmunoassay. A small molecule, T4 cannot by itself be used as an antigen; but by using it as a hapten conjugated to albumin or as the native thyroglobulin conjugate, antibodies can be produced. The dextro isomer of T4 generally binds as well to antibody, as does the naturally occurring levo isomer, but the dextro isomer is not naturally present in biological fluids in measurable quantities. Antibody affinity for T3 is several fold less than for T4, and since the plasma concentration of T3 is only one-fiftieth that of T4, T3 induced error is very small. Other derivatives or precursors of T4, such as 3,5 L-diiodothyronine, 3 monoiodothyronine, L-thyronine, diiodotyrosine, and monoiodotyrosine, react negligibly with the antibody. Iodide has essentially no effect on the T4 antibody interaction. Two infrequently encountered compounds that displace circulating T4 from TBG binding sites, diphenylhydantoin and salicylic acid, also have no effect on the T4 antibody interaction. For clinical purposes, the T4 antibodies have adequate specificity.

The variable number of high-affinity TBG binding sites in serum would tend to abort the precisely controlled interaction of a limited quantity of antibody binding sites with unknown amounts of T4. Salicylic acid and 8-anilino-1-naphthaline sulfuric acid (ANS) block binding of T4 to TBG and do not appreciably bind to antibody. Their addition to the reaction allows accurate estimates of T4 without extraction procedures designed to separate T4 from native TBG. The binding of T4 to antibody is marked with trace amounts of ^{125}I-T4, and separation of bound from free is accomplished by methods common to all immunoassays. Concentration of total T4 by radioimmunoassay is normally between 5 and 12 μg/dl with females being slightly higher than males. Intraassay coefficient of variation is on the order of 5%; interassay variation is approximately 7%.

Irrespective of how precise the quantification of T4 is, there is appreciable overlap of the values between normal subjects and hypothyroid or hyperthyroid patients. This is illustrated in Figure 142.1, which schematically represents values typically found in various states of thyroid function.

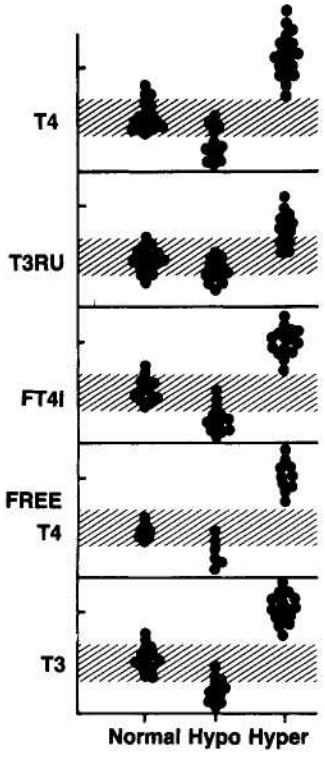

Figure 142.1
Thyroid function studies in normal subjects and in hypothyroid and hyperthyroid patients. Cross-hatched areas represent the respective normal ranges. T4: total serum thyroxine. T3RU: T3 resin uptake. FT4I: free thyroxine index. Free T4: total serum free T4. T3: total serum triiodothyronine. Hypo: Hypothyroid. Hyper: hyperthyroid.

A very significant fraction of hypothyroid individuals will have values of T4 that fall within the normal range.

Triiodothyronine Resin Uptake

Patients with high or low T4 values often have increased or decreased concentrations of TBG rather than abnormal rates of T4 production. As a T4 radioimmunoassay cannot distinguish between the two possibilities, these patients are in jeopardy of having their abnormal laboratory values falsely attributed to thyroid dysfunction.

The T3RU is an indirect measure of TBG binding capacity. It is performed by mixing a comparatively low affinity solid phase T3 resin binder with an aliquot of the patient's serum to which is added a trace amount of ^{125}I-labeled T3. The ^{125}I-T3 partitions between solid-phase resin binder and serum binding sites, and at the end of the incubation the supernatant serum is removed. The ^{125}I-T3 activity remaining on the resin binder divided by total ^{125}I-T3 added and then multiplied by 100 defines percentage RU. Normal values vary between 25 and 50% and are a function of the type of solid phase binder used and the conditions employed for incubation. Intraassay coefficient of variation is 1 to 2%; the interassay value is between 2 and 5%.

In hyperthyroidism the patient's TBG is relatively saturated, so a higher than usual fraction of ^{125}I-T3 goes to the resin binder. Conversely, in hypothyroidism less label is bound to the resin binder because of the reduced amount of endogenous T4 competing for a normal complement of TBG sites. With a primary increase in TBG concentration the RU is also reduced, but now because of an excess of TBG rather than a reduction of T4. With low TBG the RU as expected is high.

As illustrated in Figure 142.1, the large overlap between hypothyroid and normal and hyperthyroid and normal patients precludes use of the T3RU as an estimator of thyroid function. Taken in conjunction with the total T4, however, the T3RU offers insight into the cause of any given deviation of T4 concentration. Thus a patient with an elevation of total T4 due primarily to an increase in TBG would be expected to have a low T3RU. Conversely, a patient with reduced concentration of TBG and a corresponding low concentration of total T4 would have a high T3RU. These changes are outlined in Table 142.1.

Free Thyroxine Index

Inasmuch as changes in the T3RU caused by binding abnormalities are discordant with changes in T4, the T3RU has been used to correct the total T4. This corrected T4, the free T4 index (FT4I), is generally calculated as follows:

$$\text{FT4I} = \text{Total T4 patient} \times \frac{\text{T3RU patient}}{\text{T3RU mean control}}$$

The resultant number is expressed without units and is illustrated by the following example. A woman with increased TBG due to pregnancy has a T4 of 15 (5 to 12 μg/dl) and a T3RU of 20% (25 to 35%). The mid–normal control T3RU for the laboratory on that day is 30%, so the FT4I is 15 × 20/30, or 10. On the other hand, a high total T4 associated with increased production leads to oversaturation of TBG with an increase in both total T4 concentration and T3RU. As both multiplicands are increased, the derived FT4I would tend to exaggerate the increase above normal. Similar constructs for low binding states versus hypothyroidism can be developed from Table 142.1.

In Figure 142.1, comparison of the total T4 with the FT4I shows that the latter test discriminates better between normals and abnormals, but there is still overlap between subsets of patients. No single estimation of T4, corrected or otherwise, can reliably identify the status of thyroid function in all patients.

Table 142.1
T3 Resin Uptakes and T4 Concentrations Expected with Various Conditions

Hyperthyroidism	
	High T4
	High T3 resin uptake
Hypothyroidism	
	Low T4
	Low T3 resin uptake
High TBG	
	High T4
	Low T3 resin uptake
Low TBG	
	Low T4
	High T3 resin uptake

Free Thyroxine

A more accurate method of assessing thyroid function is to measure the concentration of free T4 using an *equilibrium dialysis technique*. By employing this technique, TBG's variable effect on total T4 concentration is eliminated, thereby affording more precise estimates of thyroid status.

Patient's sera is enriched with a trace amount of ^{125}I-labeled T4 and dialyzed against a protein-free buffer overnight. At equilibrium the free ^{125}I-T4 concentration is the same in the retentate and the diffusate, while all bound ^{125}I-T4 is in the retentate. Unfortunately, iodine–125 contaminating ^{125}I-T4 also passes into the diffusate. The diffusate is enriched with serum that binds the ^{125}I-T4 but not the iodine–125. Contaminating iodine–125 is removed by precipitation and washing of the protein-bound ^{125}I-T4. Alternatively, the serum diffusate mixture is dialyzed a second time against an iodide-binding resin. The ratio of purified diffusate ^{125}I-T4 to the original retentate ^{125}I-T4 defines the free fraction. Absolute values of free T4 are calculated as (^{125}I-T4 diffusate/^{125}I-T4 retentate) × T4 concentration and are generally on the order of 1.5 to 5.9 ng/dl. In some laboratories, the T4 in the original diffusate is simply measured by radioimmunoassay. In skilled hands, the intraassay coefficient of variation will be approximately 5%, while the interassay variation will be 5 to 8%.

The technique is time-consuming, demands much technical expertise, and is not generally available. Nonetheless, the test usually provides excellent separation between normal and hyperthyroid individuals and is the surest way to obtain a reliable estimate of total free T4 in binding abnormalities (Figure 142.1). Unfortunately, there is overlap between hypothyroid and euthyroid patients.

Modifications of the free T4 have been developed to circumvent the difficulties of performing the equilibrium dialysis free T4. *The analog free T4* employs a radiolabeled derivative of T4 that is reported not to bind to TBG, but that does compete with T4 for binding sites on the T4 antibody. Labeled analog and a limited quantity of T4 antibody are mixed directly with patient's serum or standards, and quantification of antibody-bound analog is accomplished by routine radioimmunoassay techniques. Specificity of the T4 antibody is similar to that described for the T4 radioimmunoassay. With a normal concentration of TBG and no abnormal binding substances, the test affords acceptable separation of various states of thyroid function; however, in certain clinical situations, the results are discordant from those of the equilibrium dialysis free T4. For example, with a high concentration of TBG, the analog free T4 is lower than the equilibrium dialysis free T4. Following heparin administration, the dialysis free T4 increases; the analog free T4 may decrease. In the familial syndrome of dysalbuminemic hyperthyroxinemia, the equilibrium dialysis T4 is normal; the analog free T4 is increased. With hereditary analbuminemia, values of the analog free T4 are low, while values of the equilibrium dialysis free T4 are normal; and in the unusual patient having T4 binding antibodies, the analog free T4 is high, while the equilibrium dialysis free T4 is normal.

A second modification of the free T4 employs a reaction tube coated with T4 antibody. Patient's sera are incubated in the tube with appropriate buffer for a short time and the tube is washed. The amount of patient's T4 binding to antibody is presumed to be proportional to the free T4 concentration. Next, ^{125}I-T4 in buffer is added and allowed to come to equilibrium with the antibody and the patient's bound T4. The tube is washed again and counted. The fraction of ^{125}I-T4 remaining in the tube is a function of the number of antibody binding sites and the relative degree of their occupancy by unlabeled patient T4. Published interassay variation is between 16 and 19%. Discordances between equilibrium dialysis free T4 and the *antibody coated tube technique* are similar to those described for the analog free T4.

A third method employs ^{125}I-T4 saturated antibody emulsified and coated with material having the property of dialysis membranes. The *microencapsulated antibody* is mixed directly with patient's sera, and displacement of antibody bound ^{125}I-T4 is proportional to free T4 concentration. Published coefficient of variation suggests an intraassay variation of 3 to 8% and an interassay variation of 3 to 10.5%.

This technique seems less influenced by changes in TBG concentration and yields normal values in the state of dysalbuminemic hyperthyroxinemia. In the desperately ill and in patients with a variety of disorders such as cirrhosis and chronic renal failure, there may be minimal to remarkable differences from one free T4 method to another. None of the modifications of the free T4 yields results that exactly agree with the equilibrium dialysis technique, which remains the best means of obtaining a reliable estimate of the concentration of free T4. The concentration of free T3 can also be determined employing equilibrium dialysis methodology. As with T4, good separation between hyperthyroid and euthyroid individuals is achievable.

Triiodothyronine

Serum concentration of total T3 is measured by radioimmunoassay, and methods for producing T3 antibodies are similar to those employed for T4. Antibody reacts significantly with the dextro isomer of T3 and with triiodothyroacetic acid, a naturally occurring conjugation product of T3. However, and most important, binding affinity for T4 is less than 1% of that for T3. Monoiodotyrosine, diiodotyrosine, 3,5 L-diiodothyronine, and thyronine bind negligibly. Since triiodothyroacetic acid is present in small concentration and T4 reacts little despite its fifty-fold greater concentration, the specificity of the T3 antibody is adequate for clinical purposes. Intraassay coefficient of variation is 3.7 to 8%. Normal values range between 80 and 230 ng/dl depending upon assay conditions and the antibody employed.

A small overlap between normal and hyperthyroid patients is observed (Figure 142.1). Many hyperthyroid patients have less elevation of T4 than T3, and the diagnosis will be more readily appreciated by obtaining the latter determination. An occasional patient with hyperthyroidism, so called T3 toxicosis, has normal T4 and free T4 values but a diagnostic elevation of T3. For these reasons T3 concentration should be obtained in any patient in whom the suspicion of hyperthyroidism is not clearly verified by the T4.

A significant fraction of patients who are hypothyroid and not otherwise ill have normal values of serum T3 (Figure 142.1). Conversely, in many acutely or chronically ill euthyroid patients, the concentration of T3 is reduced; patients taking any one of a growing number of drugs have a similarly reduced concentration of T3. The mechanism of reduction is thought to be secondary to reduced peripheral conversion of T4 to T3 by the 5′ deiodinase enzyme. The prevalence of nonthyroidal causes of low T3 concen-

tration and the frequency of normal T3 concentration in hypothyroid patients make use of this determination hazardous for establishing the diagnosis of hypothyroidism, and it is not recommended for this purpose.

Thyroid Stimulating Hormone

Recently, a new immunoassay methodology has been applied to the measurement of TSH. Depending on the type of label attached to the TSH antibody, the assay is variously called the immunoradiometric assay, the immunofluorimetric assay, the immunochemiluminometric assay, or the immunoenzymometric assay. Briefly, excess monoclonal-labeled TSH antibody is mixed with standard or unknown TSH samples. After equilibrium, a solid-phase TSH antibody having the property of reacting with a different epitope of the TSH molecule is added, and the reaction is again allowed to go to completion. At this juncture TSH bound to labeled TSH antibody is also bound to solid-phase antibody, which allows for separation of TSH with its attendant antibodies and label from the reaction mixture. After washing, the concentration of label is measured by techniques appropriate for the label. In the case of the immunoradiometric assay, the antibody is labeled with ^{125}I, and this is counted. The original concentration of TSH is directly proportional to the ^{125}I activity separating with the bound solid-phase antibody. Published sensitivities of the various assays range from 0.004 to 0.6 μU/ml. Intraassay coefficient of variation is from 12 to 15% for low values and 2 to 3% for high values. This new methodology appears to be a reliable means of separating hyperthyroid from euthyroid patients. With very few exceptions, hyperthyroid patients have zero or low values, whereas normal subjects have measurable values of TSH. There is in general an excellent correlation between zero values of TSH and TRH-TSH nonresponsiveness.

For these reasons the sensitive TSH determination is increasingly being used as a first-line test to confirm or screen for the diagnosis of hyperthyroidism. In otherwise healthy individuals this approach has merit; however, a disconcerting number of sick, hospitalized euthyroid patients are found to have low or unmeasurable TSH values (Table 142.2). Most have sustained trauma or have a severe illness, often treated with steroids or dopamine; but some have acute psychiatric disorders and others are depressed. Low or unmeasurable values for TSH and flat TRF–TSH responses have also been found in otherwise well patients with nontoxic nodular goiters, patients recently treated for thyrotoxicois, patients with "euthyroid" Graves' disease or exophthalmous, normal subjects in the first trimester of pregnancy, and patients with pituitary or hypothalomic hypothyroidism. Finally, a small fraction of apparently well people are found to have low TSH values which sometimes are normal several weeks later but sometimes remain low in the absence of recognizable thyroid disease. Conversely, a fraction of euthyroid hospitalized patients have elevated TSH concentrations. Some of these are in renal failure, whereas others are severely ill with a variety of nonthyroidal diseases.

Table 142.2
Euthyroid Conditions Associated with Low and High Sensitive TSH

Low sensitive TSH
Recent treatment for thyrotoxicosis
Euthyroid Graves' ± exophthalmos
Nontoxic nodular goiter
First trimester of pregnancy
Depression
Acute psychiatric disorder
Severe illness ± steroid Rx ± Dopamine Rx
Trauma
High sensitive TSH
Severe illness ± steroid therapy
Renal failure

In the setting of thyroid failure induced by ^{131}I treatment of hyperthyroidism or external irradiation to the neck for treatment of lymphoma, the concentration of TSH often rises long before T4 falls below normal. In fact, TSH is sometimes elevated in patients who have few or no symptoms, and the question of whether these subjects have minimal hypothyroidism or "compensated" thyroid dysfunction is difficult to resolve. This latter circumstance has raised a number of issues, the most important of which has been the question of whether to treat.

With an elevated TSH, it is reasonable to assume that the T4 concentration is at or below the lowest acceptable limit for that patient. If the patient is symptomatic, and especially when there are signs of hypothyroidism, treatment is indicated even if the value of T4 is within the "normal" range. Patients who have normal values of T4 and no symptoms or signs of hypothyroidism, but in whom the TSH is clearly elevated, pose a special problem that may best be resolved by a period of observation.

While TSH is an extremely reliable marker for the state of primary hypothyroidism, it is not invariably low in pituitary or hypothalamic hypothyroidism. If there is discordance between the expected concentration of T4 and TSH, the possibility of pituitary or hypothalamic disease should be considered. Thus, in a patient who has a low or low normal T4 and a TSH that is either normal, low, or

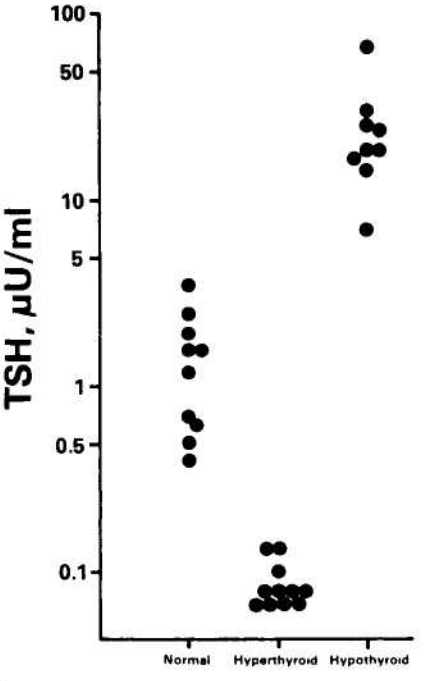

Figure 142.2
Serum TSH in various states of thyroid function.

just minimally elevated, a TRF challenge should be used to measure pituitary secretory reserve.

TSH Response to Thyrotropin Releasing Hormone

For a variety of reasons, the measurement of TRH in serum as a test of thyroid function has not been generally achieved. A reliable assay would be of great clinical interest as it might provide a powerful means of distinguishing between various levels of dysfunction along the pituitary–hypothalamic axis.

Intravenous administration of TRH is followed by a measurable rise in TSH which peaks within 20 to 30 minutes. The TRH stimulation test is performed by giving 500 μg of TRH as an intravenous bolus. Side effects are nausea, a peculiar taste, chest discomfort, urinary urgency, and a modest increase in blood pressure. Timing of blood samples is predicated on the questions being asked (Figure 142.3). If hyperthyroidism or primary hypothyroidism are diagnostic possibilities, samples are drawn at injection and 20 and 30 minutes afterward. The "flat line" response of hyperthyroidism and supranormal response of primary hypothyroidism will be evident at these time points (Figure 142.3). If pituitary or hypothalamic hypothyroidism is suspected, samples should be obtained at injection and 20, 40, 60, 90, and 120 minutes. These will illustrate the reduced response of pituitary hypothyroidism and the delayed response of hypothalamic hypothyroidism. Unfortunately, a fraction of patients with pituitary hypothyroidism have a delayed TSH release, so this finding is consistent with both pituitary and hypothalamic dysfunction. In females at any age, a normal peak response is greater than 5 μU/ml. In males up to age 39, a similar value applies; but after age 40, peak increases as small as 2 μU/ml are found in some normal men.

A fraction of multinodular goiters and follicular adenomas are autonomous and make sufficient hormone to suppress TSH production and thereby blunt the TSH response to TRH. In this setting a flat TSH response is not necessarily diagnostic of hyperthyroidism. On the other hand, a normal TSH response excludes hyperthyroidism. Frequently, patients with Graves' ophthalmopathy have a flat response, and they are not necessarily hyperthyroid. A normal response does not exclude the diagnosis of Graves' ophthalmopathy. Other euthyroid nonresponders are patients treated for hyperthyroidism within the last several months, patients with severe illness, and especially those treated with dopamine and steroids. Finally, some patients with depression and acute psychiatric disorders have a flat response.

In a patient who has a low T4 concentration and a normal-to-low concentration of TSH, a blunted or flat-line TSH response to TRH indicts the pituitary as the cause of hypothyroidism. A delayed and sometimes exaggerated TSH peak suggests either pituitary or hypothalamic disease. Unfortunately, a number of hypopituitary subjects will have a significant TSH increase that cannot be distinguished from a low normal response, and this is especially true in older males. In this setting, and as isolated TSH deficiency is unusual, a complete evaluation of the pituitary gland will establish the presence of pituitary disease.

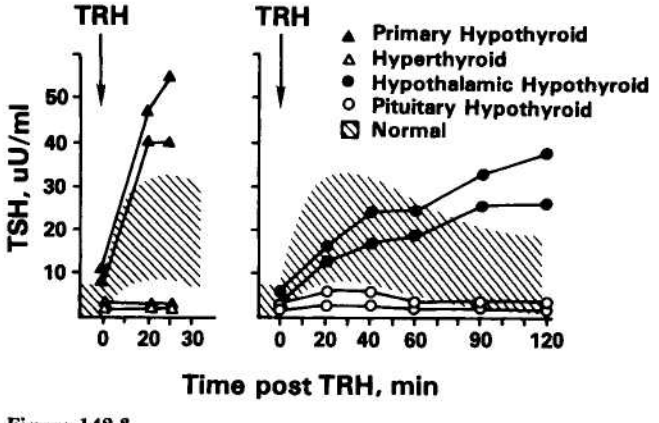

Figure 142.3
TSH response to TRH in various types of hypothyroidism.

Radioactive Iodine Uptake

The thyroid's avidity for iodide and the ease of measuring it externally give the clinician another indirect measurement of T4 production. By using appropriate columnation and suitable standards, fractional thyroid accumulation of an oral dose of ^{131}I can be measured. Activity measured over the thyroid following oral administration of the radionuclide is characterized by an early, rapid uptake phase followed by a plateau of radioactivity in the gland that is most conveniently measured at 24 hours (Figure 142.4).

The fractional uptake of a given amount of ^{131}I depends on the production rate of thyroid hormone, thyroid iodide stores, and dietary iodide intake. Iodide stores and dietary iodide are usually closely inter-related, but since the dietary iodide is only 150 to 600 μg/day, pharmacologic iodide loads (certain drugs or dyes) may artificially reduce the radioiodine uptake to very low values.

Despite its susceptibility to these hazards, the radioactive iodine uptake is an excellent adjunctive test. As with the

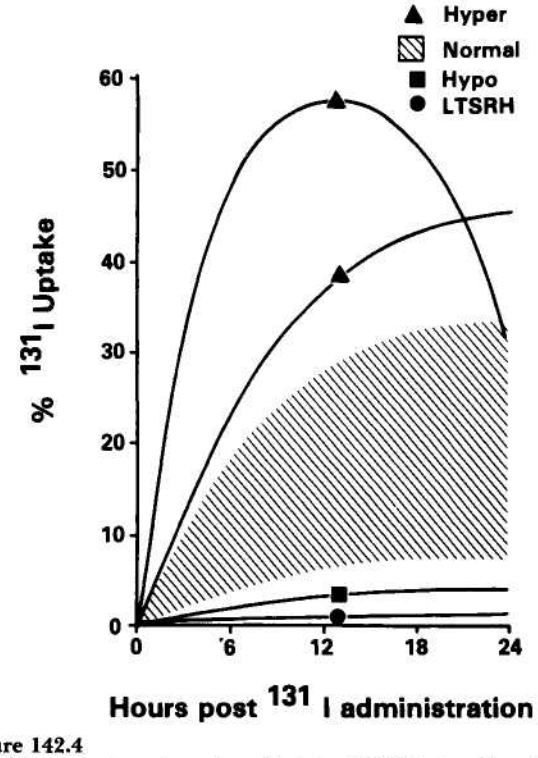

Figure 142.4
The ^{131}I uptake in various thyroid states. LTSRH: lymphocytic thyroiditis with spontaneously resolving hyperthyroidism.

estimation of T4, there is overlap in various states of thyroid function as assessed by the radioactive iodine test. Major disadvantages are cost and the requirement for at least two visits to the hospital. Because of its reduced use, it is increasingly unavailable. For these reasons, the radioactive iodine uptake is not routinely used to assess thyroid function, but in some circumstances it still has great utility. For example, if lymphocytic thyroiditis with spontaneously resolving hyperthyroidism is suspected, low iodine uptake during the hyperthyroid phase will greatly assist in diagnosis. In addition, if a patient is hyperthyroid from ingesting pharmacological doses of thyroid hormone, or if the patient has the rare struma ovarii with hyperthyroidism, the radioactive iodine uptake will again be low. In the latter case, scanning of the abdomen will reveal the excessive T4 production site. In patients with symptoms of hyperthyroidism and nondiagnostic studies of thyroid hormone concentration, a significant elevation of the ^{131}I uptake helps establish the diagnosis.

Basic Science

Pyroglutamyl-histadyl-prolinamide, the TSH-releasing hormone, or TRH, is synthesized in anterior hypothalamic neurons and released in the region of the median eminence (Figure 142.5). After circulating down the neurohypophyseal portal plexus, TRH binds to cell membrane receptors on anterior pituitary thyrotropes and causes production and release of TSH. The number of TRH receptors is in part regulated by T3 nuclear receptor occupancy. High T3 nuclear receptor occupancy is associated with reduced TRH receptor numbers, and low T3 nuclear receptor occupany the converse. This reciprocal relationship between T3 nuclear occupancy and TRH receptors modules the thyrotrope's response to TRH.

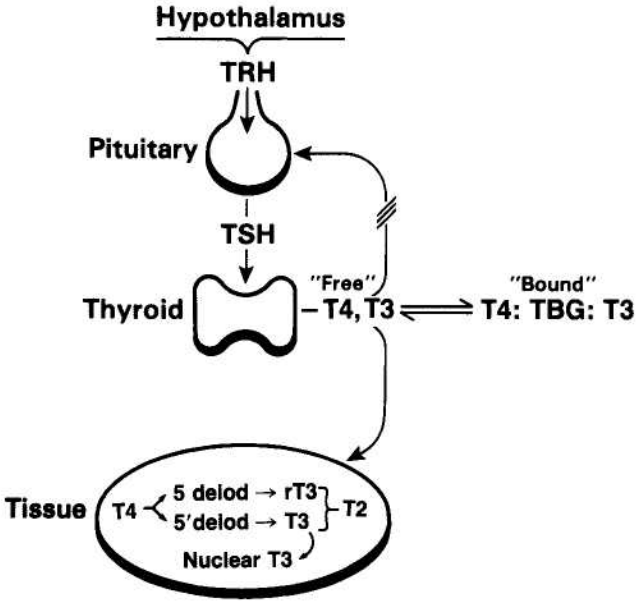

Figure 142.5
Normal thyroid homeostasis. TRH released in the hypothalamus stimulates synthesis and release of TSH by anterior pituitary thyrotropes. TSH, in turn, stimulates the thyroid to produce and release T4 and T3, which on entering the extracellular compartment, reversibly bind to TBG. "Free" T4 enters tissue and is enzymatically deiodinated at the 5 or 5′ position producing reverse T3 or T3 respectively. The binding of T3 to nuclear receptors is followed by hormone action. In the pituitary, nuclear receptor binding by T3 leads to downregulation of TRH receptors and blunting of the TRH effect. TRH: thyrotropin releasing hormone. TSH: thyroid stimulating hormone. T4: thyroxine. T3: triiodothyronine. TBG: thyroxine binding globulin. 5 deiod: 5 deiodinase. 5′ deiod: 5′ deiodinase. rT3: reverse T3. T_2: diiodothyronine.

TSH, a basic glycoprotein with a molecular weight of approximately 28,000 daltons, is composed of alpha and beta chains linked by sulfhydryl bonds. The alpha chain is common to TSH, luteinizing hormone, follicle stimulating hormone, and human chorionic gonadotropin while the beta chain is unique to each. In the case of TSH, it provides important physicochemical properties promoting interaction of TSH with its plasma membrane receptor. TSH binding leads to endocytosis of colloid followed by fusion of the endocytic vacuole with intracellular lysosomes, which leads to digestion of the contained thyroglobulin and release of bound T4 and T3 in a 10 : 1 ratio.

Exiting T4 and T3 enter the circulation and reversibly bind to circulating proteins, the most important of which is TBG. Others are thyroid binding prealbumin and albumin. TBG, an inter-alpha-globulin, has the highest affinity for the iodothyronines, but because of its low concentration, it has a limited capacity. Both T4 and T3 are tightly bound; T4 is only 0.03% free, while T3 is 0.3% free. Bound hormone is at equilibrium with the free or dialyzable fraction of hormone. This equilibrium minimizes perturbations in concentration of the free hormone that could occur as a consequence of abrupt changes in utilization or production.

It is presumed that the unbound T4 enters cells and is deiodinated at one of two potential sites. Removal of carbon 5′ iodine of the outer ring, 5′ deiodination, forms biologically active T3, while removal of carbon 5 iodine of the inner ring, 5 deiodination, forms biologically inactive "reverse T3" (Figure 142.6). About 70 to 80% of T3 production is accounted for by this peripheral conversion of T4 to T3. Binding of T3 to the nuclear T3 receptor of the target cell is followed by hormone action.

From the above, it is apparent that the plasma concentrations of T4 and T3 are a function of TRH, TSH, thyroid production of T4 and T3, TBG, peripheral deiodinative conversion of T4 to T3, and deiodinative clearance of T3. Perturbation in any of these determinants may lead to changes in others, and these changes may be reflected by changes in thyroid function studies. For instance, a primary increase in the production of TBG causing an increased plasma TBG concentration would lead to a transiently reduced free T4 because of an increase in T4 binding and thereby a reduced intracellular T4 concentration. As a consequence, intracellular T3 production and T3 nuclear binding would be reduced. When this occurs in the pituitary

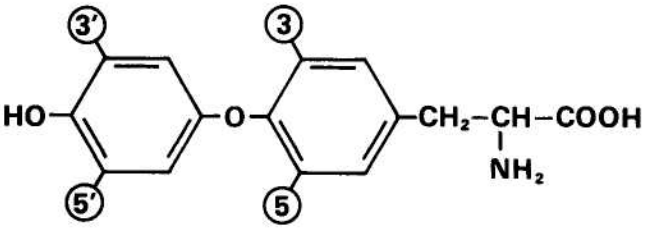

Figure 142.6
Thyroxine. Iodine is represented by circles designating the ring carbon number.

thyrotrope, augmented TSH release in response to TRH would follow and thereby an increase in the production of T4 until the concentration of free and intracellular hormones was again normal. This would be associated with an increased saturation of the enlarged pool of TBG and a final steady-state increase in extracellular T4 and T3 concentration.

Clinical Significance

Alteration of Thyroid Function Studies in Hyperthyroidism

In the great majority of instances, hyperthyroidism is associated with glandular overproduction of hormone; it is accompanied by increased T4, T3RU, FT4I, free T4, T3, and ^{131}I uptake, while TSH is low and TRH-TSH responsiveness is blunted (Table 142.2). With a low ^{131}I uptake, it is most likely that excessive hormone derives from inflammatory disruption of gland accompanying lymphocytic thyroiditis with spontaneously resolving hyperthyroidism, but subacute (viral) thyroiditis, struma ovarii, exogenous iodide, and surreptitious ingestion of T4 should be considered. Hyperthyroidism caused by a pituitary tumor is accompanied by an elevated TSH. When hyperthyroidism is secondary to hydatiform mole or embryonal carcinoma, HCG is diagnostically increased. Occasionally hyperthyroidism is caused by overproduction of T3 and measurements relating to T4 are normal. If hyperthyroidism is from oral administration of T3, the ^{131}I uptake is reduced and the T4 concentration is low.

Table 142.3
Thyroid Function Studies in Hyperthyroidism

Usually high: T4, T3 resin uptake, free T4 index, free T4, T3, ^{131}I uptake
Low TSH, TRH–TSH response
- Hyperthyroid Graves' disease
- Hyperthyroid Hashimoto's disease
- Toxic multinodular goiter
- Hyperfunctioning follicular adenoma
- Thyroid cancer

Low ^{131}I uptake
- Lymphocytic thyroiditis with spontaneously resolving hyperthyroidism
- Subacute thyroiditis
- Struma ovarii
- Iodide-induced hyperthyroidism
- Factitious T4-induced hyperthyroidism

High TSH
- TSH-secreting pituitary tumor

High HCG
- Embryonal carcinoma of testis
- Hydatiform mole

High T3, ^{131}I uptake; normal T4, T3 resin uptake, free T4 index, free T4
Low TSH, TSH response to TRH
"T3 toxicosis"
- Graves' disease
- Toxic multinodular goiter
- Hyperfunctioning follicular adenoma

Low ^{131}I uptake, low T4
- Factitious T3-induced hyperthyroidism

Alteration of Thyroid Function Studies in Hypothyroidism

Hypothyroidism most often is caused by glandular hypofunction. T4, T3 RU, free T4 index, free T4, and T3 fall within the normal range or are low (Figure 142.1 and Table 142.4). TSH and/or TRH–TSH responsiveness are high. When pituitary or hypothalamic disease is at fault, the TSH may be low or normal. TRH-TSH responsiveness will be blunted in the case of pituitary disease or delayed and possibly exaggerated in hypothalamic and pituitary disease.

Nonthyroidal Factors Associated with Changes in Thyroid Function Studies

INCREASED CONCENTRATION OF SERUM T4

Familial increases in TBG concentration are not associated with abnormalities in thyroid function. These patients have supranormal concentrations of TBG with high total T4, total T3, and a decrease in the T3RU. The free T4 and TRH–TSH responsiveness are normal (Table 142.5). Except with very high elevations of TBG, the FT4I can be expected to be normal. More recently an abnormal albumin-like protein found in subjects with the syndrome of familial dysalbuminemic hyperthyroxinemia has been found to cause an increase in T4 and FT4I. Because the abnormal protein does not bind T3, the total T3 and T3RU are normal. Free T4 by equilibrium dialysis and the microencapsulated free T4 are normal; however, the analog free T4 may be remarkably elevated. TSH and TRH-TSH responsiveness are normal. The rare familial elevation of thyroid binding prealbumin is accompanied by an increased T4 and FT4I, but as T3 is little bound by thyroid binding prealbumin, its concentration is normal.

Among *acquired causes of increased binding*, pregnancy and hydatiform mole are associated with increased TBG concentrations secondary to a hyperestrogenic state. The changes in T4, T3RU, and T3 occurring as a result of the increased TBG in pregnancy can be expected to return to normal several weeks postpartum. Free T4 may be increased and TRH-TSH responsiveness decreased during the first trimester of pregnancy. Thyrotoxicosis sometimes

Table 142.4
Thyroid Function Studies in Hypothyroidism

Usually low T4, T3 resin uptake, free T4 index, free T4, T3
High TSH, TSH response to TRH
- Hashimoto's thyroiditis
- ^{131}I treatment for hyperthyroidism
- Subtotal thyroidectomy for hyperthyroidism or thyroid cancer
- Mantel irradiation for lymphoproliferative disease
- Neck irradiation following radical neck surgery
- Iodine
- Lithium
- Propylthiouracil
- Methimazole
- Amiodarone
- Sulfonurea

Usually low T4, T3 resin uptake, free T4 index, free T4, T3
Normal or low TSH, blunted TSH response to TRH
- Pituitary hypothyroidism

Delayed and sometimes exaggerated TRH–TSH response
- Hypothalamic hypothyroidism, pituitary hypothyroidism

Table 142.5
Nonthyroidal Conditions Associated with Changes in T4 and T3

Increased concentration of serum T4
Increased binding
Congenital
Familial increase in TBG
Dysalbumic hyperthyroxinemia
Familial increase in TBPA
Acquired
Pregnancy
Hydatiform mole
Hepatitis (viral, chronic active, alcohol)
Lymphocytoma
Acute intermittent porphyria
Psychiatric disturbance
Antibodies to T3 and T4
Drugs
Estrogen
Heroin
Methadone
Clofibrate
5-Flurouracil
Peripheral resistance to thyroid hormone
Nonthyroid illness
Drugs inhibiting conversion of T4 to T3
Propranolol
Amiodarone
Ipodate
Iopanoate
Thyroxine treatment
Decreased concentration of serum T4
Decreased binding
Familial decrease in TBG
Pseudohypoparathyroidism
Nonthyroid illness including uremia
Prolactinoma
Drugs
Androgen
Heparin
Salicylates
Fenclofenac
Diazepam
Diphenylhydantoin
Sulfonurea
Sulfonamides
Iodide
Lithium
Carbamazepine
Phenobarbital
Sodium nitroprusside
Amiodarone
Phenothiazine
Furosemide
Decreased concentration of serum T3
Nonthyroidal illness
Protein-calorie malnutrition
Uncontrolled diabetes mellitus
Cirrhosis
Hyperparathyroidism
Drugs
Propylthiouracil
Methimazole
Propranolol
Glucocorticoids
Ipodate
Iopanoate
Amiodarone
Colistipal

complicates hydatiform mole. These latter conditions are a consequence of the thyroid-stimulating properties of placental and tumor HCG. Diseases of the liver, such as viral or alcoholic hepatitis, chronic active hepatitis, and biliary cirrhosis, are associated with rises in TBG. Similar changes have been described with lymphocytoma and acute intermittent porphyria.

Poorly understood, but no less important, is the transient increase in TBG found in some acutely disturbed psychiatric patients. Further, some of these patients have elevated T4, FT4I, and usually normal but occasionally elevated T3 that is not related to a TBG increase. These abnormalities and the frequent occurrence of a blunted TRH–TSH response in acutely ill psychiatric patients are potential sources of misdiagnosis. The blunted TRH-TSH may return to normal, but it remains flat in a fraction of patients.

A very rare group of patients have antibodies to the thyroid that also bind T3 and T4. In one patient with T3 binding antibodies, immunoassay values for T3 were spuriously low when propylene glycol was used to separate bound from free; however, when the double antibody technique was employed, T3 was increased. In this particular case the TSH was elevated and the patient hypothyroid. In those cases where antibody binds T4, the T4 will likewise be low if propylene glycol is used to separate bound from free. T3, T3RU, free T4 by equilibrium dialysis, TSH, and TRH stimulation are normal. Radioligand free T4 is high.

Occurring on a familial basis and sometimes appearing sporadically, the syndrome of euthyroid hyperthyroxinemia due to *peripheral resistance to thyroid hormone* is characterized by increased T4, T3, T3RU, and FT4I. TSH is normal or slightly increased and the response to TRH is augmented, but the patients are clinically euthyroid or hypothyroid. The increased thyroid function tests plus the goiter have led on occasion to inadvertent treatment for thyrotoxicosis. Occasionally, patients with the familial syndrome will have deafmutism and stippled epiphysis.

Hyperthyroxinemia can occur in nonthyroidal illness. Severely ill patients occasionally have elevations of total T4 and T3RU. The T3 is frequently low as a consequence of the acute illness (see below), and the TRH–TSH test may be blunted for the same reasons. More common in elderly patients, the findings return to normal if the patient recovers. Follow-up is indicated, as an occasional patient in this group has thyrotoxicosis. If the high T4 is accompanied by a normal T3 and the TRH–TSH response is reduced, follow-up is mandatory because the incidence of thyrotoxicosis in this group is appreciable. Perhaps associated with a similar mechanism are the changes in T3 and T4 that follow acute myocardial infarction. A reduction in T3 will generally be maximum around the third postinfarct day and return to normal a few days thereafter. Between 6 and 7 days after the infarction, T4 concentration may be increased but subsequently returns to normal.

Numerous substances can cause *pharmacological increases in T4*. Estrogen, heroin, methadone, clofibrate, and 5-fluorouracil cause a primary increase in the concentration of TBG with the expected changes in total T4, T3RU, and T3. Some drugs cause a transient rise in T4 and reduction in T3 by inhibiting conversion of T4 to T3. Propranolol is the most common offender of this type. The iodinated antiarrhythmic drug amiodarone and the iodinated contrast agents ipodate and iopanoate increase T4 by a similar mechanism, but they have diverse and poorly understood effects on the thyroid gland as well. Amiodarone has been asso-

ciated with transient thyrotoxicosis and temporary or permanent hypothyroidism, both of which have been attributed to its iodide content. Iopanoate has also been associated with thyrotoxicosis. Ipodate and iopanoate inhibit organification and have enjoyed limited use in the treatment of thyrotoxicosis. At least in the case of ipodate, the organification inhibition has been dissociated from its iodide content. Hyperthyroxinemia with or without elevated T3 has been noted in patients with goiters who were treated with iodide. Replacement thyroxine therapy is often associated with increased T4 and free T4 and normal concentrations of T3. At least in part this may relate to the earlier notion that 0.2 to 0.4 mg/day was an appropriate daily replacement dose. Now it is appreciated that 0.05 to 0.15 mg/day is generally adequate.

STATES WITH LOW THYROXINE CONCENTRATION

Relatively common in *severe illness* and a grim prognostic sign is the confusing array of changes in thyroid tests characterized by decreased T4, increased or normal T3RU, low T3, normal or low free T4 index, low, normal, or high free T4 by equilibrium dialysis, low or normal free T4 by the nondialysis techniques, normal TSH, and blunted or normal TRH–TSH response. In part, the findings may relate to a reduction in T4 production rate and reduced concentration of TBG. The patients are often on dopamine, and this inhibits TSH response to TRH. The major abnormality appears to be an inhibition of T4 binding present in the serum of these patients. Another drug used frequently in this setting, furosemide, inhibits binding of T4, but it is not certain if this is the putative binding inhibitor. If the patient survives, studies will return to normal. A modest elevation of TSH may be noted during the recovery phase.

Patients with *renal failure* often have a number of alterations in thyroid function tests. The T4, free T4 index, and free T4 by equilibrium dialysis are sometimes low. Free T4 by the nondialytic technique is often low, and the T3 is commonly low. TSH is usually normal, but may be modestly elevated. The TRH–TSH response is blunted or normal.

Congenital low TBG concentration is characterized by a low T4, low T3, high T3RU, normal TSH, and normal TRH–TSH responsiveness. Free T4 by equilibrium dialysis is normal. With extremely low concentrations of TBG, the FT4I may be low.

Patients with *prolactin-secreting tumors* sometimes have low T4 concentrations with discordantly normal TSH and TRH responsiveness. The mechanism of the low T4 has been speculated to be secondary to suppression of TSH secretion, which in turn is secondary to excessive hypothalamic dopamine secretion induced by the high prolactin concentration. Dopamine blockade in this setting leads to supernormal basal concentrations of TSH and significantly increased TRH responsiveness.

The very rare syndrome of *pseudohypoparathyroidism*, Type I, characterized by deficiency of guanine nucleotide regulatory protein, can be accompanied by a reduction in the concentration of T4, free T4, and T3, while the TSH and TRH–TSH responsiveness are increased. The question of whether these patients are hypothyroid is not easy to resolve.

PHARMACOLOGICAL DEPRESSION OF T4 CONCENTRATION

Androgenic compounds reduce T4 production and consequently the concentration of TBG with the expected changes in thyroid function studies. A reduction in T4 secondary to displacement from its serum-binding sites is observed in patients treated with heparin, phenylbutazone, large doses of salicylates, fenclofenac, and diazepam. Salicylates and fenclofenac also lower T3. Diphenylhydantoin lowers T4 and T3 by a similar mechanism, and causes a modest increase in T4 clearance by enzyme induction. Diphenylhydantoin may also reduce TSH as well as TRH–TSH responsiveness by a mechanism not yet elucidated. Sulfonureas displace T4 from TBG and, in susceptible individuals, cause an organification defect and reversible hypothyroidism. Sulfonamides, probably through a similar organification inhibition, lower T4 and T3. Rarely iodides, but lithium in as many as 25% of treated patients, cause reversible hypothyroidism. Goiter may accompany pharmacologically induced hypothyroidism. In addition to diphenylhydantoin, carbamazepine and phenobarbital may cause slight lowering of T4 by enzyme induction, leading to more rapid peripheral clearance of hormone. Sodium nitroprusside inhibits thyroid function and reduces T4. The hypothyroxinemia associated with amiodarone and iodide therapy are described above.

Heavy cigarette smoking may be associated with a mild reduction of T4, FT4I, and T3, which is unaccompanied by changes in T3RU and TSH. This may relate to thiacyanate derived from the burning tobacco. Phenothiazine lowers T4 concentration by an unknown mechanism.

STATES CHARACTERIZED BY A LOW T3 CONCENTRATION

Protein-calorie malnutrition, severe illness, and uncontrolled diabetes are associated with low T3 concentration secondary to reduced 5′ deiodinase activity. T4, T3RU, and FT4I are usually normal, while TSH and TRH-TSH responsiveness are normal or low; in protein-calorie malnutrition there may be a modest but significant increase in T4 and free T4. Patients with anorexia nervosa sometimes have low T4 levels.

A low T3 is frequently found in patients with cirrhosis. The T4 and free T4 may be low, while in some patients the free T4 is high. TSH is high or normal and the TRH–TSH response is normal. The mechanism for these confusing changes is not known. Hyperparathyroidism has been associated with low T3; the mechanism of this change is likewise unexplained.

Pharmacological lowering of T3 occurs with a number of drugs. Propylthiouracil, but not methimazole, inhibits 5′ deiodinase activity. Since both drugs inhibit organification in the thyroid, both are associated with a decrease in T4 and T3 concentration. Propranolol causes only slight 5′ deiodinase inhibition and a small reduction in T3 concentration, while glucocorticoids, sodium ipodate, iopanoate, amiodarone, and colistipal effect significant reductions in the enzyme activity and in T3 concentrations.

Strategy for the Diagnosis of Hyperthyroidism

Hyperthyroidism presents in remarkably diverse ways. On the one hand is the anxious young woman with classic signs and symptoms, while on the other is an elderly male with only goiter and proximal muscle weakness. In the former, a confirmatory test is adequate; in the latter, a number of equivocal tests may necessitate protracted follow-up or a therapeutic trial. In either event, it is critical to establish the diagnosis of hyperthyroidism. Table 142.6 is intended to offer guidance to the physician seeking to diagnose hyper-

Table 142.6
Hyperthyroidism

In the otherwise healthy patient
sensitive TSH
confirm with free T4 index, or T4, T3 resin uptake; if T4 measurement does not confirm, T3
validate sensitive TSH if necessary with TRF–TSH
In the hospitalized sick patient
free T4 index or T4, T3 resin uptake, sensitive TSH and T3

thyroidism. It will not replace thorough knowledge of the patient, nor can it be a substitute for rigorous thinking.

For otherwise healthy patients, the sensitive TSH immunoassay is the best first-line test to evaluate thyroid function. However, some assays have appreciably better sensitivity and specificity than others and it behooves the user to know the limitations of the one employed. A low value is consistent with but not necessarily diagnostic of hyperthyroidism, as some patients with nontoxic nodular goiter, euthyroid Graves' disease with or without exophthalmos and others treated for hyperthyroidism within the last several months may have low sensitive TSH values and also a flat TRF–TSH response. Moreover, some elderly and some depressed patients may have low values with low or marginal TSH responses to TRH as well. If a low TSH is found in a patient in whom there is doubt as to whether hyperthyroidism is present, elevation of the T4 and T3RU or the FT4I, or these being normal, the T3 radioimmunoassay would strongly support the diagnosis. In fact, it is always wise to confirm the diagnosis of hyperthyroidism with at least two tests, and a measure of thyroid hormone concentration is an excellent compliment to the TSH test. To evaluate patients taking T4, the sensitive TSH is clearly the test of choice. In this setting the T4, T3RU, and FT4I can be particularly misleading, sometimes being high when the patient is receiving appropriate amounts of T4 and normal when the patient is receiving excessive hormone. If there is doubt about the validity of a low TSH, a TRF infusion with 20- and 30-minute post injection TSH values is recommended. A flat response validates the low TSH, but again is only consistent with thyrotoxicosis for reasons cited above. A normal response excludes hyperthyroidism.

The available information suggests that the FT4I is at least as good as the sensitive TSH for the diagnosis of hyperthyroidism in sick hospitalized patients. This because low TSH values are not uncommon in patients who have sustained trauma, have an acute psychiatric emergency, or have a variety of medical disorders which often have led to treatment with steroids or dopamine. Because of the clinical urgency in the sick patient and the attendant cost for each hospital day, it is expedient to simultaneously obtain both the sensitive TSH and an FT4I and even a T3RIA as the combination of changes are likely to afford rapid insight into the cause of any given abnormal result. For instance, a severely ill patient with a low TSH accompanied by a low FT4I and T3RIA would fall readily into the "nonthyroid illness" category whereas a clinically similar patient with a low TSH, a high FT4I and T3RIA would likely have thyrotoxicosis. On recovery an occasional patient noted initially to have low TSH and normal FT4I will develop an FT4I level diagnostic of hyperthyroidism. Similarly, a sick patient with a low TSH, a normal FT4I, and a normal T3 should be followed as a significant number of these have been reported to develop thyrotoxicosis.

Table 142.7
Hypothyroidism

1. Physical examination and history unequivocal or suggestive
T4, T3 resin uptake, TSH ± AMA
or
Free T4 index, TSH ± AMA
2. Lab equivocal
TRH–TSH response
3. Low T4, T3 resin uptake, or low free T4 index; low or normal TSH
TRH–TSH response flat or delayed
Pituitary evaluation

If the studies are equivocal, a therapeutical trial may be warranted. Time permitting, repeated observations and testing at monthly intervals will often be diagnostic.

Strategy for the Diagnosis of Hypothyroidism

The patient presenting with severe myxedema is fortunately becoming a historical anachronism. Thanks to physician awareness and the inclusion of thyroid function studies in screening programs, the number of severely myxedematous patients is much reduced. Nonetheless, the incidence of hypothyroidism is appreciable and screening of all adults, especially the elderly, is warranted. When a reasonable clinical suspicion exists, a full work-up should be undertaken. The recommended approach is outlined in Table 142.7.

A low T4 and T3RU, or a low FT4I with a high TSH is diagnostic of primary hypothyroidism. Antimicrosomal antibodies establish that the patient has autoimmune thyroid disease. In a patient with equivocal findings, an exaggerated 20- to 30-minute TSH response to TRH is consistent with primary hypothyroidism, or at least a compensated state of thyroid dysfunction.

A low T4 and T3RU or FT4I accompanied by a normal or low TSH suggests pituitary hypothyroidism. TRH stimulation in this setting should be done with samples at the time of injection and 20, 40, 60, 90, and 120 minutes afterward. Pituitary hypothyroidism will be associated with a blunted TSH response, the very rare hypothalamic hypothyroidism, and some cases of pituitary hypothyroidism with an elevation that is highest 1 to 2 hours after injection.

Thyroid function studies usually provide an accurate assessment of the patient's status. In the person ill from a variety of causes or taking any one of a growing number of drugs, abnormal results are most often secondary to these nonthyroidal conditions, and knowing the changes imposed by these conditions enables the physician to interpret most abnormal tests appropriately. Thyroid function studies are not wanting in accuracy so much as they are in need of interpretation, the responsibility of the physician caring for the patient.

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