Evaluation of Thyroid Function in Health and Disease

Last Updated: September 21, 2000

Authors

- **Jayne Franklyn**
- **Michael Shephard**

Archived

This chapter has been superceeded by 4 newer chapters, 6a, 6b, 6c, and 6d. However this Chapter, written originally by Dr Samuel Refetoff and updated by Drs Franklyn and Shephard, remains a treasure trove of information on many now-obscure thyroid tests, and references. For that reason we maintain it as a part of our Archive for use of MDs who may wish to investigate a bit of the history of thyroid testing. L De Groot, MD

The possibility of thyroid disease is considered when signs or symptoms suggest hyper- or hypothyroidism or some physical abnormality of the thyroid gland. Evaluation of the patient should include a thorough history and physical examination. Since most thyroid diseases require prolonged periods of treatment, it is crucial that a firm diagnosis be established before embarking on such a program. Further, a number of medications, in particular those used in the treatment of thyroid disease, may alter the results of thyroid function tests in such a way that reinvestigation after therapy has begun may provide ambiguous results.

EVALUATION BY LABORATORY TESTS

During the past three decades, clinical thyroidology has witnessed the introduction of a plethora of diagnostic procedures. These laboratory procedures provide greater choice, sensitivity, and specificity which have enhanced the likelihood of early detection of occult thyroid diseases presenting with only minimal clinical findings or obscured by coincidental nonthyroid diseases. They also assist in the exclusion of thyroid dysfunction when symptoms and signs closely mimic a thyroid ailment. On the other hand, the wide choice of complementary and overlapping tests indicates that each procedure has its limitations and that no single test is always reliable.

Thyroid tests can be classified into broad categories according to the information they provide at the functional, etiologic, or anatomic levels (Table 6-1).

1. Tests that directly assess the level of the gland activity and integrity of hormone biosynthesis. These tests such as thyroidal radioiodide uptake and perchlorate discharge are carried out in vivo.

2. Tests that measure the concentration of thyroid hormones and their transport in blood. They are performed in vitro and provide indirect assessment of the level of the thyroid

hormone dependent metabolic activity.

3. Another category of tests attempts to more directly measure the impact of thyroid hormone on peripheral tissues. Unfortunately, tests available to assess this important parameter are nonspecific, since they are often altered by a variety of nonthyroidal processes.

4. The presence of several substances, such as thyroid autoantibodies, usually absent in healthy individuals, are useful in establishing the etiology of some thyroid illnesses.

5. Invasive procedures, such as biopsy, for histological examination or enzymatic studies are occasionally required to establish a definite diagnosis. Gross abnormalities of the thyroid gland, detected by palpation, can be assessed by scintiscanning and by ultrasonography.

6.The integrity of the hypothalamo-pituitary-thyroid axis can be evaluated by (a) the response of the pituitary gland to thyroid hormone excess or deficiency; (b) the ability of the thyroid gland to respond to thyrotropin (TSH); and (c) the pituitary responsiveness to thyrotropin-releasing hormone (TRH). These tests are intended to identify the primary organ affected by the disease process that manifests as thyroid dysfunction; in other words, primary (thyroid), secondary (pituitary), or tertiary (hypothalamic) malfunction.

7.Lastly, a number of special tests will be briefly described. Some are valuable in the elucidation of the rare inborn errors of hormone biosynthesis, and others are mainly research tools.

Each test has inherent limitations, and no single procedure is diagnostically adequate for the entire spectrum of possible thyroid abnormalities. The choice, execution, application and interpretation of each test requires the understanding of thyroid physiology and biochemistry dealt with in the preceding chapters. Thyroid tests serve not only in the diagnosis and management of thyroid illnesses but also to better understand the pathophysiology underlying a specific disease.

Table 6-1. Tests of Thyroid Function and Aids in the Diagnosis of Thyroid Diseases

In Vivo Tests of Thyroid Gland Activity and Integrity of Hormone Synthesis and Secretion Thyroidal Radioiodide Uptake (RAIU) Early Thyroid RAIU and 99mPertechnetate Uptake Measurements Perchlorate Discharge Test Saliva to Plasma Radioiodide Ratio Measurement of Hormone Concentration and Other Iodinated Compounds and Their Transport in Blood Measurement of Total Thyroid Hormone Concentration in Serum Iodometry Radioligand and Immunometric Assays TT4 TT3 Measurement of Total and Unsaturated Thyroid Hormone-Binding Capacity in Serum In vitro Uptake Tests TBG Measurement Estimation of Free	Tests Related to Cardiovascular Function Miscellaneous Biochemical and Physiologic Changes Related to the Action of Thyroid Hormone on Peripheral Tissues Measurement of Substances Absent in Normal Serum Thyroid Autoantibodies Thyroid-Stimulating Immunoglobulins (TSI) Thyroid Stimulation Assays Standard in vivo Mouse Bioassay (LATS) In vitro Bioassays (animal or human tissue and recombinant TSH Receptor) Thyrotropin Binding Assays Thyroid Growth-Promoting Assay Other Substances with Thyroid-Stimulating Activity Exophthalmos-Producing Substance (EPS) Tests of Cell-Mediated Immunity (CMI) Anatomic and Tissue Diagnoses Thyroid Scintiscanning Radioiodide and 99mPertechnitate Scans Other Isotope Scans Fluorescent Scans Ultrasonography X-Ray and Related Procedures Computed Tomography (CT

Thyroid Hormone Concentration Dialysable T4 and T3 by Isotopic Equilibrium Free T4 and T3 Index Methods Estimation of FT4 and FT3 by TBG Measurement Two-step Immunoassays Analogue (one-step) Immunoassays Measurements of Iodine-Containing Hormone Precursors and Products of Degradation 3.3′,5′-triiodothyronine of Reverse T3 (rT3) 3,5,-diiodothyronine (3,5-T2) 3,3′,-diiodothyronine (3,3′-T2) 3′,5′,-diiodothyronine (3′,5′,-T2) 3′-monoiodothyronine (3′-T1) 3-monoiodothyronine (3-T1) Tetra- and triiodothyroacetic acid (TETRAC and TRIAC) 3,5,3′-T3 sulfate (T3S) di- and monoiodityrosine (MIT and DIT) Thyroglobulin (Tg) Measurement of Thyroid Hormone and Its Metabolites in Other Body Fluids and in Tissues Urine Amniotic Fluid (AF) Cerebrospinal Fluid (CSF) Milk Saliva Effusions Tissues Tests Assessing the Effects of Thyroid Hormone on Body Tissues Basal Metabolic Rate (BMR) Deep Tendon Reflex Relaxation Time (Photomotogram) Scanning) Angiography Lymphography Thermography Magnetic Resonance Imaging (MRI) Biopsy of the Thyroid Gland Core Biopsy (Open od Closed) Percutaneous Fine-needle Aspiration (FNA) Evaluation of the Hypothalamic-Pituitary-Thyroid Axis Thyrotropin (TSH) Thyrotropin-Releasing Hormone (TRH) Test Other Tests of TSH Reserve Thyroid Stimulation Test Thyroid Suppression Test Specialized Thyroid Tests Iodotyrosine Deiodinase Activity Test for Defective Hormonogenesis Iodine Kinetic Studies Absorption of Thyroid Hormone Turnover Kinetics of T4 and T3 Metabolic Kinetics of Thyroid Hormones and Their Metabolites Measurement of the Production Rate and Metabolic Kinetics of Other Compounds Transfer of Thyroid Hormone from Blood to Tissues Applications of Molecular Biology in the Diagnosis of Thyroid Diseases

In Vivo Tests of Thyroid Gland Activity and Integrity of Hormone Synthesis and Secretion

Common to these tests is the administration to the patient of radioisotopes that cannot be distinguished by the body from the naturally occurring stable iodine isotope (127I). In contrast to all other tests, these procedures provide a means to directly evaluate thyroid gland function. Formerly these tests were used in the diagnosis of hypothyroidism and thyrotoxicosis, but this application has been supplanted by measurement of serum TSH and thyroid hormone concentrations in blood. Also, alterations of thyroid gland activity and in handling of iodine are not necessarily coupled to the amount of hormone produced and secreted. The tests are time consuming, relatively expensive and expose the patient to irradiation. Nevertheless, they still have some speccific applications including the diagnosis of inborn errors of thyroid hormonogenesis. Administration of isotopes is required for thyroid gland scanning used to demonstrate ectopic thyroid tissue and to establish the etiology of some forms of thyrotoxicosis. Finally, measurement of the thyroidal radioiodide uptake can be used as a means for estimating the dose of radioiodide to be delivered in the therapy of thyrotoxicosis and thyroid carcinoma.

To understand the physiological basis of this category of tests, one should remember the following facts. Iodine is an integral part of the thyroid hormone molecule. Although several other tissues (salivary glands, mammary glands, lacrimal glands, the choroid plexus, and the parietal cells of the stomach) can extract iodide from blood and generate a positive tissue to serum iodide gradient, only the thyroid gland stores iodine for an appreciable period of time. [1] Since the kidneys continually filter

blood iodide, the final fate of most iodine atoms is either to be trapped by the thyroid gland or to be excreted in the urine. When a tracer of iodide is administered to the patient, it rapidly becomes mixed with the stable extrathyroidal iodide pool and is thereafter handled identically as the stable isotope. Thus, the thyroidal content of radioiodine gradually increases and that in the extrathyroidal body pool gradually declines, until virtually no free iodide is left. Normally this end point is reached between 24 and 72 hours.

From data of the radioiodide uptake by the thyroid gland and/or urinary excretion and/or stable iodide concentration in plasma and urine, the following parameters can be derived: (1) the rate of thyroidal iodine uptake (thyroid iodide clearance), (2) the fractional thyroid radioactive iodide uptake (RAIU), (3) the absolute iodide uptake (AIU) by the thyroid gland, and (4) the urinary excretion of radioiodide, or iodide clearance. After the complete removal of the administered radioiodide from the circulation, depletion of the radioisotope from the thyroid gland can be monitored by direct counting over the gland. Reappearance of the radioiodine in the circulation in protein-bound form can be measured and can be used to estimate the intrathyroidal turnover of iodine and the secretory activity of the thyroid gland.

The foregoing tests can be combined with the administration of agents known either to normally stimulate or to inhibit thyroid gland activity thus providing information on the control of thyroid gland activity. Administration of radioiodide followed by scanning allows us to examine the anatomy of functional tissue. The latter two applications of in vivo tests utilizing radioiodide will be discussed under their respective headings.

The potential hazard of irradiation resulting from the administered radioisotopes should always be kept in mind. Children are particularly vulnerable, and doses of X-rays as small as 20 rads to the thyroid gland are associated with increased risk of developing thyroid malignancies. [2] However, it must be noted that there is no proven danger from isotopes used for the diagnosis of thyroid diseases. [3] In vivo administration of radioisotopes is absolutely contraindicated during pregnancy and in breast-feeding mothers because of placental transport of isotope and excretion into breast milk.

A number of radioisotopes are now available. Furthermore, provision of more sophisticated and sensitive detection devices has substantially decreased the dose required for the completion of the studies. Table 6-2 [5-7] lists the most commonly used isotopes for in vivo studies of the thyroid. Isotopes with slower physical decay, such as 125I and 131I, are particularly suitable for long-term studies. Isotopes with faster decay, such as 123I and 132I, usually deliver a lower irradiation dose and are advantageous in short-term and repeated studies. The peak photon energy gamma emission differs among isotopes, allowing the execution of simultaneous studies with two isotopes.

Table 6-2. Commonly Used Isotopes for In Vivo Studies and Radiation Dose Delivered

Nuclide	Principal Photon Energy (keV)	Physical Decay		Estimated Radiation Dose (m rads/ÂµCi)		Average Dose Given for Scanning Purposes (ÂµCi)
		Mode	Half-Life (Days)	Thyroida	Total Body	
131I-	364	ÃŸ (0.606 Mev)	8.1	1,340	0.08	50
125I-	28	Electron capture	60	835	0.06	50
123I-	159	Electron capture	0.55	13	0.03	200
132I-	670	ÃŸ (2.12	0.10	15	0.1	50b

		MeV)				
99mTc04-	141	Isometric transition	0.25	0.2	0.01	2,500

aCalculations take into account the rate maximal uptake, and residence time of the isotope as well as gland size. For the iodine isotopes, average data for adult euthyroid persons used were: t-1/2 of uptake 5 hours, biologic t-1/2 50 days, maximal uptake 20%, and gland size 15 g (see also refs. [Quimby, 1970 #628;MIRD, 1975 #629;MIRD, 1976 #630]). bDose used for early thyroidal uptake studies.

Thyroidal Radioiodide Uptake (RAIU)

This is the most commonly used thyroid test requiring the administration of a radioisotope. It is usually given orally in a capsule or in liquid form and the quantity accumulated by the thyroid gland at various intervals of time is measured using a gamma scintillation counter. Correction for the amount of isotope circulating in the blood of the neck region, by subtracting counts obtained over the thigh, is of particular importance during the early periods following its administration. A dose of the same radioisotope, usually 10%, placed in a neck “phantom” is also counted as a “standard”. The percentage of thyroidal radioactive iodide uptake (RAIU) is calculated from the counts cumulated per constant time unit.

The percentage of RAIU 24 hours after the administration of radioiodide is most useful, since in most instances the thyroid gland has reached the plateau of isotope accumulation, and because it has been shown that at this time, the best separation between high, normal, and low uptake is obtained. Normal values for 24-hour RAIU in most parts of North America are 5 to 30 percent. In many other parts of the world, normal values range from 15 to 50 percent. Lower normal values are due to the increase in dietary iodine intake following the enrichment of foods, particularly mass produced bread (150 Âµg of iodine per slice), with this element. [8] The inverse relationship between the daily dietary intake of iodine and the RAIU test is clearly illustrated in Figure 6-1. The intake of large amounts of iodide (>5 mg/day), mainly from the use of iodine-containing radiologic contrast media, antiseptics, vitamins, and drugs such as amiodarone, suppresses the RAIU values to a level hardly detectable using the usual equipment and doses of the isotope. Depending upon the type of iodine preparation and the period of exposure, depression of RAIU can last for weeks, months, or even years. Even external application of iodide may suppress thyroidal radioiodide uptake. The need to inquire about individual dietary habits and sources of excess iodide intake is obvious.

Figure 6-1. Relation of 24 hour thyroidal radioiodide (I131) uptake (RAIU) to dietary content of stable iodine (I12 7). The uptake increases with decreasing dietary iodine. With iodine intake below the amount provided from thyroid hormone degradation, the latter contributes a larger proportion of the total iodine taken up by the thyroid. Under current dietary habits in the United States, the average 24-hour thyroidal RAIU is below 20 percent.

The test does not measure hormone production and release but merely the avidity of the thyroid gland for iodide and its rate of clearance relative to the kidney. Disease states resulting in excessive production and release of thyroid hormone are most often associated with increased thyroidal RAIU and those causing hormone underproduction with decreased thyroidal RAIU (Figure 6-2, below).

Important exceptions include high uptake values in some hypothyroid patients and low values in some hyperthyroid patients. Increased thyroidal RAIU with hormonal insufficiency co-occur in the presence of severe iodide deficiency and in the majority of inborn errors of hormonogenesis (see Chapter 20 and 16). In the former, lack of substrate, and in the latter, a specific enzymatic block of hormone synthesis cause hypothyroidism poorly compensated by TSH-induced thyroid gland overactivity. Decreased thyroidal RAIU with hormonal excess is typically encountered in the syndrome of transient thyrotoxicosis (both de Quervain's and painless thyroiditis), [9] ingestion of exogenous hormone (thyrotoxicosis factitia), iodide-induced thyrotoxicosis (Jod-Basedow disease), [10] and in patients with thyrotoxicosis on moderately high intake of iodide (see Table 6-3). High or low thyroidal RAIU as a result of low or high dietary iodine intake, respectively, may not be associated with significant changes in thyroid hormone secretion.

Figure 6-2. Examples of thyroidal RAIU curves under various pathological conditions. Note the prolonged uptake in renal disease due to decreased urinary excretion of the isotope and the early decline in thyroidal radioiodide content in some patients with thyrotoxicosis associated with a small but rapidly turning over intrathyroidal iodine pool.

Various factors including diseases that affect the value of the 24-hour thyroidal RAIU are listed in Table 6-3 . Several variations of the test have been devised which have particular value under special circumstances. Some of these are briefly described.

Table 6-3. Diseases and Other Factors That Affect the 24-Hour Thyroidal RAIU

Increased RAIU
Hyperthyroidism (Graves' disease, Plummer's disease, toxic adenoma, trophoblastic disease, pituitary resistance to thyroid hormone, TSH-producing pituitary adenoma)
Non-toxic goiter (endemic, inherited biosynthetic defects, generalized resistance to thyroid hormone, Hashimoto's thyroiditis)
Excessive hormonal loss (nephrosis, chronic diarrhea, hypolipidemic resins, diet high in soybean)
Decreased renal clearance of iodine (renal insufficiency, severe heart failure)
Recovery of the suppressed thyroid (withdrawal of thyroid hormone and anti-thyroid drug administration, subacute thyroiditis, iodine-induced myxedema)
Iodine deficiency (endemic or sporadic dietary deficiency, excessive iodine loss as in pregnancy or in the dehalogenase defect)
TSH administration

Decreased RAIU

Hypothyroidism (primary or secondary)

Defect in iodide concentration (inherited “trapping” defect, early phase of subacute thyroiditis, transient hyperthyroidism)

Suppressed thyroid gland caused by thyroid hormone (hormone replacement, thyrotoxicosis factitia, struma ovarii)

Iodine excess (dietary, drugs and other iodine contaminants)

Miscellaneous drugs and chemicals (see Tables 39-10 and 39-12)

Early Thyroid RAIU and 99mPertechnetate Uptake Measurements

In some patients with severe thyrotoxicosis and low intrathyroidal iodine concentration, the turnover rate of iodine may be accelerated causing a rapid initial uptake of radioiodide, reaching a plateau before 6 hours, followed by a decline through release of the isotope in hormonal or other forms (Figure 6-2, above). Although this phenomenon is rare, some laboratories choose to routinely measure early RAIU, usually at 2, 4 or 6 hours. Early measurements require the accurate determination of background activity contributed by the circulating isotope. Radioisotopes with a shorter half-life, such as 123I and 132I, are more suitable.

Since thyroidal uptake in the very early period following administration of radioiodide reflects mainly iodide trapping activity, 99mTc as the pertechnetate ion (99mTcO4-) may be used. In euthyroid patients, thyroid trapping is maximal at about 20 minutes and is approximately 1% of the administered dose [11] . This test, when coupled with the administration of T3, can theoretically be used to evaluate thyroid gland suppressibility in thyrotoxic patients treated with antithyroid drugs (see below).

Perchlorate Discharge Test

This test is used to detect defects in intrathyroidal iodide organification. It is based on the following physiological principle. Iodide is “trapped” by the thyroid gland through an energy-requiring active transport mechanism. Once in the gland, it is rapidly bound to thyroglobulin and retention no longer requires active transport. Several ions, such as thiocyanate (SCN-) and perchlorate (ClO4-), inhibit active iodide transport and cause the release of the intrathyroidal iodide not bound to thyroid protein. Thus, measurement of intrathyroidal radioiodine loss following the administration of an inhibitor of iodide trapping would indicate the presence of an iodide-binding defect.

In the standard test, epithyroid counts are obtained at frequent intervals (every 10 or 15 minutes) following the administration of radioiodide. Two hours later, 1g of KClO4 is administered orally and repeated epithyroid counts continue to be obtained for an additional 2 hours. In normal individuals, radioiodide accumulation in the thyroid gland ceases after the administration of the iodide transport inhibitor but there is little loss of the thyroidal radioactivity accumulated prior to induction of the

“trapping” block. A loss of 5% percent or more indicates an organification defect (see Chapter 16). [12] The severity of the defect is proportional to the extent of radioiodide discharged from the gland and is complete when virtually all the activity accumulated by the gland is lost (see Fig. 16-2, below). The test is positive in the inborn defect of iodide organification, which can be associated with deafness (Pendred’s syndrome), during the administration of iodide organification blocking agents, in many patients with thyroiditis, or following treatment with radioactive iodide.

Figure 6-2. Examples of thyroidal RAIU curves under various pathological conditions. Note the prolonged uptake in renal disease due to decreased urinary excretion of the isotope and the early decline in thyroidal radioiodide content in some patients with thyrotoxicosis associated with a small but rapidly turning over intrathyroidal iodine pool.

Measurement of Hormone Concentration and Other Iodinated Compounds and Their Transport in Blood

Measurements of T4 and T3 in serum and the estimation of their free concentration have become the most commonly used tests for the evaluation of the thyroid hormone-dependent metabolic status. This approach results from the development of simple, sensitive, and specific methods for measuring these iodothyronines and because of the lack of specific tests for the direct measurement of the metabolic effect of these hormones. Other advantages are the requirement of only a small blood sample and the large number of determinations that can be completed by a laboratory during a regular workday.

The thyroid gland is the principal source of all hormonal iodine-containing compounds or their precursors and peripheral tissue are the source of the products of degradation. Their chemical structures, and normal concentrations in serum are given in Figure 6-3. It is important to note that the concentration of each substance is dependent not only upon the amount synthesized and secreted but also upon its affinity for carrier serum proteins, distribution in tissues, rate of degradation, and finally, clearance.

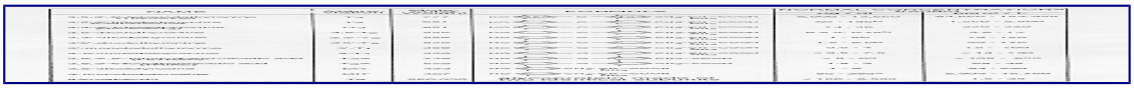

Figure 6-3: Iodine-containing compounds in serum of healthy adults. a. Iodothyronine concentration in the euthyroid population are not normally distributed. Thus, calculation of the normal range on the basis of 95% confidence limits for a Gaussian distribution is not accurate. b. Significant decline with old age. c. Probably an overestimation due to cross-reactivity by related substances.

The main secretory product of the thyroid gland is t4t3 being next in relative abundance. Both compounds are metabolically active when administered vivo. They synthesized and stored as a part larger moleculethyroglobulin.

[18] Under normal circumstances, only minute amounts of Tg escape into the circulation. On a molar basis, it is the least abundant iodine-containing compound in blood. With the exception of T4, Tg, and small amounts of DIT and MIT, all other iodine-containing compounds found in the serum of normal man are produced mainly in extrathyroidal tissues by a stepwise process of deiodination of T4. [19] An alternative pathway of T4 metabolism that involves deamination and decarboxylation but retention of the iodine residues gives rise to TETRAC and TRIAC. [20,21] Conjugation to form sulfated iodoproteins also occurs. [22] Circulating iodoalbumin is generated by intrathyroidal iodination of serum albumin. [23] Small amounts of iodoproteins may be formed in peripheral tissues [24] or in serum [25,26] by covalent linkage of T4 and T3 to soluble proteins. Although the physiological function of circulating iodine compounds other than T4 and T3 remains unknown, measurement of changes in their concentration is of research interest.

Measurement of Total Thyroid Hormone Concentration in Serum

Iodometry. Iodine constitutes an integral part of the thyroid hormone molecule. It is thus not surprising that determination of iodine content in serum was the first method suggested almost six decades ago for the identification and quantitation of thyroid hormone. [27] Measurement of the Protein-Bound Iodine (PBI) was the earliest method used routinely for the estimation of thyroid hormone concentration in serum. This test measured the total quantity of iodine precipitable with the serum proteins, [28] 90% of which is T4. The normal range was 4 – 8 Âµg I/dl of serum.

Efforts to measure serum thyroid hormone levels with greater specificity and with lesser interference from nonhormonal iodinated compounds, led to the development of the butanol extractable iodine (BEI) and T4I by column techniques. All such chemical methods for the measurement of thyroid hormone in serum have been replaced by the ligand assays which are devoid of interference by even large quantities of nonhormonal iodine-containing substances.

Radioimmunoassays. Concentrations of thyroid hormones in serum can be measured by radioimmunoassays (RIA). The principle of these assays is the competition of a hormone (H), being measured, with the same isotopically labeled compound (H*) for binding to a specific class of IgG molecules present in the antiserum [antibody (Ab)]. H is the ligand and the Ab is either a polyclonal antiserum to H or a monoclonal IgG. The reaction obeys the law of mass action. Thus, at equilibrium, the amount of H* bound to Ab to form the complex Ab-H* is inversely proportional to the concentration of H, forming the complex Ab-H, provided the amounts of Ab and H* are kept constant.

AbH* + [H] AbH + H*

The radioisotope content in Ab-H* or in the unbound (free) H* is determined after their separation by precipitation of the antibody-ligand complex or adsorption of the free ligand. Some RIAs are carried out with the Ab fixed to a solid support, reacting with H and H* in solution. Increments of known amounts of H are added to a series of reactions to construct a standard curve that describes the curvilinear stoichiometric relationship between Ab-H* and H. It can be converted to a straight line by a number of mathematical transformations, such as the logit-log plot. Blank reactions contain H* but not specific Ab or, a large excess of H in a full reaction. [29] The sensitivity of the assay is dependent upon the affinity of the Ab and specific activity of H*. Under optimal conditions, as little as 1 pg of H can be measured.

In assays for thyroid hormones, the hormone needs to be liberated from serum binding proteins, mainly TBG. Methods to achieve this include extraction, competitive displacement of the hormone being measured, or inactivation of thyroxine-binding globulin (TBG). [31-34] Rarely, some patients develop circulating antibodies against thyronines that interfere with the RIA carried out on unextracted serum samples. Depending on the method used for the separation of bound from free ligand, values obtained may be either spuriously low or high in the presence of such antibodies. [38,39]

A wide choice of commercial kits is available for most RIA procedures, making these assays accessible to all medical centers. RIAs have been adapted for the measurement of T4 in small samples of dried blood spots on filter paper and are used in screening for neonatal hypothyroidism. [40]

Non-radioactive Methods. More recently, assays have been developed that are based on the principle of the radioligand assay but do not use radioactive material. These assays, which use ligand conjugated to an enzyme have largely replaced RIAs. The enzyme-linked ligand competes with the ligand being measured for the same binding sites on the antibody. Quantitation is carried out by spectrophotometry of the color reaction developed after the addition of the enzyme substrate. [42] Both homogeneous [enzyme-multiplied immunoassay technique (EMIT)] and heterogeneous [enzyme-linked immunosorbent assay (ELISA)] assays for T4 have been developed. [43-45] In the homogeneous assays, no separation step is required, thus providing easy automation. [43] In one such assay, T4 is linked to malate dehydrogenase, inhibiting the enzyme activity. The enzyme is activated when the T4-enzyme conjugate is bound to T4-specific antibody. Active T4 conjugates to other enzymes, such as peroxidase [44] and alkaline phosphatase, [45] have also been developed. The assay has been adapted for the measurement of T4 in dried blood samples used in mass screening programs for neonatal hypothyroidism. [45] Other non-radioisotope immunoassays use fluorescence excitation for detection of the labeled ligand, a technique which is finding increasing application. Such assay methods utilize a variety of chemiluminescent molecules such as 1,2-dioxetanes, luminol and derivatives, acridinium esters, oxalate esters and firefly luciferins, as well as many sensitizers and fluorescent enhancers. [45a] One such assay which employes T4 conjugated to ÃŸ-galactosidase and fluoresence measurements of the hydrolytic product of 4-methyl-umbelliferyl-ÃŸD-galactopyranoside has been adapted for use in a microanalytical system requiring only 10Âµl of serum. [45b]

Serum Total Thyroxine (TT4). The usual concentration of TT4 in adults ranges from 5 to 12 Âµg/dl (64 – 154 nmol/L). When concentrations are below or above this range in the absence of thyroid dysfunction, they are usually the result of an abnormal level of serum TBG. The hyperestrogenic state of pregnancy and administration of estrogen-containing compounds are the most common causes of a significant elevation of serum TT4 levels in euthyroid persons. Less commonly, TBG excess is inherited. [50] Serum TT4 is virtually undetectable in the fetus until midgestation. Thereafter, it rapidly increases, reaching high normal adult levels during the last trimester. A further acute but transient rise occurs within hours after delivery. [51] Values remain above the adult range until 6 years of age, but subsequent age related changes are minimal so that in clinical practice, the same normal range of TT4 applies to both sexes and all ages.

Small seasonal variations and changes related to high altitude, cold, and heat have been described. Rhythmic variations in serum TT4 concentration are of two types: variations related to postural changes in serum protein concentration [56] and true circadian variation. [31] Postural changes in protein concentration do not alter the free T4 (FT4) concentration.

Although levels of serum TT4 below the normal range are usually associated with hypothyroidism, and above this range with thyrotoxicosis, it must be remembered that the TT4 level may not always

correspond to the FT4 concentration which represents the metabolically active fraction (see below). The TT4 concentration in serum may be altered by independent mechanisms: (1) an increase or decrease in the supply of T4 , as seen in most cases of thyrotoxicosis and hypothyroidism, respectively; (2) changes due solely to alterations in T4 binding to serum proteins; and (3) compensatory changes in serum TT4 concentration due to high or low serum levels of T3. Conditions associated with changes in serum TT4 and their relationship to the metabolic status of the patient are listed in Table 6-4.

Table 6-4. Conditions Associated with Changes in Serum TT4 Concentration and Relation to the Metabolic Status

Metabolic Status	Serum TT4 Concentration		
	High	Low	Normal
Thyrotoxic	Hyperthyroidism (all causes, including Graves disease, Plummer's disease, toxic thyroid adenoma, early phase of subacute thyroiditis)Thyroid hormone leak (early stage of subacute thyroiditis, transient thyrotoxicosis) Excess of exogenous or ectopic T4 (thyrotoxicosis factitia, struma ovarii) Predominantly Pituitary resistance to thyroid hormone	Intake of excessive amounts of T3 (thyrotoxicosis factitia)	Low TBG (congenital or acquired)T3 thyrotoxicosis (untreated or recurrent post therapy); morecommon in iodine deficient areas Drugs competing with T4-binding to serum proteins (see also entry under euthyroid with low TT4) Hypermetabolism of nonthyroidal origin (Luft's syndrome)
Euthyroid	High TBG (congenital or acquired)T4-binding albumin-like variant Endogenous T4 antibodies Replacement therapy with T4 only Treatment with D-T4 Generalized resistance to thyroid hormone	Low TBG (congenital or acquired)Endogenous T4 antibodies Mildly elevated or normal T3 T3 replacement therapy Iodine deficiency Treated thyrotoxicosis Chronic thyroiditis Congenital goiter Drugs competing with T4-binding to serum proteins (see Table 39-4)	Normal state
Hypothyroid	Severe generalized resistance to thyroid hormone	Thyroid gland failurePrimary (all causes, including gland destruction, severe iodine deficiency, inborn error of	High TBG (congenital or acquired)?Isolated peripheral tissue resistance to thyroid hormone

hormonogenesis)

Secondary (pituitary failure)

Tertiary (hypothalamic failure)

Serum TT4 levels are low in conditions associated with decreased TBG concentration, the presence of abnormal TBG's with reduced binding affinity (see Chapter 16) or when the available T4-binding sites on TBG are partially saturated by competing drugs present in blood in high concentrations (see Table 5-2). Conversely, TT4 levels are high when the serum TBG concentration is high. The person remains euthyroid provided the feedback regulation of the thyroid gland is intact.

Although changes in transthyretin (TTR) concentration rarely give rise to significant alterations in TT4 concentration, [57] the presence of a variant serum albumin with high affinity for T4 [58,59] or antibodies against T4 [38,39] produce apparent elevations in the measured TT4 concentration, whereas the metabolic status remain normal. The variant albumin is inherited as an autosomal dominant trait termed familial dysalbuminemic hyperthyroxinemia (FDH) (see Chapter 16).

Another possible cause of discrepancy between the observed serum TT4 concentration and the metabolic status of the patient is divergent changes in the serum TT3 and TT4 concentrations with alterations in the serum T3/T4 ratio. The most common situation is that of elevated TT3 concentration. The source of T3 may be endogenous, as in T3 thyrotoxicosis, or exogenous, as during ingestion of T3. In the former situation, contrary to the common variety of thyrotoxicosis, elevation in the serum TT3 concentration is not accompanied by an increase in the TT4 level. In fact, the serum TT4 level is normal and occasionally low. [60] This finding indicates that in T3 thyrotoxicosis the hormone is predominantly secreted as such rather than arising from the peripheral conversion of T4 to T3. Ingestion of pharmacologic doses of T3 results in thyrotoxicosis associated with severe depression of the serum TT4 concentration. A moderate hypersecretion of T3 can be associated with euthyroidism and a low serum TT4 concentration. This circumstance, occasionally referred to as T3 euthyroidism, may be more prevalent than T3 thyrotoxicosis. It is believed to constitute a state of compensatory T3 secretion as a physiologic adaptation of the failing thyroid gland, such as after treatment for thyrotoxicosis, in some cases of chronic thyroiditis, or during iodine deprivation. [61,62] Serum TT4 concentration is also low in normal persons receiving replacement doses of T3. Conversely, serum TT4 levels are above the upper limit of normal in 15-50% of patients treated with exogenous T4. [63] Because of the relatively slow rate of metabolism and large extrathyroidal T4 pool, the serum concentration of the hormone varies little with the time of sampling in relation to ingestion of the daily dose. [64]

Serum Total Triiodothyronine (TT3). Normal serum TT3 concentrations in the adult are 80-190 ng/dl (1.2 – 2.9 nmol/L). While sex differences are small, those with age are more dramatic. In contrast to serum TT4, TT3 concentration at birth is low, about one-half the normal adult level. It rises within 24 hours to about double the normal adult value followed by a rapid decrease over the subsequent 24 hours to a level in the upper adult range, which persists for the first year of life. [51] A decline in the mean TT3 level has been observed in old age, although not in healthy subjects. [52,53] so that a fall in TT3 may refelct the prevalence of nonthyroidal illness rather than to age alone. [67] Although a positive correlation between serum TT3 level and body weight has been observed, it may be related to overeating. [68] Rapid and profound reductions in serum TT3 level can be produced within 24-48 hours

of total calorie or only carbohydrate deprivation. [69-71]

Most conditions causing serum TT4 levels to increase are associated with high TT3 concentrations. Thus, serum TT3 levels are usually elevated in thyrotoxicosis and reduced in hypothyroidism. However, in both conditions the TT3/TT4 ratio is elevated relative to normal euthyroid persons. This elevation is due to the disproportionate increase in serum TT3 concentration in thyrotoxicosis and a lesser diminution in hypothyroidism relative to the TT4 concentration. [72] Accordingly, measurement of the serum TT3 level is a more sensitive test for the diagnosis of hyperthyroidism, and that of TT4 more useful in the diagnosis of hypothyroidism.

There are circumstances in which changes in the serum TT3 and TT4 concentrations are either disproportionate or in opposite direction (Table 6-5). These include the syndrome of thyrotoxicosis with normal TT4 and FT4 levels (T3 thyrotoxicosis). In some patients, treatment of thyrotoxicosis with antithyroid drugs may normalize the serum TT4 but not TT3 level, producing a high TT3/TT4 ratio. In areas of limited iodine supply [62] and in patients with limited thyroidal ability to process iodide, [61] euthyroidism can be maintained at low serum TT4 and FT4 levels by increased direct thyroidal secretion of T3. Although these changes have a rational physiologic explanation, the significance of discordant serum TT4 and TT3 levels under other circumstances is less well understood.

Table 6-5. Conditions That May be Associated with Discrepancies Between the Concentration of Serum TT3 and TT4

Serum(+ = up, – = down, N=normal)			Metabolic Status		
TT3/TT4 Ratio	TT3	TT4	Thyrotoxic	Euthyroid	Hypothyroid
+	+	N	T3-thyrotoxicosis (endogenous)	Endemic iodine deficiency (T3 autoantibodies)a	—-
+	N	-	Treated thyrotoxicosis (T4 autoantibodies)	Endemic cretins (severe iodine deficiency)	
+	+	-	Pharmacologic doses of T3 (exogenous T3-toxicosis) Partially treated thyrotoxicosis	T3 replacement (especially 1 to 3 h after ingestion) Endemic iodine deficiency	(T3 autoantibodies)
-	-	N	Most conditions associated with reduced conversion of T4 to T3 Chronic or severe acute illness b Trauma (surgical, burns) Fasting and malnutrition Drugs c (T3 autoantibodies)a		
-	N	+	Severe nonthyroidal illness associated with thyrotoxicosis	Neonates (first three weeks of life) T4 replacement Familial hyperthyroxinemia due to T4-binding albumin-like variant (T4 autoantibodies)a	
-	-	+	At birth Acute nonthyroidal illness withtransient hyperthyroxinemia	(T4 autoantibodies)a	

a Artifactual values dependent upon the method of hormone determination in serum. b Hepatic and

renal failure, diabetic ketoacidosis, myocardial infarction, infectious and febrile illness, malignancies c
Glucocorticoids, iodinated contrast agents, amiodarone, propranolol, propylthiouracil

The most common cause of discordant serum concentrations of TT3 and TT4 is a selective decrease of serum TT3 due to decreased conversion of T4 to T3 in peripheral tissues. This reduction is an integral part of the pathophysiology of a number of nonthyroidal acute and chronic illnesses and calorie deprivation (see Chapter 5). In these conditions, the serum TT3 level is often lower than that commonly found in patients with frank primary hypothyroidism. Yet, these persons do not present clear clinical evidence of hypometabolism. In some individuals, decreased T4 to T3 conversion in the pituitary gland [75] or in peripheral tissues [76] is thought to be an inherited condition.

A variety of drugs may also produce changes in the serum TT3 concentration without apparent metabolic consequences (see Chapter 6). Drugs that compete with hormone binding to serum proteins decrease serum TT3 levels, generally without affecting the free T3 concentration (Table 5-5). Some drugs, such as glucocorticoids, [77] depress the serum TT3 concentration by interfering with the peripheral conversion of T4 to T3. Others, such as phenobarbital, [78] depress the serum TT3 concentration by stimulating the rate of intracellular hormone degradation. The majority have multiple effects. These effects are combinations of those described above, as well as inhibition of the hypothalamic-pituitary axis or thyroidal hormonogenesis. [79]

Changes in serum TBG concentration have an effect on the serum TT3 concentration similar to that on TT4 (see Chapter 16). The presence of endogenous antibodies to T3 may result in apparent elevation of the serum TT3 but as in the case of high TBG, it does not cause hypermetabolism. [38]

Administration of commonly used replacement doses of T3, usually in the order of 75 Âµg/day or 1 Âµg/kg body weight per day, [80] results in serum TT3 levels in the thyrotoxic range. Furthermore, because of the rapid gastrointestinal absorption and relatively fast degradation rate, the serum level varies considerably according to the time of sampling in relation to hormone ingestion. [64]

Measurement of Total and Unsaturated Thyroid Hormone-Binding Capacity in Serum

Because the concentration of thyroid hormone in serum is dependent on its supply as well as on the abundance of hormone-binding sites on serum proteins, the estimation of the latter has proved useful in the correct interpretation of values obtained from the measurement of the total hormone concentration. These results have been used to provide an estimate of the free hormone concentration, which is important in differentiating changes in serum total hormone concentration due to alterations of binding proteins in euthyroid patients from those due to abnormalities in thyroid gland activity giving rise to hypermetabolism or hypometabolism.

In Vitro Uptake Tests: In vitro uptake tests measure the unoccupied thyroid hormone-binding sites on TBG. They use labeled T3 or T4 and some form of synthetic absorbent to measure the proportion of radiolabeled hormone that is not tightly bound to serum proteins. Because ion exchange resins are often used as absorbents, the test became known as the resin T3 or T4 uptake test (T3U or T4U), describing the technique rather than the entity measured.

The test is usually carried out by incubating a sample of the patient’s serum with a trace amount of labeled T3 or T4. The labeled hormone, not bound to available binding sites on TBG present in the serum sample, is absorbed onto an anion exchange resin and measured as resin-bound radioactivity.

Values correlate inversely with the concentration of unsaturated TBG. Various methods use different absorbing materials to remove the hormone not tightly bound to TBG. [83] Labeled T3 is usually used because of its less firm yet preferential binding to TBG. Depending upon the method, typical normal results for T3U are 25-35% or 45-55%. Thus, it is more valuable to express results of the uptake tests as a ratio of the result obtained in a normal control serum run in the same assay as the test samples. Normal values will then range on either side of 1.0, usually 0.85-1.15.

The uptake of the tracer by the absorbent is inversely proportional to the amount of unsaturated binding sites (unoccupied by endogenous thyroid hormone) in serum TBG. Thus, the uptake is increased when the amount of unsaturated TBG is reduced as a result of excess endogenous thyroid hormone or a decrease in the concentration of TBG. In contrast, the uptake is decreased when the amount of unsaturated TBG is increased as a result of a low serum thyroid hormone concentration or an increase in the concentration of TBG. Since the test can be affected by either or both independent variables, serum total thyroid hormone and TBG concentrations, the results cannot be interpreted without knowledge of the hormone concentration. As a rule, parallel increases or decreases in both serum TT4 concentration and the T3U test indicate hyperthyroidism and hypothyroidism, respectively, whereas discrepant changes in serum TT4 and T3U suggest abnormalities in TBG binding. However, abnormalities in hormone and TBG concentrations may coexist in the same patient. For example, a hypothyroid patient with a low TBG level will typically show a low TT4 level and normal T3U result (Figure 6-4). Several nonhormonal compounds, due to structural similarities, compete with thyroid hormone for its binding site on TBG. Some are used as pharmacologic agents and may thus alter the in vitro uptake test as well as the total thyroid hormone concentration in serum. A list is provided in Table 5-2 .

Figure 6-4. Graphic representation of the relationship between the serum total T4 concentration, the RT3U test, and the free T4 (FT4) concentration in various metabolic states and in association with changes in TBG. The principle of communicating vessels is used as an illustration. The height of fluid in the small vessel represents the level of FT4; the total amount of fluid in the large vessel, the total T4 concentration; and the total volume of the large vessel, the TBG capacity. Dots represent resin beads and black dots, those carrying the radioactive T3 tracer (T3*). The RT3U test result (black dots) is inversely proportional to the unoccupied TBG binding sites represented by the unfilled capacity of the large vessel. (From S. Refetoff, Endocrinology, L.J. DeGroot (ed). 1979, Grune & Straton Inc.)

TBG and TTR Measurements.

The concentrations of TBG and TTR in serum can be either estimated by measurement of their total T4-binding capacity at saturation or more usually measured directly by immunologic techniques. [87,88]

TBG concentration in serum can be determined by RIA, [88] and both TBG and TTR can be measured by Laurell's rocket immunoelectrophoresis, [89,90] by radial immunodiffusion, [91] or by enzyme immunoassay; [87] commercial methods are available. The true mean value for TBG is 1.6 mg/dl (260 nmol/L), with a range of 1.1 – 2.2 mg/dl(180 – 350 nmol/L) serum. In adults, the normal range for TTR is 16 – 30 mg/dl (2.7 – 5.0 Âµmol/L). The concentrations of TBG and TTR in serum vary with age, sex, pregnancy, and posture. Determination of the concentration of these proteins in serum is

particularly helpful in evaluation of extreme deviations from normal, as in congenital abnormalities of TBG. In most instances, however, the in vitro uptake test, in conjunction with the serum TT4 level, gives an approximate estimation of the TBG concentration.

Estimation of Free Thyroid Hormone Concentration

A minute amount of thyroid hormone circulates in the blood in a free form, not bound to serum proteins. It is in reversible equilibrium with the bound hormone and represents the diffusible fraction of the hormone capable of traversing cellular membranes to exert its effects on body tissues. [94] Although changes in serum hormone-binding proteins affect both the total hormone concentration and the corresponding fraction circulating free, in the euthyroid person the absolute concentration of free hormone remains constant and correlates with the tissue hormone level and its biologic effect. Information concerning this value is probably the most important parameter in the evaluation of thyroid function as it relates to the metabolic status of the patient.

With few exceptions, the free hormone concentration is high in thyrotoxicosis, low in hypothyroidism, and normal in euthyroidism even in the presence of profound changes in TBG concentration, [97] provided the patient is in a steady state (see Fig. 5-4). Notably, free T4 (FT4) concentration may be normal or even low in patients with T3 thyrotoxicosis and in those ingesting pharmacologic doses of T3. On occasion, the concentration of FT4 may be outside the normal range in the absence of an apparent abnormality in the thyroid hormone-dependent metabolic status. This is frequently observed in severe nonthyroidal illness during which both high and low values have been reported. [98-100] As expected, when a euthyroid state is maintained by the administration of T3 or by predominant thyroidal secretion of T3, the FT4 level is also depressed. More consistently, patients with a variety of nonthyroidal illnesses have low FT3 levels. [101] This decrease is characteristic of all conditions associated with depressed serum TT3 concentrations due to a diminished conversion of T4 to T3 in peripheral tissues (see Chapter 5). Both FT4 and FT3 values may be out of line in patients receiving a variety of drugs (see below). Marked elevations in both FT4 and FT3 concentrations in the absence of hypermetabolism are typical of patients with resistance to thyroid hormone (see Chapter 16). The FT3 concentration is usually normal or even high in hypothyroid persons living in areas of severe endemic iodine deficiency. Their FT4 levels are, however, normal or low. [62]

Direct Measurement of Free T4 and Free T3. Direct measurements of the absolute FT4 and FT3 concentrations are technically difficult and have, until recently, been limited to research assays. In order to minimize perturbations of the relationship between the free and bound hormone, these must be separated by ultrafiltration or by dialysis involving minimal dilution and little alteration of the pH or electrolyte composition. The separated free hormone is then measured directly by radioimmunoassay or chromatography. [97,97a] These assays are probably the most accurate available, but small, weakly bound, dialyzable substances or drugs may be removed from the binding proteins and the free hormone concentration measured in their presence may not fully reflect the free concentration in vivo.

Isotopic Equilibrium Dialysis. This method has been the “gold standard” for the estimation of the FT4 or FT3 concentration for almost 30 years. It is based on the determination of proportion of T4 or T3 that is unbound, or free, and is thus able to diffuse through a dialysis membrane, i.e., the dialyzable fraction (DF). To carry out the test, a sample of serum is incubated with a tracer amount of labeled T4 or T3. The labeled tracer rapidly equilibrates with the respective bound and free endogenous hormones. The sample is then dialyzed against buffer at a constant temperature until the concentration of free

hormone on either side of the dialysis membrane has reached equilibrium. The DF is calculated from the proportion of labeled hormone in the dialysate. The contribution from radioiodide present as contaminant in the labeled tracer hormone should be eliminated by purification [98] and by various techniques of precipitation of the dialyzed hormone.102 FT4 and FT3 levels can be measured simultaneously by addition to the sample of T4 and T3 labeled with two different radioiodine isotopes.103 Ultrafiltration is a modification of the dialysis technique. [98] Results are expressed as the fraction (DFT4 or DFT3) or percent (%FT4 or %FT3) of the respective hormones which dialyzed and the absolute concentrations of FT4 and FT3 are calculated from the product of the total concentration of the hormone in serum and its respective DF. Typical normal values for FT4 in the adult range from 1.0 to 3.0 ng/dl (13 – 39 pmol/L) and for FT3 from 0.25 to 0.65 ng/dl (3.8 – 10 nmol/L).

Results by these techniques are generally comparable to those determined with the direct, one step, methods (see below) but are more likely to differ with extremely low or extremely high TBG concentrations or in the presence of circulating inhibitors of protein binding, especially in situations of non-thyroidal illness. [104, 104a,104b] The measured DF may be altered by the temperature at which the assay is run, the degree of dilution, the time allowed for equilibrium to be reached and the composition of the diluting fluid. [105] The calculated value is dependent on an accurate measurement of total T4 or T3 and may be incorrect in patients with T4 or T3 autoantibodies. Some of these problems, particularly those arising from dilution, may be superceded by commercially available dialysis methods or ultrafiltration methods of free from bound hormone which do not necessitate serum dilution.

Index Methods. As the determination of free hormone by equilibrium dialysis is cumbersome and technically demanding, many clinical laboratories have used a method by which a free T4 index (FT4I) or free T3 index (FT3I) is derived from the product of the TT4 or TT3 (determined by immunoassay) and the value of an in vitro uptake test (see below). While not always in agreement with the values obtained by dialysis, these techniques are rapid and simple. They are more likely to fail at extremely low or extremely high TBG concentrations, in the presence of abnormal binding proteins, in the presence of circulating inhibitors of protein binding , and their reliability has been questioned in patients with non-thyroidal illness.

The theoretical contention that the FT4I is an accurate estimate of the absolute FT4 concentration can be confirmed by the linear correlation between these two parameters. This is true provided results of the in vitro uptake test (T3U or T4U) are expressed as the thyroid hormone binding ratio (THBR), determined by dividing the tracer counts bound to the solid matrix by counts bound to serum proteins. [106] Values are corrected for assay variations using appropriate serum standards and are expressed as the ratio of a normal reference pool. [106,107] The normal range is slightly narrower than the corresponding TT4 in healthy euthyroid patients with a normal TBG concentration. It is 6.0 – 10.5 Âµg/dl or 77 – 135 nmol/l when calculated from TT4 values measured by RIA. In thyrotoxicosis, FT4I is high and in hypothyroidism it is low irrespective of the TBG concentration. Euthyroid patients with TT4 values outside the normal range as a result of TBG abnormalities have a normal FT4I. [83] Lack of correlation between the FT4I and the metabolic status of the patient has been observed under the same circumstances as those described for similar discrepancies when the FT4 concentration was measured by dialysis.

Methods for the estimation of the FT3I are also available [103] but are rarely used in routine clinical evaluation of thyroid function. Like the FT4I, it correlates well with the absolute FT3 concentration. The test corrects for changes in TT3 concentration resulting from variations in TBG concentration.

Estimation of FT4 and FT3 Based on TBG Measurements. Since most T4 and T3 in serum are bound to TBG, their free concentration can be calculated from their binding affinity constants to TBG and molar

concentrations of hormones and TBG. [109,110] A simpler calculation of the T4/TBG and T3/TBG ratios yields values that are similar to but less accurate than the FT4I and FT3I, respectively. [106]

Two-step Immunoassays. In these assays, the free hormone is first immunoextracted by a specific bound antibody (first step), frequently fixed to the tube (coated tube). [111,112] After washing, labeled tracer is added and allowed to equilibrate between the unoccupied sites on the antibody and those of serum thyroid hormone-binding proteins. The free hormone concentration will be inversely related to the antibody bound tracer and values are determined by comparison to a standard curve. Values obtained with this technique are generally comparable to those determined with the direct methods. They are more likely to differ in the presence of circulating inhibitors of protein binding and in sera from patients with non-thyroidal illness.

Analog (One-Step) Immunoassays. In these assays, a labeled analog of T4 or T3 directly competes with the endogenous free hormone for binding to antibodies. [113] In theory, these analogs are not bound by the thyroid hormone binding proteins in serum. However, various studies have found significant protein binding to the variant albumin-like protein, [113a] to transthyretin and to iodothyronine autoantibodies. [114] This results in discrepant values to other assays in a number of conditions including non-thyroidal illness, pregnancy and in individuals with familial dysalbuminemic hyperthyroxinemia (FDH). [113a] A growing number of commercial kits is available some of which have been modified to minimize these problems, [113b] . Nonetheless, their accuracy remains controversial, although such comercial methods are being increasingly adopted in the routine clinical chemistry laboratory. [112]

Considerations in Selection of Methods for the Estimation of Free Thyroid Hormone Concentration. None of the available methods for the estimation of the free hormone concentration in serum is infallible in the evaluation of the thyroid hormone-dependent metabolic status. Each test possesses inherent advantages and disadvantages depending upon specific physiologic and pathologic circumstances. For example, methods based on the measurement of the total thyroid hormone and TBG concentrations cannot be used in patients with absent TBG due to inherited TBG deficiency. Under such circumstances, the concentration of free thyroid hormone is dependent upon the interaction of the hormone with serum proteins that normally play a negligible role (TTR and albumin). When alterations of thyroid hormone binding do not equally affect T4 and T3, discrepant results of FT4I are obtained when using labeled T4 or T3 in the in vitro uptake test. For example, euthyroid patients with the inherited albumin variant (FDH) or having endogenous antibodies with greater affinity for T4 will have high TT4 but a normal T3U test which will result in an overestimation of the calculated FT4I. In such instances, calculation of the FT4I from a T4U test may provide more accurate results. Conversely, reduced overall binding affinity for T4 which affects T3 to a lesser extent will underestimate the FT4I derived from a T3U test. Similarly, use of the T4U and T3U for estimation of the free hormone concentration, is satisfactory in the presence of alterations in TBG concentration but not alterations of the affinity of TBG for the hormone. [116,117]

Methods based on equilibrium dialysis are most appropriate in the estimation of the free thyroid hormone level in patients with all varieties of abnormal binding to serum proteins provided the true concentration of total hormone has been accurately determined. All methods for the estimation of the FT4 concentration may give either high or low values in patients with severe nonthyroidal illness. [96-100] , [119] , [120] This has been attributed to the presence of inhibitors of thyroid hormone binding to serum proteins as well as to the various adsorbents used in the test procedures. [121,122] Some of these inhibitors have been postulated to leak from the tissues of the diseased patient. [123,124] Such discrepancies are even more pronounced during transient states of hyperthyroxinemia or hypothyroxinemia associated with acute illness, after withdrawal of treatment with thyroid hormone and in acute changes in TBG

concentration (see Chapters 5 and 16).

The contribution of various drugs that interfere with binding of thyroid hormone to serum proteins or with the in vitro tests should also be taken into account in the choice and interpretation of tests (see Table 5-2). Although the free thyroid hormone concentration in serum seems to determine the amount of hormone available to body tissues, factors that govern their uptake, transport to the nucleus and functional interactions with nuclear receptors ultimately determine their biological effects.

Measurements of Iodine-Containing Hormone Precursors and Products of Degradation

The last two decades have witnessed the development of RIAs for the measurement of a number of naturally occurring, iodine-containing substances that possess little if any thyromimetic activity. Some of these substances are products of T4 and T3 degradation in peripheral tissues. Others are predominantly, if not exclusively, of thyroidal origin. Since they are devoid of significant metabolic activity, measurement of their concentration is of value only in the research setting in detecting abnormalities in the metabolism of thyroid hormone in peripheral tissues, as well as defects of hormone synthesis and secretion.

3,3′,5′-Triiodothyronine or Reverse T3 (rT3). rT3 is principally a product of T4 degradation in peripheral tissues (see Chapter 3). It is also secreted by the thyroid gland, but the amounts are practically insignificant. [126] Thus, measurement of rT3 concentration in serum reflects both tissue supply and metabolism of T4 and identifies conditions that favor this particular pathway of T4 degradation.

When total rT3 (TrT3) is measured in unextracted serum, a competitor of rT3 binding to serum proteins must be added. [127] Several chemically related compounds may cross-react with the antibodies. The strongest cross-reactivity is observed with 3,3′-T2 but this does not present a serious methodologic problem because of its relatively low levels in human serum. Though cross-reactivity with T3 and T4 is lesser, these compounds are more often the cause of rT3 overestimation due to their relative abundance, particularly in thyrotoxicosis. [128] Free fatty acids interfere with the measurement of rT3 by RIA. [129]

The normal range in adult serum for TrT3 is 14-30 ng/dl (0.22 – 0.46 nmol/L) although varying values have been reported. It is elevated in subjects with high TBG and in some individuals with FDH. [132] Serum TrT3 levels are normal in hypothyroid patients treated with T4, indicating that peripheral T4 metabolism is an important source of circulating rT3. [126, 133] Values are high in thyrotoxicosis and low in untreated hypothyroidism. High values are normally found in cord blood and in newborns. [133,134]

With only a few exceptions, notably uremia, serum TrT3 concentrations are elevated in all circumstances that cause low serum T3 levels in the absence of obvious clinical signs of hypothyroidism. These conditions include, in addition to the newborn period, a variety of acute and chronic nonthyroidal illnesses, calorie deprivation, and the influence of a growing list of clinical agents and drugs (see Table 5-3).

Current clinical application of TrT3 measurement in serum is in the differential diagnosis of conditions associated with alterations in serum T3 and T4 concentrations when thyroid gland and metabolic abnormalities are not readily apparent.

The dialyzable fraction of rT3 in normal adult serum is 0.2 – 0.32%, or approximately the same as that of T3. The corresponding serum FrT3 concentration is 50 – 100 pg/dl (0.77 – 1.5 pmol/L). In the

absence of gross TBG abnormalities, variations in serum FrT3 concentration closely follow those of TrT3. [101]

3,5-Diiodothyronine (3,5-T2). The normal adult range for total 3,5-T2 in serum measured by direct RIAs is 0.20 – 0.75 ng/dl (3.8 – 14 pmol/L). [135] That 3,5-T2 is derived from T3 is supported by the observations that conditions associated with high and low serum T3 levels have elevated and reduced serum concentrations of 3,5-T2, respectively. [136] Thus, high serum 3,5-T2 levels have been reported in hyperthyroidism, and low levels in serum of hypothyroid patients, newborns, during fasting, and in patients with liver cirrhosis.

3,3′-Diiodothyronine (3,3′-T2). Normal concentrations in adults probably range from 1 to 8 ng/dl (19 – 150 pmol/L). [137] Levels are clearly elevated in hyperthyroidism and in the newborn. Values have been found to be either normal or depressed in nonthyroidal illnesses, [137] in agreement with the demonstration of reduced monodeiodination of rT3 to 3,3′-T2. [138] In vivo turnover kinetic studies and measurement of 3,3′-T2 in serum after the administration of T3 and rT3 have clearly shown that 3,3′-T2 is the principal metabolic product of these two triiodothyronines.

3′,5′-Diiodothyronine (3′,5′-T2). Reported concentrations in serum of normal adults have a mean overall range of 1.5 – 9.0 ng/dl (30 – 170 pmol/L). [139,140] The substances that principally cross react in the assay are rT3, 3,3-LT2 and 3-T1. Values are high in hyperthyroidism and in the newborn. [139,140] Being the derivative of rT3 monodeiodination, [139] 3′,5′-T2 levels are elevated in serum during fasting [140,141] and in chronic illnesses [133] in which the level of the rT3 precursor is also high. Administration of dexamethasone also produces an increase in the serum 3′,5′-T2 level. [139]

3′-Monoiodothyronine (3′-T1). The concentration of 3′-T1 in serum of normal adults, measured by RIA, has been reported to range from 0.6 to 2.3 ng/dl (15 – 58 pmol/L) [133] and from <0.9 to 6.8 ng/dl (<20 – 170 pmol/L). Its two immediate precursors, 3,3,'-T2 and 3′,5′-T2 are the main cross-reactants in the RIA. Serum levels are very high in hyperthyroidism and low in hypothyroidism. The concentration of 3′-T1 in serum is elevated in all conditions associated with high rT3 levels, including newborns, nonthyroidal illness, and fasting. [134] This finding is not surprising since the immediate precursor at 3′-T1 is 3′,5′-T2, [142] a product of rT3 deiodination, which is also present in serum in high concentration under the same circumstances. The elevated serum levels of 3′-T1 in renal failure are attributed to decreased clearance since the concentrations of its precursors are not increased.

3-Monoiodothyronine (3-T1). Experience with the measurement of 3-T1 in serum is limited. Normal values in serum of adult humans using 3H labeled 3-T1 in a specific RIA ranged from <0.5 – 7.5 ng/dl (<13 – 190 pmol/L). [143] The mean concentration of 3-T1 in serum of thyrotoxic patients and in cord blood was significantly higher. 3-T1 appears to be a product of in vivo deiodination of 3,3′-T2.

Tetraiodothyroacetic Acid (TETRAC or T4A) and Triiodothyroacetic Acid (TRIAC or T3A). The iodoamino acids T4A and T3A, products of deamination and oxidative decarboxylation of T4 and T3, respectively, have been detected in serum by direct RIA measurements. [21, 76, 144] Reported mean concentrations in the serum of healthy adults have been 8.7 ng/dl [144] and 2.6 ng/dl (range, 1.6 – 3.0 ng/dl or 26 – 48 pmol/L)) [21] for T3A and 28 ng/dl (range <8 – 60 mg/dl or <105 – 800 pmol/L) [76] for T4A. Serum T4A levels are reduced during fasting and in patients with severe illness, [145] although the percentage of conversion of T4 to T4A is increased. [20, 146] The concentration of serum T3A remains unchanged during the administration of replacement doses of T4 and T3. [21] It has been suggested that intracellular rerouting of T3 to T3A during fasting is responsible for the maintenance of normal serum

TSH levels in the presence of low T3 concentrations. [147]

3,5,3′-T3 Sulfate (T3S). A RIA procedure to measure T3S in ethanol extracted serum samples is available. [22] Concentrations in normal adults range from 4-10 ng/dl (50-125 pmol/L). Although the principal source of T3S is T3, and the former binds to TBG, values are high in newborns and low in pregnancy. This suggests different rates of T3S generation or metabolism in mother and fetus. T3S values are high in thyrotoxicosis and in nonthyroidal illness.

Diiodotyrosine (DIT) and Monoiodotyrosine (MIT). Although RIA methods for the measurement of DIT and MIT have been developed, due to limited experience, their value in clinical practice remains unknown. Early reports gave a normal mean value for DIT in serum of normal adults of 156 ng/dl (3.6 nmol/L), [148] with progressive decline due to refinement of techniques to values as low as 7 ng/dl with a range of 1 – 23 ng/dl (0.02 – 0.5 nmol/L). [149] Thus, the normal range for MIT of 90 – 390 ng/dl (2.9 – 12.7 nmol/L) [150] is undoubtedly an overestimation. Iodotyrosine that has escaped enzymatic deiodination in the thyroid gland appears to be the principal source of DIT in serum. Iodothyronine degradation in peripheral tissues is probably a minor source of iodotyrosines since administration of large doses of T4 to normal subjects produces a decline rather than an increase in the serum DIT level. [149] DIT is metabolized to MIT in peripheral tissues. Serum levels of DIT are low during pregnancy and high in cord blood.

Thyroglobulin (Tg). RIA methods were those first used routinely for measurement of Tg in serum, [151] , although other assays methods employing IRMA, ICMA, and ELISA technology have been reported [151a-d] and are gaining increasing popularity. They are specific and, depending upon the sensitivity of the assay, capable of detecting Tg in the serum of approximately 90% of the euthyroid healthy adults. When antisera are used in high dilutions, there is virtually no cross-reactivity with iodothyronines or iodotyrosines. Results obtained from the analysis of sera containing Tg autoantibodies may be inaccurate, depending upon the antiserum employed. [152] The presence of thyroid peroxidase antibodies does not interfere with the Tg RIA. Despite the relaibility of measurements of serum Tg, it is clear that different assay methods may result in values discrepant by up to 30%, even though refernce preparations are available. [152a] Typically, IMA methods underestimate the serum Tg value, while RIA methods overestimate it, so it is essential that clinical decisions are based upon serial measurements using the same assay.

Tg concentrations in serum of normal adults range from <1 to 25 ng/ml (<1.5 – 38 pmol/L), with mean levels of 5 – 10 ng/ml. [151] , [153-155] On a molar basis, these concentrations of Tg are minute relative to the circulating iodothyronines; 5,000-fold lower than the corresponding concentration of T4 in serum. Values tend to be slightly higher in women than in men. [151] In the neonatal period and during the third trimester of pregnancy, mean values are approximately 4- and 2-fold higher. [154,156] They gradually decline throughout infancy, childhood and adolescence. [157] The positive correlation between the levels of serum Tg and TSH indicates that pituitary TSH regulates the secretion of Tg.

Elevated serum Tg levels reflect increased secretory activity by stimulation of the thyroid gland or damage to thyroid tissue, whereas values below or at the level of detectability indicate a paucity of thyroid tissue or suppressed activity. Tg levels in a variety of conditions affecting the thyroid gland have been reviewed [158] and are listed in Table 6-6.

Table 6-6 Conditions Associated with Changes in Serum Tg Concentration Listed According to the Presumed Mechanism

IncreasedTSH mediated	Acute and transient (TSH and TRH administration, neonatal period)

Chronic stimulation Iodine deficiency, endemic goiter, goitrogens Reduce thyroidal reserve (lingual thyroid) TSH-producing pituitary adenoma Generalized resistance to thyroid hormone TBG deficiency

Non-TSH mediated Thyroid stimulators IgG (Graves' disease) hCG (trophoblastic disease) Trauma to the thyroid (needle aspiration and surgery of the thyroid gland, 131I therapy) Destructive thyroid pathology Subacute thyroiditis "Painless thyroiditis" Postpartum thyroiditis Abnormal release Thyroid nodules (toxic, nontoxic, multinodular goiter) Differentiated nonmedullary thyroid carcinoma Ab normal clearance (renal failure)

DecreasedTSH suppression Administration of thyroid hormone

Decreased synthesis Athyreosis (postoperative, congenital) Tg synthesis defect

Interpretation of a serum Tg value should take into account the fact that Tg concentrations may be high under normal physiologic conditions or altered by drugs. Administration of iodine and antithyroid drugs increase the serum Tg level, as do states associated with hyperstimulation of the thyroid gland by TSH or other substances with thyroid-stimulating activity. This is due to increased thyroidal release of Tg rather than changes in its clearance. [159] Administration of TRH and TSH also transiently increases the serum level of Tg. [160] Trauma to the thyroid gland, such as that occurring during diagnostic and therapeutic procedures including percutaneous needle biopsy, surgery, or 131I therapy, can produce a striking, although short-lived, elevation in the Tg level in serum. [154, 161,162] Pathological processes with destructive effect on the thyroid gland also produce transient, though more prolonged increases. [163] Tg is undetectable in serum after total ablation of the thyroid gland as well as in normal persons receiving suppressive doses of thyroid hormone. [158] It is thus a useful test in the differential diagnosis of thyrotoxicosis factitia, [164] especially when transient thyrotoxicosis with a low RAIU or suppression of thyroidal RAIU by iodine are alternative possibilities.

The most striking elevations in serum Tg concentrations have been observed in patients with metastatic differentiated nonmedullary thyroid carcinoma even after total surgical and radioiodide ablation of all normal thyroid tissue. [154, 165] It usually persists despite full thyroid hormone suppressive therapy, suggesting excessive autonomous release of Tg by the neoplastic cells. The determination is thus of particular value in the follow-up and management of metastatic thyroid carcinomas, particularly when they fail to concentrate radioiodide. [153, 165] Follow-up of such patients with sequential serum Tg determinations helps the early detection of tumor recurrence or growth and the assessment of the efficacy of treatment. Measurement of serum Tg is also useful in patients with metastases, particularly to bone, in whom there is no evidence of a primary site and thyroid malignancy is being considered in the differential diagnosis. [154, 165] On the other hand, serum Tg levels are of no value in the differential diagnosis of primary thyroid cancer because levels may be within the normal range in the presence of differentiated thyroid cancer and high in a variety of benign thyroid diseases. [153,155, 165] Whether early detection of recurrent thyroid cancer after initial ablative therapy could be achieved by serum Tg measurement without cessation of hormone replacement therapy is debated because Tg secretion by the tumor is modulated by TSH and is suppressed by the administration of thyroid hormone. [166-168] Detectable serum thyroglobulin during thyroid hormone suppression reliably indicated the presence residual or recurrent disease.

Tg levels are high in the early phase of subacute thyroiditis. [163] Declining serum Tg levels during the

course of antithyroid drug treatment of patients with Graves' disease may indicate the onset of a remission. [162] , [169] Tg may be undetectable in the serum of neonates with dyshormonogenetic goiters due to defects in Tg synthesis [170] but are very high in some hypothyroid infants with thyromegaly or ectopy. [171] Measurement of serum Tg in hypothyroid neonates is useful in the differentiation of infants with complete thyroid agenesis from those with hypothyroidism due to other causes, and thus in most cases obviates the need for the diagnostic administration of radioiodide. [171] , [172]

Measurement of Thyroid Hormone and Its Metabolites in Other Body Fluids and in Tissues

Clinical experience with measurement of thyroid hormone and its metabolites in body fluids other than serum and in tissues is limited for several reasons. Analyses carried out in urine and saliva do not appear to give additional information, not obtained from measurements carried out in serum. Amniotic fluid, cerebrospinal fluid, and tissues are less readily accessible for sampling. Their likely application in the future will depend on information they could provide beyond that obtained from similar analyses in serum.

Urine

Because thyroid hormone is filtered in the urine predominantly in free form, measurement of the total amount excreted over 24 hours offers an indirect method for the estimation of the free hormone concentration in serum. The 24-hour excretion of T4 in normal adults ranges from 4 to 13 Âµg and from 1.8 to 3.7 Âµg, depending upon whether total or only conjugated T4 is measured. Corresponding normal ranges for T3 are 2.0 – 4.0 Âµg and 0.4 – 1.9 Âµg. [173-175] Striking seasonal variations have been shown for the urinary excretion of both hormones, with a nadir during the hot summer months, in the absence of significant changes in serum TT4 and TT3. As expected, values are normal in pregnancy and in nonthyroidal illnesses, and are high in thyrotoxicosis and low in hypothyroidism. [174] , [175] The test may not be valid in the presence of gross proteinuria and impairment of renal function. [176]

Amniotic Fluid (AF)

All iodothyronines measured in blood have also been detected in AF. With the exception of T3, 3,3′-T2 and 3′-T2, the concentration at term is lower than that in cord serum. [139,140] , [142] , [177-179] This fact cannot be fully explained by the low TBG concentration in AF. Although the source of iodothyronines in AF is unknown, the general pattern more closely resembles that found in the fetal than in the maternal circulation.

The TT4 concentration in AF average 0.5 Âµg/dl (65 nmol/L) with a range of 0.15 – 1.0 Âµg/dl and is thus very low when compared to values in maternal and cord serum. [177-179] The FT4 concentration is, however, twice as high in AF relative to serum. The TT3 concentration is also low relative to maternal serum being on the average 30 ng/dl (0.46 nmol/L) in both AF and cord serum. [179] rT3, on the other hand, is very high in AF, on average 330 ng/dl (5.1 nmol/L) during the first half of gestation, declining precipitously at about the 30th week of gestation to an average of 85 ng/dl (1.3 nmol/L) which is also found at term. [178,179]

Cerebrospinal Fluid (CSF)

T4, T3, and rT3 concentrations have been measured in human CSF. [180-182] The concentrations of both TT4 and TT3 are approximately 50-fold lower than those found in serum. However, the concentrations of these iodothyronines in free form are similar to those in serum. In contrast, the level of TrT3 in CSF is only 2.5-fold lower than that of serum, whereas that of FrT3 is 25-fold higher. This is probably due to the presence in CSF of a larger proportion of TTR which has high affinity for rT3. [181] All the thyroid hormone-binding proteins present in serum are also found in CSF, although in lower concentrations. [181] The concentrations of TT4 and FT4 are increased in thyrotoxicosis and depressed in hypothyroidism. Severe nonthyroidal illness gives rise to increased TrT3 and FrT3 levels. [182]

Milk

TT4 concentration in human milk is of the order of 0.03 – 0.5 Âµg/dl. [183] Analytical artifacts were responsible for the much higher values formerly reported. [183,184] TT3 concentrations range from 10 to 200 ng/dl (015 – 3.1 nmol/L). [184,185] The concentration of TrT3 ranges from 1 – 30 ng/dl (15 – 460 pmol/L). [184] Thus, it is unlikely that milk would provide a sufficient quantity of thyroid hormone to alleviate hypothyroidism in the infant.

Saliva

It has been suggested that only the free fraction of small nonpeptide hormones which circulate predominantly bound to serum proteins would be transferred to saliva and that their measurement, in this easily accessible body fluid, would provide a simple and direct means to determine their free concentration in blood. This hypothesis was confirmed for steroid hormones, [186] not tightly bound to serum proteins. Levels of T4 in saliva range from 4.2 – 35 ng/dl (54 – 450 pmol/L) and do not correlate with the concentration of free T4 in serum. [187] This finding is, in part, due to the transfer of T4 bound to the small but variable amounts of serum proteins that reach the saliva.

Effusions

TT4 measured in fluid obtained from serous cavities bears a direct relationship to the protein content and the serum concentration of T4. Limited experience with Tg measurement in pleural effusions from patients with thyroid cancer metastatic to lungs suggests that it may be of diagnostic value. [165]

Tissues

Since the response to thyroid hormone is expressed at the cell level, it is logical to assume that hormone concentration in tissues should correlate best with its action. Methods for extraction, recovery, and measurement of iodothyronines from tissues have been developed but, for obvious reasons, data from thyroid hormone measurements in human tissues are limited. Preliminary work has shown that under several circumstances, hormonal levels in tissues such as liver, kidney, and muscle usually correlate with those found in serum. [188]

Measurements of T3 in cells most accessible for sampling in humans, namely, red blood cells gave values of 20 – 45 ng/dl (0.31 – 0.69 nmol/L) or one-fourth those found in serum. [189] They are higher in thyrotoxicosis and lower in hypothyroidism.

The concentrations of all iodothyronines have been measured in thyroid gland hydrolysates. [18] , [133] , [139] In normal glands, the molar ratios relative to the concentration of T4 are on average as follows: T4/T3 = 10; T4/rT3 = 80; T4/3,5′-T2 = 1,400; T4/3,3′-T2 = 350; T4/3′,5′-T2 = 1,100; and T4/3′-T1 = 4,400. Information concerning the content of iodothyronines in hydrolysates of abnormal thyroid tissue is limited, and the diagnostic value of such measurements has not been established.

Measurement of Tg in metastatic tissue obtained by needle biopsy may be of value in the differential diagnosis, especially when the primary site is unknown and the histological diagnosis is not conclusive.

Tests Assessing the Effects of Thyroid Hormone on Body Tissues

Thyroid hormone regulates a variety of biochemical reactions in virtually all tissues. Thus, ideally, the adequacy of hormonal supply should be assessed by the tissue responses rather than by parameters of thyroid gland activity or serum hormone concentration which are several steps removed from the site of thyroid hormone action. Unfortunately, the tissue responses (metabolic indices) are nonspecific because they are altered by a variety of physiologic and pathologic mechanisms unrelated to thyroid hormone deprivation or excess. The following review of biochemical and physiologic changes mediated by thyroid hormone has a dual purpose: (1) to outline some of the changes that may be used as clinical tests in the evaluation of the metabolic status, and (2) to point out the changes in various determinations commonly used in the diagnosis of a variety of nonthyroidal illnesses, which may be affected by the concomitant presence of thyroid hormone deficiency or excess.

Basal Metabolic Rate (BMR)

The BMR has a long history in the evaluation of thyroid function. It measures the oxygen consumption under basal conditions of overnight fast and rest from mental and physical exertion. Since standard equipment for the measurement of BMR may not be readily available, it can be estimated from the oxygen consumed over a timed interval by analysis of samples of expired air. [190] The test indirectly measures metabolic energy expenditure or heat production.

Results are expressed as the percentage of deviation from normal after appropriate corrections have been made for age, sex, and body surface area. Low values are suggestive of hypothyroidism, and high values reflect thyrotoxicosis. The various nonthyroidal illnesses and other factors affecting the BMR, including sources of errors, have been reviewed. [191] Although this test is no longer a part of the routine diagnostic armamentarium, it is still useful in research.

Deep Tendon Reflex Relaxation Time (Photomotogram)

Delay in the relaxation time of the deep tendon reflexes, visible to the experienced eye, occurs in

hypothyroidism. Several instruments have been devised to quantitate various phases of the Achilles tendon reflex. Although normal values vary according to the phase of the tendon reflex measured, the apparatus used and individual laboratory standards, the approximate adult normal range for the half-relaxation time is 230-390 msec. Diurnal variation, differences with sex, and changes with age, cold exposure, fever, exercise, obesity, and pregnancy have been reported. However, the main reason for the failure of this test as a diagnostic measure of thyroid dysfunction is the large overlap with values obtained in euthyroid patients and alterations caused by nonthyroidal illnesses. [192]

Tests Related to Cardiovascular Function

Thyroid hormone induced changes in the cardiovascular system can be measured by noninvasive techniques. One such test measures the time interval between the onset of the electrocardiographic QRS complex (Q) and the arrival of the pulse wave at the brachial artery, detected by the Korotkoff sound (K) at the antecubital fossa. [193] Related tests which determine the systolic time interval (STI) measure the preejection period (PEP), obtained by subtraction of the left ventricular ejection time (LVET) from the total electromechanical systole (Q-A2). [194] The left ventricular ejection time (LVET) which is also affected by the thyroid status can be measured by the M mode echocardiogram [195] (Figure 6-5). The PEP/LVET ratio is also useful in the assessment of thyroid hormone action in the cardiovascular system. [196] As with other tests of thyroid hormone action, the principal deficiency of these measurements is their alteration in a variety of nonthyroidal illnesses.

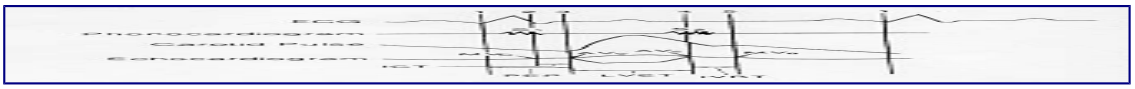

Figure 6-5: Simultaneous tracings of electrocardiogram (ECG), phonocardiogram, carotid pulse and echocardiogram. Measurements of the systolic pre-ejection period (PEP), isovolemic contraction time (ICT), left ventricular ejection time (LVET) and isovolumic relaxation time (IVRT) are indicated. (From I Kline, The thyroid, L.E. Braverman & R.D. Utiger (eds). 1991, J.B. Lippincot Co.)

Miscellaneous Biochemical and Physiologic Changes Related to the Action of Thyroid Hormone on Peripheral Tissues

Thyroid hormone affects the function of a variety of peripheral tissues. Thus, hormone deficiency or excess may alter a number of determinations used in the diagnosis of illnesses unrelated to thyroid hormone dysfunction. Knowledge of the determinations which may be affected by thyroid hormone is important in the interpretation of laboratory data (Table 6-7).

Table 6-7. Biochemical and Physiologic Changes Related to Thyroid Hormone Deficiency and Excess (+ = up, – = down, N = normal)

Entity Measured	During Hypothyroidism	During Thyrotoxicosis
Metabolism of various substances and drugs Fractional	-	+

turnover rate (antipyrine,197 dipyrone,198 PTU, and methimazole,197 albumin,199 low-density lipoproteins,200 cortisol,201,202 and Fe203,204)		
Serum		
Amino Acids Tyrosine (fasting level and after load)205,206	-	+
Glutamic acid205	N	+
Proteins		
Albumin207	-	-
Sex hormone- binding globulin14,208,209	-	++
Ferritin210,211	-	+
Low-density lipoproteins200	-	+
Fibronectin212		+
Factor VIII-related antigen212		+
Tissue-plasminogen activator212		+
TBG83	+	-
TBPA213	N	-
Hormones		
Insulin		
Response to glucose214	-	-
Response to glucagon215	+	-
Estradiol-17ÃŸ216 , testosterone14,208,216 and gastrin217	- or N	+
Parathyroid hormone concentration218,219	+	-
Response to PTH administration219	-	+
Calcitonin220	-	+
Calcitonin response to Ca++ infusion221	-	
Renin activity and aldosterone222,223	-	+
Catecholamines224 and noradrenaline225	+	+
Atrial naturetic peptide226,227	-	+
Erythropoietin204	N or -	+
LH216		N or +
Response to GnRH228	+	N
Prolactin and response to stimulation with TRH, arginine, and chlorpromazine229,230	+ or N	-
Growth hormone		
Response to insulin231,232	-	N or -
Response to TRH233		No change
Epidermal growth factor234		
Enzymes		
Creatine-phosphokinase,235,236 lactic dehydrogenase,236 and	+	-

glutamic oxaloacetic transminase236		
Adenylate kinase237	N	+
Dopamine ÃŸ-hydroxylase238	+	-
Alkaline phosphatase219,239 a	a	+
Malic dehydrogenase240	++	+
Angiotensin-converting enzyme,212,241 alanine aminotransferase,242 and glutathione S-transferase242,243	N	+
Coenzyme Q10244		
Others		
1,25,OH-vitamin D3245		-
Carotene, vitamin A246		
cAMP,247 cGMP,248 and Fe203,249	+ N or -	- N or +
K250		-
Na251	-	
Mg252	+	-
Ca219,253	-	+
P218,219		+
Glucose		
Concentration215,231	-	+
Fractional turnover during iv tolerance test214	-	
Insulin hypoglycemia231	prolonged	
Bilirubin254,255	+b	+
Creatinine256	N or +	-
Creatine256	N or +	+
Cholesterol,246,257 carotene,246,257 phospholipids and lethicin,246,257 and triglycerides257,258	+	-
Lipoprotein (a)259	+	-
Apolipoprotein B259	+	-
Type IV collagen260	+	+
Type III Pro-collagen 260	-	+
Free fatty acids261		+
Carcinoembryonic antigen262	+	
Osteocalcin220	-	+
Urine		
cAMP263	-	+
after epinephrine infusion264	No change	+
cGMP248	N or -	+
Mg,252	-	+
Creatinine256	N	-
Creatine256	N	+
Tyrosine206	N or -	+

MIT (after) administration of 131IMIT265		+
Glutamic acid206	N	++
Taurine266	-	
Carnitine267	-	+
Tyramine, tryptamine, and histamine268		+
17-hydroxycorticoids and ketogenic steroids269	-	+
Pyridinoline (PYD), deoxypyridinoline (DPD)270		+
Hydroxyproline,271 and hydroxylysyl glycoside272		+
Red blood cells		
Fe203,249	-	+
Na273	N	+
Zn274	N	-
Hemoglobin203,249	-	-
Glucose-6-phosphate dehydrogenase activity275	N or -	+
Reduced glutathione276 and carbonic anhydrase277	+	-
Ca-ATPase activity278	-	-
White blood cells	-	-
Alkaline phosphatase279		
ATP production in mitochondria280	?+	-
Adipose tissue	N	-
cAMP247		
Lipoprotein lipase258		
Skeletal muscle		
cAMP247		+
Sweat glands	-	+
Sweat electrolytes281	+	N
Sebium excretion rate282	-	N
Intestinal system and absorption		
Basic electrical rhythm of the duodenum283	-	+
Riboflavin absorption284		-a
Ca absorption285	+a	-
Intestinal transit and fecal fat286,287		-
Pulmonary function and gas exchange		
Dead space,288 hypoxic ventilatory drive,289 and arterial pO2288	-	
Neurologic system and CSF		
Relaxation time of deep tendon reflexes (phomotogram)290	+	-
CSF proteins291	+	
Cardiovascular and circulatory system		
Timing of the arterial sounds (QKd)193	+	-
Left ventricular ejection time (LVET), preejection period (PEP)	-	-

ratio[194]		
ECG[292,293]		
Heart rate and QRS voltage		+
Q-Tc interval	-	-
Pr interval		+
T wave	Flat or inverted	Transient abnormalities
Common arrhythmias	Atrioventricular block	Atrial fibrillation
Bones		
Osseous maturation (bone age by X-ray film)[294,295]	Delayed (epiphysial dysgenesis)	Advanced

N = normal; + = increased; – = decreased. aIn children bIn neonates.

Measurement of Substances Absent in Normal Serum

Tests that measure substances present in the circulation only under pathologic circumstances do not provide information on the level of thyroid gland function. They are of value in establishing the cause of the hormonal dysfunction or thyroid gland pathology.

Thyroid Autoantibodies

The humoral antibodies most commonly measured in clinical practice are directed against thyroglobulin (Tg) or thyroid cell microsomal (MC) proteins. The latter is principally represented by the thyroid peroxidase (TPO). [296-298] More recently, immunoassays have been developed using purified and recombinant TPO. [299, 299a, 299b] Other circulating immunoglobulins, which are less frequently used as diagnostic markers, are those directed against a colloid antigen, T4 and T3. Antibodies against nuclear components are not tissue specific. Immunoglobulins possessing the property of stimulating the thyroid gland will be discussed in the next section.

A variety of techniques have been developed for the measurement of Tg and MC antibodies. These procedures include a competitive binding radioassay, complement fixation reaction, [300] tanned red cell agglutination assay, [301] the Coon's immunofluorescent technique, [302] enzyme-linked immunosorbent assay. [299, 303] Although the competitive binding radioassay [304,305] is a sensitive test, agglutination methods combine sensitivity and simplicity and have now largely superceded other methods. Current commercial kits utilize synthetic gelatin beads rather than red cells. [305a]

In the assay of Tg and MC antibodies by hemagglutination (TgHA and MCHA), particulate material is coated with either human Tg or solubilized thyroid MC proteins (TPO) and exposed to serial dilutions of the patient's serum. Agglutination of the coated particulates occurs in the presence of antibodies specific to the antigen attached to their surface. To detect false-positive reactions, it is important to include a blank for each sample using uncoated particles. Because of the common occurrence of prozone or blocking phenomenon, it is necessary to screen all serum samples through at least six consecutive two-fold dilutions. [306] Results are expressed in terms of the highest serum dilution, or titer,

showing persistent agglutination. The presence of immune complexes, particularly in patients with high serum Tg levels, may mask the presence of Tg antibodies. Assays for the measurement of such Tg-anti-Tg immune complexes have been developed. [307]

Normally, the test response is negative but results may be positive in up to 10% of the adult population. The frequency of positive test results is higher in women and with advancing age. The presence of thyroid autoantibodies in the apparently healthy population is thought to represent subclinical autoimmune thyroid disease rather than false-positive reactions. Nonetheless, it is difficult to compare results from such studies since some laboratories using agglutination methods report low titres (1/100-1/400) as positive. It is important when reporting values that a method-specific normal range is utilized and assays calibrated against internationally available refernce preparations. The availability of such preparations allows the reporting of results in International Units. [305a] TPO antibodies are detectable in approximately 95% of patients with Hashimoto's thyroiditis and 85% of those with Graves' disease, irrespective of the functional state of the thyroid gland. Similarly, Tg antibodies are positive in about 60 and 30% of adult patients with Hashimoto's thyroiditis and Graves' disease, respectively. [305,306] , [308,309] Tg antibodies are less frequently detected in children with autoimmune thyroid disease. [310] Although higher titers are more common with Hashimoto's thyroiditis, quantitation of the antibody titer carries little diagnostic implication. The tests are of particular value in the evaluation of patients with atypical or selected manifestations of autoimmune thyroid disease (ophthalmopathy and dermopathy). Positive antibody titers are predicative of post partum thyroiditis. [311] Low antibody titers occur transiently in some patients after an episode of subacute thyroiditis. [312] There is no increased incidence of thyroid autoantibodies in patients with multinodular goiter, thyroid adenomas, or secondary hypothyroidism. In some patients with Hashimoto's thyroiditis and undetectable thyroid autoantibodies in their serum, intrathyroidal lymphocytes have been demonstrated to produce TPO antibodies.

Other antibodies directed against thyroid components or other tissues have been described in the serum of some patients with autoimmune thyroid disease. They are less frequently measured, and their diagnostic value in thyroid disease has not been fully evaluated. Circulating antibodies capable of binding T4 and T3 have also been demonstrated in patients with autoimmune thyroid diseases which may interfere with the measurement of T4 and T3 by RIA techniques. [38,39] , [314]

Antibodies reacting with nuclear components, which are not tissue specific, and with cellular components of parietal cells and adrenal, ovarian, and testicular tissues are more commonly encountered in patients with autoimmune thyroid disease. [315] Their presence reflects the frequency of coexistence of several autoimmune disease processes in the same patient (see Chapter 7).

Thyroid-Stimulating Immunoglobulins (TSI)

A large number of names have been given to tests which measure abnormal ?-globulins present in the serum of some patients with autoimmune thyroid disease, in particular Graves' disease. [317] The interaction of these unfractionated immunoglobulins with thyroid follicular cells usually results in a global stimulation of thyroid gland activity and only rarely causes inhibition. It has been recommended that these assays all be called TSH receptor antibodies (TRAb) with a phrase "measured by …… assay" to identify the type of method used for their determination. [106] The tests will be described under three general categories: (1) those measuring the thyroid stimulating activity using in vivo or in vitro bioassays; (2) tests based on the competition of the abnormal immunoglobulin with binding of TSH to its receptor; and (3) measurement of thyroid growth promoting activity of immunoglobulins. Tests employ both human and animal tissue material or cell lines.

Thyroid-Stimulation Assays.

The earliest assays employed various modifications of the McKenzie mouse bioassay. [318,319] The abnormal ?-globulin with TSH-like biological properties has relatively longer in vivo activity, hence its name, long-acting thyroid stimulator (LATS). The assay measures the LATS induced release of thyroid hormone from the mouse thyroid gland prelabeled with radioiodide. The presence of LATS in serum is pathognomonic of Graves' disease. However, depending upon the assay sensitivity, a variable percent of untreated patients will show a positive LATS response. LATS may be found in the serum of patients with Graves' disease even in the absence of thyrotoxicosis. Although it is more commonly present in patients with ophthalmopathy, especially when accompanied by pretibial myxedema, [320] LATS does not appear to correlate with the presence of Graves' disease, its severity, or course of complications. LATS crosses the placenta and may be found transiently in newborns from mothers possessing the abnormal ? globulin. [321]

Attempts to improve the ability to detect thyroid stimulating antibodies (TSAb) in autoimmune thyroid disease lead to the development of several in vitro assays using animal as well as human thyroid tissue. The ability of the patient's serum to stimulate endocytosis in fresh human thyroid tissue is measured by direct count of intracellular colloid droplets formed. Using such a technique, human thyroid stimulator (HTS) activity has been demonstrated in serum samples from patients with Graves' disease that were devoid of LATS activity measured by the standard mouse bioassay. [322] TSAb can be detected by measuring the accumulation of cyclic adenosine monophosphate (cAMP) or stimulation of adenylate cyclase activity in human thyroid cell cultures and thyroid plasma membranes, respectively. [323] Accumulation of cAMP in the cultured rat thyroid cell line FRTL5 has also been used as an assay for TSAb. [324] Stimulation of release of T3 from human [325] and porcine [326] thyroid slices is another form of in vitro assay for TSAb. An in vitro bioassay using a cytochemical technique depends upon the ability of thyroid-stimulating material to increase lysosomal membrane permeability to a chromogenic substrate, leucyl-ÃŸ-naphthylamide, which then reacts with the enzyme naphthylamidase. Quantitation is by scanning and integrated microdensitometry. [327]

The cloning of the TSH receptor [328,329] lead to the development of an in vitro assay of TSab using cell lines that express the recombinant TSH receptor. [330,331] This assay, based on the generation if cAMP, is specific for the measurement of human TSH receptor antibodies that have thyroid stimulating activity and thus contrasts with assays based on binding to the TSH receptor (see below) that cannot distinguish between antibodies with thyroid-stimulating and TSH-blocking activity. Accordingly, the recombinant human TSH receptor assay measures antibodies relevant to the pathogenesis of autoimmune thyrotoxicosis and is more sensitive than formerly used TSab assays. [331a] For example, 94% of serum samples were positive for TSab compared to 74% when the same samples were assayed using FRTL5 cells. [332]

Thyrotropin-Binding Inhibition Assays. The principal of binding-inhibition assays dates to the discovery of another class of abnormal immunoglobulins in patients with Graves' disease; those which neutralize the bioactivity of LATS tested in the mouse. [333] This material, known as LATS protector (LATS-P), is species specific having no biologic effect on the mouse thyroid gland but capable of stimulating the human thyroid. [334] The original assay was cumbersome, limiting its clinical application.

Techniques used currently, which may be collectively termed radioreceptor assays, are based on the competition of the abnormal immunoglobulins and TSH for a common receptor-binding site on thyroid cells. The test is akin in principle to the radioligand assays, in which a natural membrane receptor takes

the place of the binding proteins or antibodies. Various sources of TSH-receptors are employed including, human thyroid cells, [335] their particulate or solubilized membrane, [336,337] and cell membranes from porcine thyroids [338] or from guinea pig fat cells [339] or recombinant human TSH receptor expressed in mammalian cells. [340] Since the assays do not directly measure thyroid-stimulating activity, the abnormal immunoglobulins determined have been given variety of names, such as thyroid binding inhibitory immunoglobulins (TBII) or antibodies (TBIAb) and thyrotropin-displacing immunoglobulins (TDI). This type of assay has indicated that not all the antibodies detected do stimulate the thyroid, and some are inhibitory. Even using modern techniques, [305a] the presence of inhibitory antibody is less sensitive and specific for Graves' disease than the presence of styimulatory antibody activity. [331] The stimulatory and inhibitory effects can be differentiated only by functional assays, typically measuring the production of cyclic AMP.

Thyroid Growth-Promoting Assays.

Assays have been also developed that measure the growth promoting activity of abnormal immunoglobulins. One such assay is based on the staining by the Feulgen reaction of nuclei from guinea pig thyroid cells in S-phase. [341] Another assay measures the incorporation of 3H-thymidine into DNA in FRTL cells. [342] Whether the thyroid growth stimulating immunoglobulins (TGI) measured by these assays represent a population of immunoglobulins distinct from that with stimulatory functional activity remains a subject of active debate.

Clinical Applications. Measurement of abnormal immunoglobulins that interact with thyroid tissue by any of the methods described above is not indicated as a routine diagnostic test for Graves' disease. It is useful, however, in a few selected clinical conditions: (1) in the differential diagnosis of exophthalmos, particularly unilateral exophthalmos, when the origin of this condition is otherwise not apparent; the presence of TSI would obviate the necessity to undertake more complex diagnostic procedures described elsewhere; [343] (2) in the differential diagnosis of pretibial myxedema, or other forms of dermopathy, when the etiology is unclear and it is imperative that the cause of the skin lesion be ascertained; (3) in the differentiation of Graves' disease from toxic nodular goiter, when both are being considered as the possible cause of thyrotoxicosis, when other tests such as thyroid scanning and thyroid autoantibody tests have been inconclusive, and particularly when such a distinction would play a role in determining the course of therapy; (4) when non-autoimmune thyrotoxicosis is suspected in a patient with hyperthyroidism and diffuse or nodular goiter [344,345] ; (5) in Graves' disease during pregnancy, when high maternal levels of TSAb are a warning for the possible occurrence of neonatal thyrotoxicosis; (6) in neonatal thyrotoxicosis, where serial TSAb determinations showing gradual decrease may be helpful to distinguish between intrinsic Graves' disease in the infant and transient thyrotoxicosis resulting from passive transfer of maternal TSAb. [321] , [346] Some investigators have found the persistence of TSAb's to be predicative of the relapse of Graves' thyrotoxicosis following a course of antithyroid drug therapy. [347]

Other Substances with Thyroid-Stimulating Activity

Some patients with trophoblastic disease develop hyperthyroidism as a result of the production and release of a thyroid stimulator which has been termed molar or trophoblastic thyrotropin or big

placental TSH. [348] It is likely that the thyroid-stimulating activity in patients with trophoblastic disease is entirely due to the presence of high levels of human chorionic gonadotropin (hCG). [350] Thus, the RIA of hCG can be useful in the differential diagnosis of thyroid dysfunction.

Exophthalmos-Producing Substance (EPS)

A variety of tests have been developed for measuring exophthalmogenic activity in serum. [351-354] Although a great uncertainty still exists regarding the pathogenesis of thyroid associated eye disease, the role of the immune system appears to be central. Exophthalmogenic activity has also been detected in IgG fractions of some patients with Graves' ophthalmopathy. The role of assays to detect specific antibodies is discussed further in Chapter 7 .

Tests of Cell-Mediated Immunity (CMI)

Delayed hypersensitivity reactions to thyroid antigens are present in autoimmune thyroid diseases (see Chapters 7). CMI was measured in several ways: (1) the migration inhibition test, which measured the inhibition of migration of sensitized leukocytes when exposed to the sensitizing antigen; (2) the lymphotoxic assay, which measured the ability of sensitized lymphocytes to kill target cells when exposed to the antigen; (3) the blastogenesis assay, which scored the formation of blast cells after exposure of lymphocytes to a thyroid antigen; and (4) thymus-dependent (T) lymphocyte subset quantitation utilizing monoclonal antibodies. More recently, measures of T-cell proliferation, determined by uptake of 3Hthymidine, has become the standard test of CMI employed in the research setting. [354a, 354b] The tests require fresh leukocytes from the patient, are variable in their response, and are difficult to perform.

Anatomic and Tissue Diagnoses

The purpose of the procedures described in this section is to evaluate the anatomic features of the thyroid gland, localize and determine the nature of abnormal areas and eventually provide a pathologic or tissue diagnosis. All of these tests are performed in vivo.

Thyroid Scintiscanning

Normal and abnormal thyroid tissue can be externally imaged by three scintiscanning methods: (1) with radionuclides that are concentrated by normal thyroid tissues such as iodide isotopes, and 99mTc given as the pertechnetate ion; (2) by administration of radiopharmaceutical agents which are preferentially concentrated by abnormal thyroid tissues; and (3) fluorescent scanning, which uses an external source of 241Am and does not require administration of radioactive material. Each has specific indications, advantages, and disadvantages.

The physical properties, dosages, and radiation delivered by the most commonly used radioisotopes are listed in Table 6-2 . The choice of scanning agents depends on the purpose of the scan, the age of the patient, and the equipment available. Radioiodide scans cannot be performed in patients who have recently ingested iodine-containing compounds. 123I and 99mTcO4- are the radionuclides of choice because of the low radiation exposure. [355-357] Iodine-131 is still used for the detection of functioning

metastatic thyroid carcinoma by total body scanning.

Radioiodide and 99mPertechnetate Scans. 99mTcO4- is concentrated, and all iodide isotopes are concentrated and bound, by thyroid tissue. Depending upon the isotope used, scans are carried out at different times after administration: 20 minutes for 99mTcO4-, 4 or 24 hours for 123I-; 24 hours for 125I- and 131I-; and 48, 72, and 96 hours when 131I- is used in the search for metastatic thyroid carcinoma. The appearance of the normal thyroid gland on scan may be best described as a narrow-winged butterfly. Each “wing” represents a thyroid lobe, which in the adult measures 5 ? 1 cm in length and 2.3 ? 0.5 cm in width. [358] Common variants include the absence of a connecting isthmus, a large isthmus, asymmetry between the two lobes, and trailing activity extending to the cricoid cartilage (pyramidal lobe). The latter is more commonly found in conditions associated with diffuse thyroid hyperplasia. Occasionally, collection of saliva in the esophagus during 99mTcO4- scanning may simulate a pyramidal lobe, but this artifact can be eliminated by drinking water.

The indications for scanning are listed in Table 6-8 . In clinical practice, scans are most often requested for evaluation of the functional activity of solitary nodules. Normally, the isotope is homogeneously distributed throughout both lobes of the thyroid gland. This distribution occurs in the enlarged gland of Graves’ disease and may be seen in Hashimoto’s thyroiditis. A mottled appearance may be noted in Hashimoto’s thyroiditis and can occasionally be seen in Graves’ disease especially after therapy with radioactive iodide. Irregular areas of relatively diminished and occasionally increased uptake are characteristic of large multinodular goiters. The traditional nuclear medicine jargon classifies nodules as “hot”, “warm,” and “cold,” according to their isotope-concentrating ability relative to the surrounding normal parenchyma (Figure 6-6). Hot, or hyperfunctioning, nodules are typically benign, although the presence of malignancy has been reported. [359,360] Cold, or hypofunctioning, nodules may be solid or cystic. Some may prove to be malignant, but the great majority are benign. This differentiation cannot be made by scanning. [77 , 361] Occasionally, a nodule which is functional on a 99mTcO4- scan will be found to be cold on an iodine scan; this pattern is found with both benign and malignant nodules. The scan is of particular value in identifying autonomous thyroid nodules since the remainder of the gland is suppressed. Search for functioning thyroid metastases is best accomplished using 2-10 mCi of 131I after ablation of the normal thyroid tissue and cessation of hormone therapy to allow TSH to increase above the upper limit of normal. Recent studies have addressed the question of whether recombinant human TSH allows scanning without requiring cessation of hormone therapy. [362] Uptake is also found outside the thyroid gland in patients with lingual thyroids and in the rare ovarian dermoid tumor containing functioning thyroid tissue.

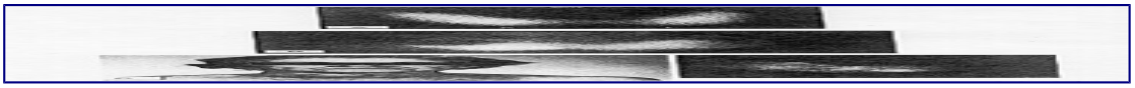

Figure 6-6. Thyroid Scans. (a) Normal thyroid imaged with 123I. (b) Cold nodule in the right lobe imaged by 99mTc. (c) Elderly woman with obvious multinodular goiter and the corresponding radioiodide scan on the right.

Table 6-8. Indications for Radionuclide Scanning

Detection of anatomic variants and search for ectopic thyroid tissue (thyroid hemiagenesis, lingual thyroid, struma ovarii) Diagnosis of congenital athyreosis Determination of the nature of abnormal neck or chest (mediastinal) masses Evaluation of solitary thyroid nodules (functioning or non-functioning) Evaluation of thyroid remnants after surgery Detection of functioning thyroid metastases Evaluation of focal functional thyroid abnormalities (suppressed or nonsuppressible tissue)

The scan can be used as an adjunct during TSH stimulation and T3 suppression tests to localize suppressed normal thyroid tissue or autonomously functioning areas, respectively (see below). Applications other than those listed in Table 6-8 are of doubtful benefit and are rarely justified considering the radiation exposure, expense, and inconvenience. 123I single photon emission computed tomography (SPECT) may also be useful in the evaluation of thyroid abnormalities. [363]

Other Isotope Scans. Because most test procedures, short of direct microscopic examination of thyroid tissue, fail to detect thyroid malignancy with any degree of certainty, efforts have been made to find other radioactive materials that would hopefully be of diagnostic use. Several such agents that are concentrated by metabolically active tissues, irrespective of whether they have iodide-concentrating ability, have been tried. However, despite claims to the contrary, they have either had only limited value or their diagnostic usefulness has not been fully evaluated. These agents include 75Se methionine, 125Ce, 67Ga, citrate, 32P, pyrophosphate 99mTc, and 201Thallium. [364]

Scanning with 131I-labeled anti-TG for the detection of occult metastatic thyroid malignancy that fails to concentrate 131I showed early promising results. [365] However, the procedure has not proved clinically useful.

Ultrasonography

Ultrasonography, or echography, is used to outline the thyroid gland and to characterize lesions differing in density from the surrounding tissue. The technique differentiates interphases of different acoustic densities, using sound frequencies in the megahertz range that are above the audible range. A transducer fitted with a piezoelectric crystal produces and transmits the signal and receives echo reflections. Interfaces of different acoustic densities reflect dense echoes, liquid transmits sound without reflections, and air-filled spaces do not transmit the ultrasound. [368]

One of the most useful applications of the ultrasonogram is the differentiation of solid from cystic lesions. [368,369] Purely cystic lesions are entirely sonolucent, whereas solid lesions produce multiple echoes due to multiple sonic interphases. Many lesions, however, are mixed (solid and cystic) called complex lesions. Some tumors may have the same acoustic characteristics as the surrounding normal tissue thus, escaping echographic detection. While high-resolution ultrasonography can detect thyroid nodules of the order of few millimeters, [370] lesions need to be larger than 0.5 cm to allow differentiation between solid and cystic structures. A sonolucent pattern is frequently noted in glands with Hashimoto's thyroiditis, but this has also been described in multinodular glands and in patients with Graves' disease. [368 , 371 , 372]

Because sonography localizes the position as well as the depth of lesions, the procedure has been used to guide the needle during aspiration biopsy. [373] In complex lesions, the sonographic guiding insures sampling from the solid portion of the nodule. With experience and proper calibration, sonography can be used for the estimation of thyroid gland size. [374,375] Several recent reports have described treatment of toxic nodules by the injection of alcohol under sonographic guidance. [376] Although ultrasonography has found virtually the same applications as scintiscanning, claims that the former may differentiate benign from malignant lesions are unfounded. Also, ultrasonography cannot be used for the assessment of substernal goiters because of interference from overlying bone.

The procedure is simple and painless, and at the frequencies of sound used, do not produce tissue damage. Since it does not require the administration of isotopes, it can be safely used in children and during pregnancy. Also, because the procedure is independent of iodine-concentrating mechanisms, it is

valuable in the study of suppressed glands.

X-Ray Procedures

A simple X-ray film of the neck and upper mediastinum may provide valuable information regarding the location, size, and effect of goiter on surrounding structures. X-rays may show an asymmetric goiter, an intrathoracic extension of the gland, and displacement or narrowing of the trachea. If there is any suggestion of posterior extension of the mass, it is useful to take films during the swallow of X-ray contrast material. The soft tissue X-ray technique may disclose calcium deposits. Large deposits in flakes or rings are typical of an old multinodular goiter, whereas foci of finely stippled flecks of calcium are suggestive of papillary adenocarcinoma.

Information, not related to anatomic abnormalities of the thyroid gland may be obtained from X-ray studies. In children with a history of hypothyroidism, an X-ray film of the hand to determine the bone age could aid in estimating the onset and duration of thyroid dysfunction. [294,295] Hypothyroidism leads to retardation in bone age and in infants produces a dense calcification of epiphyseal plates most easily seen at the distal end of the radius. Long-standing myxedema produces pituitary hypertrophy which, especially in children but also in adults, causes enlargement of the sella turcica demonstrable on imaging of the pituitary region.

Computed Tomography (CT) and Magnetic Resonance Imaging (MRI). These techniques provide useful information on the location and architecture of the thyroid gland as well as its relationship to surrounding tissues. [378] They are, however, too costly relative to other procedures which provide similar information. An important application of CT is the assessment and delineation of obscure mediastinal masses and large substernal goiters. [379] The necessity to infuse iodine containing contrast agents limits the application of CT in patients being considered for radioiodide therapy. CT and MRI have found firm application in another area of thyroid diseases, namely, in the evaluation of ophthalmopathy [343] and mediastinal masses. [379]

Other Procedures

A barium swallow may be useful in evaluating impingement of a goiter on the esophagus, while a flow volume loop [380] may be useful in documenting functional impingement on the upper airway.

Biopsy of the Thyroid Gland

Histologic examination of thyroid tissue for diagnostic purposes requires some form of an invasive procedure. The biopsy procedure depends on the intended type of microscopic examination. Core biopsy for histologic examination of tissue with preservation of architecture is obtained by closed needle or open surgical procedure; aspiration biopsy is performed to obtain material for cytologic examination.

Core Biopsy. Closed core biopsy is an office procedure carried out under local anesthesia. A large (about 15-gauge) cutting needle of the Vim-Silverman type is most commonly used. [384] The needle is introduced under local anesthesia through a small skin nick and firm pressure is applied over the puncture site for 5-10 minutes after withdrawal of the needle. In experienced hands, complications are rare, but may include transient damage to the laryngeal nerve, puncture of the trachea, laryngospasm,

jugular vein phlebitis, and bleeding.385 Because of the fear of disseminating malignant cells, biopsy was restricted for many years to the differential diagnosis of diffuse benign diseases. With the improvement of cytology and biopsy techniques, open biopsy carried out under local or general anesthesia has been virtually abandoned. [385]

Percutaneous Fine Needle Aspiration (FNA). The development of more sophisticated staining techniques for cytologic examination, the realization that fear of tumor dissemination along the needle tract was not well founded, and especially the high diagnostic accuracy of the technique are responsible for the increasing popularity of percutaneous fine needle aspiration. [385] , [388,388a,388b]

The procedure is exceedingly simple and safe. The patient lays supine, with the neck hyperextended by placing a small pillow under the shoulders. Local anesthesia is usually not required. The skin is prepared with an antiseptic solution. The lesion, fixed between two gloved fingers, is penetrated with a fine (22- to 27-gauge) needle attached to a syringe. Suction is then applied while the needle is moved within the nodule. A non-suction technique using capillary action has also been developed. The small amount of aspirated material, usually contained within the needle or its hub, is applied to glass slides and spread. Some slides are air dried and others are fixed before staining. As biopsy of small nodules may be technically more difficult, the use of ultrasound to guide the needle is preferred. [373] , [376] It is important that the slides be properly prepared, stained and read by a cytologist experienced in the interpretation of material from thyroid gland aspirates.

The yield of false-positive and false-negative results is variable from one center to another, but both are acceptably low. Various centers have reported that the accuracy of this technique in distinguishing benign from malignant lesions may be as high as 95%. [385] , [388] In one clinic in which the procedure is used routinely, the number of patients operated upon decreased by one-third, whereas the percentage of thyroid carcinomas among the patients who underwent surgery doubled. [389] When results are suggestive of a follicular neoplasia, surgery is required as follicular adenoma cannot be differentiated from follicular cancer by cytology alone. As the sample obtained may not always be representative of the lesion, surgical treatment is indicated for lesions highly suspicious of being malignant on clinical grounds. Other uses of aspiration biopsy include presumed lymphoma or invasive anaplastic carcinoma when biopsy may spare the patient an unnecessary neck exploration. Another application of needle aspiration is in the confirmation and treatment of thyroid cysts and autonomous thryoid nodules. [389a]

Evaluation of the Hypothalamic-Pituitary-Thyroid Axis

The development of an RIA for the routine measurement of TSH in serum and the availability of synthetic TRH [390,391] have placed increased reliance on tests assessing the hypothalamic-pituitary control of thyroid function. These tests allow the diagnosis of mild and subclinical forms of thyroid dysfunction, and provide a means to differentiate between primary, pituitary (secondary) or hypothalamic (tertiary), thyroid gland failure.

Thyrotropin (TSH)

The routine measurement of TSH in clinical practice used initially RIA techniques. These first generation assays had a sensitivity level of 1 mU/L which did not allow the separation of normal from reduced values. A major problem with early TSH RIAs was cross-reactivity with gonadotropins (LH,

FSH, and hCG) sharing with TSH a common a-subunit. [399] Nevertheless, even older RIA methods for measurement of pituitary TSH correlated well with values obtained using bioassay techniques. [401] Another uncommon source of error is the presence in the serum sample of heterophilic antibodies induced by vaccination with materials contaminated with animal serum, [402] or endogenous TSH antibodies. [403] RIA techniques for measurement of TSH in dry blood spots on filter paper are used for the screening of neonatal hypothyroidism. [33]

Newer techniques have been developed using multiple antibodies to produce a "sandwich" type assay in which one antibody (usually directed against the a subunit) serves to anchor the TSH molecule and an other (usually monoclonal antibodies directed against the ÃŸ subunit) is either radioiodinated (Immunoradiometric assay, IRMA) or is conjugated with an enzyme (Immunoenzymometric, IEMA) or a chemiluminescent compound (Chemiluminescent assay, ICMA). [112] , [404] In these assays, the signal should be directly related to the amount of the ligand present rather than being inversely related as in RIAs measuring the bound tracer. [405] This results in decreased background "noise" and a greater sensitivity, decreased interference from related compounds as well as an expanded useful range. [112] , [404] , [406] Initial improvements of the TSH assay resulted in assays with sensitivity limit of 0.1 mU/L, a normal range of approximately 0.5 – 4.5 mU/L and the ability to distinguish between low and normal TSH values. Recently, commercial assays have been developed with even higher sensitivity limit of 0.005 – 0.01 mU/L and a similar normal range but an expanded range between the lower limit of normal and the lower limit of sensitivity. [407,408]

The nomenclature for differentiating these various assays has not been standardized with manufacturers applying various combinations of "high(ly)", "ultra" and "sensitive". It has been recommended that the sensitivity limit be used in defining the assays with the early radioimmunoassays detecting values ?1 mU/L designated "first generation assays", those with a lower sensitivity limit of 0.1 mU/L designated as "second generation assays" and those with a lower sensitivity limit of ? 0.01 mU/L designated as "third generation assays". [112] The determination of the appropriate sensitivity level has also been controversial. Some define it based on the level with a coefficient of variation less than 20% and others as the lowest level which can be reliably differentiated from the zero TSH standard.112,406 At a minimum, for a TSH assay to be considered "sensitive", the overlap of TSH values in sera from clinically hyperthyroid and euthyroid individuals should be less than 5% and preferably less than 1%. [112]

In a number of these "third generation" assays, TSH detected in clinically toxic patients and elevated values found in euthyroid subjects were not confirmed when the samples were measured in other assays. In some cases, this has been attributed to the presence of antibodies directed against the animal immunoglobulins used in the assay. [409-411] These act to bind the anchoring and detecting antibodies and lead to an over-estimation of TSH. In some cases, this effect may be blocked by the addition of an excess of non-specific immunoglobulin of the same species. [411]

TSH appears abruptly in the pituitary and serum of the fetus at midgestation, and can also be detected in amniotic fluid. [51] , [412,413] The mean TSH level is higher in cord than in maternal blood. A substantial increase, to levels several fold above the upper range in adults, is observed during the first half-hour of life. [413] Levels decline to near the normal adult range by the third day of life. Minimal changes reported to occur during adult life and in early adolescence [414] have no significant effect on the overall range of normal. In the absence of pregnancy, no significant sex differences have been observed. Although early studies failed to show diurnal TSH variation, [415] significantly higher values have been recorded during the late evening and early night which are partially inhibited by sleep. [416] This diurnal

rhythm of TSH is superimposed upon continuous high-frequency, low-amplitude variations. The nocturnal TSH surge persists in patients with mild primary hypothyroidism, [417,418] and is abolished in hypothalamic hypothyroidism [417, 419] and in some patients during fasting [420] and with non-thyroidal illness. [421,422] It is enhanced by oral contraceptives, [423] and is abolished by high levels of glucocorticoids. [424] The presence of seasonal variation has not been a uniform finding, but it is unlikely to affect the clinical interpretation of serum values. [425] Various types of stressful stimuli have no significant effect on the basal serum TSH level, except for a rise during surgical hypothermia in infants but not in adults. [426] Various stimuli, such as administration of insulin, vasopressin, glucagon, bacterial pyrogens, arginine, prostaglandins, and chlorpromazine, which elicit in normal humans a secretory response of some pituitary hormones, have no effect on serum TSH. However, administration of any of a growing list of drugs has been found to alter the basal concentration of serum TSH and/or its response to exogenous TRH (see Table 5-4).

In the presence of a normally functioning hypothalamic-pituitary system, there is an inverse correlation between the serum concentration of FT4 and TSH. Changes in the serum concentration of TT4 as a result of TBG abnormalities, or drugs competing with T4 binding to TBG, have no effect on the level of serum TSH. The pituitary is exquisitely sensitive to both minimal decreases and increases in thyroid hormone concentration, with a logarithmic change in TSH levels in response to changes in T4 [404, 408, 427, 428] (Figure 6-7) Thus, serum TSH levels should be elevated in patients with primary hypothyroidism and low or undetectable in thyrotoxicosis. Indeed, in the absence of hypothalamic pituitary disease, illness or drugs, TSH is an accurate indicator of thyroid hormone status and the adequacy of thyroid hormone replacement. [404, 429]

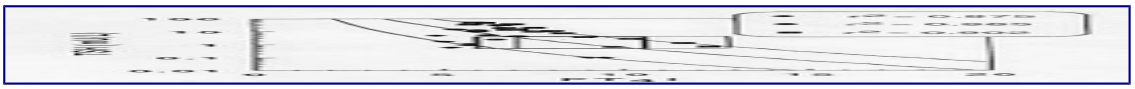

Figure 6-7. Correlation of the serum TSH concentration and the free thyroxine index (FT4I) in three individuals given increasing doses of L-T4. Note the logarithmic correlation between TSH and FT4I and the variable individual requirement of free T4 to normalize the TSH level. Normal ranges are included in the heavy lined box and those for subjects on L-T4 replacement in the light liquid box. (From D. Sarne and S. Refetoff, Endocrinology, L.J. DeGroot (ed). 1995, Grune & Straton Inc.)

In patients with primary hypothyroidism of whatever cause, levels may reach 1,000 ÂμU/ml or higher. The magnitude of serum TSH elevation grossly correlates with the severity and in part with the duration of thyroid hormone deficiency. [430,431] TSH concentrations above the upper limit of normal have been observed in the absence of clinical symptoms and signs of hypothyroidism and in the presence of serum T4 and T3 levels well within the normal range. [430, 432] This condition is most commonly encountered in patients developing hypothyroidism due to Hashimoto's thyroiditis or with limited ability to synthesize thyroid hormone because of prior thyroid surgery, radioiodide treatment, or severe iodine deficiency. [430, 433] There is disagreement on whether such patients have subclinical hypothyroidism or a "compensated state" in which euthyroidism is maintained by chronic stimulation of a reduced amount of functioning thyroid tissue through hypersecretion of TSH. Transient hypothyroidism, may occur in some infants during the early neonatal period. [434] There are two circumstances in which the usual reverse relationship between the serum level of TSH and T4 is not maintained in patients with proven primary hypothyroidism. Treatment with replacement doses of T4 may normalize or even produce serum levels of thyroid hormone above the normal range before the

high TSH levels have reached the normal range. [404] , [431] , [435] This is particularly true in patients with severe or long-standing primary hypothyroidism who may require three to six months of hormone replacement before TSH levels are fully suppressed. Conversely, serum TSH concentration may remain low or normal for up to five weeks after withdrawal of thyroid hormone replacement when serum levels of T4 and T3 have already declined to values well below the lower range of normal. [404] , [436] Causes for discrepancies between TSH and free T4 and T3 levels are listed in Table 6-9 .

Table 6-9. Discrepancies Between TSH and Free Thyroid Hormone Levels

Elevated Serum TSH Value Without Low FT4 or FT3 Values

Subclinical hypothyroidism (inadequate replacement therapy, mild thyroid gland failure)

Recent increase in thyroid hormone dosage

Drugs

Inappropriate TSH secretion syndromes

Laboratory artefact

Subnormal Serum TSH Value Without Elevated FT4 or FT3 Values

Subclinical hyperthyroidism (excessive replacement therapy, mild thyroid gland hyperfunction, autonomous nodule)

Recent decrease in suppressive thyroid hormone dosage

Recent treatment of thyrotoxicosis (Graves' disease, toxic multinodular goiter, toxic nodule)

Resolution thyrotoxic phase of thyroiditis

Nonthyroidal illness

Drugs

Central hypothyroidism

At this time, it is uncertain as to what TSH level is appropriate for suppressive thyroid hormone therapy. The frequency with which patients have subnormal, but detectable, TSH values depends on both the population studied and the sensitivity of the assay (Figure 6-8, below). Using an assay with a sensitivity limit of 0.1 mU/L, 3 to 4% of hospitalized patients have been noted to have a subnormal TSH. [432] , [437] When patients with an undetectable TSH in such an assay were re-evaluated in an assay with a sensitivity limit of 0.005 mU/L, 3 of 77 (4%) with thyrotoxicosis and 32 of 37 (86%) with non-thyroidal illness or on drugs were found to have a subnormal but detectable TSH level. [407] Thus, the more sensitive the assay, the more likely that patients with clinical thyrotoxicosis will have undetectable serum TSH while those with illness will have a subnormal but detectable level. However, with progressively more sensitive assays, the likelihood of a clinically toxic patient to have a detectable TSH will increase, and if patients on suppressive therapy are treated until the TSH is undetectable, the more likely they will have symptoms of thyrotoxicosis.

Fig ure 6-8. The effect of serum TSH assay sensitivity on the discrimination of euthyroid subject (Euth) from those with thyrotoxicosis (Toxic). (From C. Spencer, Clinical Diagnostics, Eastman Kodak Co., 1992).

A persistent absence of a reverse correlation between serum thyroid hormone and TSH concentration has a very different connotation. A low serum level of thyroid hormone without clear elevation of the

serum TSH concentration is suggestive of trophoprivic hypothyroidism, especially when associated with obvious clinical stigmata of hypothyroidism. [433] An inherited defect of the TSH receptor has been shown to produce marked persistent hyperthyrotropinemia in the presence of normal thyroid hormone levels. [438] In some cases, a mild elevation of the serum TSH level measured by RIA is probably due to the presence of immunoreactive TSH with reduced biologic activity. [397] Distinction between pituitary and hypothalamic hypothyroidism can be made on the basis of the TSH response to the administration of TRH (see below).

In another group of pathologic conditions, serum TSH levels may not be suppressed despite a clear elevation of serum free thyroid hormone levels. Because such a finding is incompatible with a normal thyroregulatory control mechanism of the pituitary, which is preserved in the more common forms of thyrotoxicosis, it has been termed inappropriate secretion of TSH. [439] It implicitly suggests a defective feedback regulation of TSH. When associated with the classical clinical and metabolic changes of thyrotoxicosis, it is usually due to TSH-secreting pituitary adenoma or isolated pituitary resistance to the feedback suppression by thyroid hormone. [439] The existence of hypothalamic hyperthyroidism can be questioned. [440] Precise diagnosis requires further studies, including radiologic examination of the pituitary gland and a TRH test. In addition, the presence of high circulating levels of the a-subunit of pituitary glycoprotein hormones (a-SU), giving rise to a disproportionately high a-SU/TSH molar ratio in serum, is characteristic, if not pathognomonic, of TSH-secreting pituitary tumors. [439, 441] Normal, and occasionally high serum TSH levels, associated with a clear elevation in serum FT4 and FT3 but no clear clinical evidence of hypothyroidism or symptoms and signs suggestive of both thyroid hormone deficiency and excess are typical of resistance to thyroid hormone (RTH) [442] (see Chapter 16).

Although TSH has been implicated in the pathogenesis of simple, nontoxic goiter, unless hypothyroidism supervenes or iodide deficiency is very severe, TSH levels are characteristically normal. Elevated TSH levels may occur in the presence of normal thyroid hormone levels and apparent euthyroidism in nonthyroidal diseases [437, 443] (see also Chapter 5) and with primary adrenal failure. [444] A more common occurrence in severe acute and chronic illnesses is a normal or low serum TSH concentration despite low levels of T3 and even low T4 levels. [407, 429, 445] TSH values may be transiently elevated during the recovery phase.446 Various hypotheses to explain these anomalous findings have been proposed, but a satisfactory explanation is not at hand.

A specific RIA for the ÃŸ subunits of human TSH is also available but has not found clinical application. [447]

Thyrotropin-Releasing Hormone (TRH)

TRH. The hypothalamic tripeptide TRH (protirelin) plays a central role in the regulation of pituitary TSH secretion. [391, 419] It is thus not surprising that attempts have been made to measure its concentration in a variety of body fluids, with the purpose of deriving information relevant to the function of the thyroid gland in health and in disease. Several methods have been used for quantitation of TRH, [448-451] but for many reasons, measurement in humans has failed to provide information of diagnostic value. These include, high dilution of TRH by the time it reaches the systemic circulation, rapid enzymatic degradation and ubiquitous tissue distribution. [448, 450,451] Mean serum TSH levels of 5 and 6 pg/ml have been reported. It is uncertain whether measurements carried out in urine truly represent TRH. [449]

TRH Test.

The TRH test measures the increase of pituitary TSH in serum in response to the administration of synthetic TRH. The magnitude of the TSH response to TRH is modulated by the thyrotroph response to active thyroid hormone and is thus almost always proportional to the concentration of free thyroid hormone in serum. The response is exquisitely sensitive to minor changes in the level of circulating thyroid hormones, which may not be detected by direct measurement. [427,428] A direct correlation between basal serum TSH values and the maximal response to TRH has been observed even in the absence of thyroid hormone abnormalities, suggesting that there may be a fine modulation of pituitary sensitivity to TRH in the euthyroid state. [452]

TRH normally stimulates pituitary prolactin secretion and, under certain pathologic conditions, the release of GH and ACTH. [391] Accordingly, the test has been used for the assessment of a variety of endocrine functions, some unrelated to the thyroid. In clinical practice, the TRH test is used mainly (1) to assess the functional integrity of the pituitary thyrotrophs and thus to aid in differentiating hypothyroidism due to intrinsic pituitary disease from hypothalamic dysfunction and (2) in the diagnosis of mild thyrotoxicosis when results of other tests are equivocal, and (3) in the differential diagnosis of inappropriate TSH secretion, in particular when a TSH-secreting adenoma is suspected.

TRH is effective when given intravenously as a bolus or by infusion, [414,453] intramuscularly, [454] or orally [455] in single or repeated doses. Doses as small as 6 Âµg can elicit a significant TSH response, and there is a linear correlation between the incremental changes in serum TSH concentrations and the logarithm of the administered TRH dose. [414] The standard test uses a single TRH dose of 400 Âµg/1.73 m2 body surface area, given by rapid intravenous injection. Serum is collected before and at 15 minutes and then at 30 minute intervals over 120-180 minutes although many clinicians chose to obtain a single post-injection sample at 15, 20 or 30 minutes. In normal persons there is a prompt increase in serum TSH, with a peak level at 15-40 minutes, which is, on the average, 16 ÂµU/ml, or fivefold the basal level. The decline is more gradual, with a return of serum TSH to the preinjection level by three to four hours. [414,453] Results can be expressed in terms of the peak level of TSH achieved, the maximal increment above the basal level (?TSH), the peak TSH value expressed as a percentage of the basal value, or the integrated area of the TSH response curve. Determination of TSH before and 30 minutes after the injection of TRH provides information concerning the presence or absence of TSH responsiveness but cannot detect delayed or prolonged responses.

The stimulatory effect of TRH is specific for pituitary TSH, its free a- and ÃŸ- subunits, [447] and prolactin. Under normal circumstances, no significant changes are observed in the serum levels of other pituitary hormones [456] or potential thyroid stimulators. [457] Responsiveness is present at birth, [458] is greater in women than in men, particularly in the follicular phase of the menstrual cycle, [459] and may be blunted in older men, [414,454,455] but this is not a consistent finding. [460] On the average, the magnitude of the response is greater at 11 P.M. than at 11 A.M., [452] in accordance with the diurnal pattern of the basal TSH level which correlates to its response to TRH. Repetitive administration of TRH to the same subject at daily intervals causes a gradual obtundation of the TSH response, [453] presumably due to the increase in thyroid hormone concentration [461] and also in part due to TSH “exhaustion”. [462] However, more than one hour must elapse between the increase in thyroid hormone concentration and TRH administration for inhibition of the TSH response to occur. A number of drugs (see Table 5-4) and nonendocrine diseases (see Chapter 5) may affect to various extents the magnitude of the response.

TRH-induced secretion of TSH is followed by a release of thyroid hormone that can be detected by direct measurement of serum TT4 and TT3 concentrations. [160] Peak levels are normally reached approximately four hours after the administration of TRH and are accompanied by an increase in serum Tg concentration. The incremental rise in serum TT3 is relatively greater, and the peak is, on the average, 50% above the basal level. Measurement of changes in serum thyroid hormone concentration after the administration of TRH has been proposed as an adjunctive test and is useful in the evaluation of the integrity of the thyroid gland or bioactivity of endogenous TSH. [463] Increase in RAIU is minimal and occurs only with high doses of TRH given orally. [455]

Side effects from the intravenous administration of TRH, in decreasing order of frequency, include nausea, flushing or a sensation of warmth, desire to micturate, peculiar taste, light-headedness or headache, dry mouth, urge to defecate, and chest tightness. They are usually mild, begin within a minute after the injection of TRH, and last for a few seconds to several minutes. A transient rise in blood pressure has been observed on occasion, but there are no other changes in vital signs, urine analysis, blood count, or routine blood chemistry tests. [456] , [464] The occurrence of circulatory collapse is exceedingly rare. [465]

The test provides a means to distinguish between secondary (pituitary) and tertiary (hypothalamic) hypothyroidism (Fig. 6-9). Although the diagnosis of primary hypothyroidism can be easily confirmed by the presence of elevated basal serum TSH levels, secondary and tertiary hypothyroidism are typically associated with TSH levels that are low or normal. On occasion the serum TSH concentration may be slightly elevated due to the secretion of biologically less potent molecules, [397] but it remains inappropriately low for the degree of thyroid hormone deficiency. Differentiation between secondary and tertiary hypothyroidism cannot be made with certainty without the TRH test. A TSH response is suggestive of a hypothalamic disorder, and a failure to respond is compatible with intrinsic pituitary dysfunction. [466] Furthermore, the typical TSH response curve in hypothalamic hypothyroidism shows a delayed peak with a prolonged elevation of serum TSH before return to the basal value (Figure 6-9). The lack of a TSH response in association with normal prolactin stimulation may be due to isolated pituitary TSH deficiency. [467] Caution should be exercised in the interpretation of test results after withdrawal of thyroid hormone replacement or after treatment of thyrotoxicosis when, despite a low serum thyroid hormone concentration, TSH may remain low and not respond to TRH for several weeks. [404] , [433] , [436] , [468]

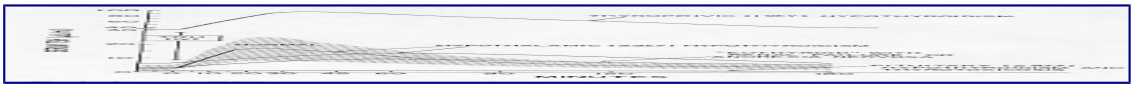

Figure 6-9. Typical serum TSH responses to the administration of a single intravenous bolus of TRH at time 0 in various conditions. The normal response is represented by the shaded area. Data used for this figure are the average of several studies. (From S. Refetoff, Endocrinology, L.J. DeGroot (ed). 1979, Grune & Straton Inc.)

In the most common forms of thyrotoxicosis, the mechanism of feedback regulation of TSH secretion is intact but is appropriately suppressed by the excessive amounts of thyroid hormone. Thus, both the basal TSH level and its response to TRH are suppressed unless thyrotoxicosis is TSH induced. [404] , [407] , [417] With the development of more sensitive TSH assays, the TRH test is generally not needed in the evaluation of a thyrotoxic patient with an undetectable TSH.407 Differential diagnosis of conditions leading to inappropriate secretion of TSH may be aided by the TRH test result. Elevated basal TSH

values that do not respond by a further increase to TRH are typical of TSH-secreting pituitary adenomas. [439] , [441] Patients with inappropriate secretion of TSH due to resistance to thyroid hormone have a normal or exaggerated TSH response to TRH that, in most instances, is suppressed with supraphysiologic doses of thyroid hormone. [442]

Because of the high sensitivity of the pituitary gland to the feedback regulation by thyroid hormone, small changes in the latter profoundly affects the response of TSH to TRH. Thus, patients with non-TSH-induced thyrotoxicosis of the mildest degree have a reduced TSH response to TRH whereas those with primary hypothyroidism exhibit an accentuated response that is prolonged (Figure 6-9, see above). These changes may occur in the absence of clinical or other laboratory evidence of thyroid dysfunction.

The TSH response to TRH, is subnormal or absent in one-third of apparently euthyroid patients with autoimmune thyroid disease, and even members of their family, may not respond to TRH. [469,470] Most, but not all patients with reduced TSH response to TRH, will also show thyroid activity that is nonsuppressible by thyroid hormone. A common dissociation between these two tests is typified by a normal TRH response in a nonsuppressible patient. This finding is not surprising since patients with nonsuppressible thyroid glands often have limited capacity to synthesize and secrete thyroid hormone, due to prior therapy or partial destruction of their glands by the disease process. Clinically, euthyroid patients, who do not respond to TRH, admittedly have a slight excess of thyroid hormone. It is less easy to reconcile the rare occurrence of TRH unresponsiveness in a patient who is suppressible by exogenous thyroid hormone. It should be remembered, however, that a suppressed pituitary may take a variable amount of time to recover, a phenomenon that may be the basis of such discrepancies. [404] , [436] , [468] Despite discrepancies between the results of the TRH and T3 suppression tests, [469,470] the use of the former is much preferred particularly in elderly patients in whom administration of T3 can produce untoward effects.

Thyroid Suppression Test

The maintenance of thyroid gland activity that is independent of TSH can be demonstrated by the thyroid suppression test. Under normal conditions, administration of thyroid hormone in quantities sufficient to satisfy the body requirement suppresses endogenous TSH resulting in reduction of thyroid hormone synthesis and secretion. Since thyrotoxicosis due to excessive secretion of hormone by the thyroid gland implies that the feedback control mechanism is not operative or has been perturbed, it is easy to understand why under such circumstances the supply of exogenous hormone would also be ineffective in suppressing thyroid gland activity. The test is of particular value in patients who are euthyroid or only mildly thyrotoxic but suspected of having abnormal thyroid gland stimulation or autonomy.

Usually the test is carried out with 100 Âµg of L-T3 (liothyronine) given daily in two divided doses over a period of 7-10 days. 24 hour RAIU is obtained before and during the last two days of T3 administration.476 Normal persons show a suppression of the RAIU by at least 50% compared to the pre-L-T3 treatment value. No change or lesser reduction is not only typical of Graves' disease but also other form of endogenous thyrotoxicosis, including toxic adenoma, functioning carcinoma, and thyrotoxicosis due to trophoblastic diseases. The presence of nonsuppressibility indicates thyroid gland activity independent of TSH but not necessarily thyrotoxicosis. Euthyroid patients with autonomous thyroid function have a normal TSH response to TRH before the administration of L-T3. However, inhibition of TSH secretion by the exogenous T3 does not suppress the autonomous activity of the thyroid gland. This is the most commonly encountered discrepancy between the results of the two related tests. When the T3 suppression test is used in conjunction with the scintiscan, localized areas of

autonomous function can be identified. The test can be carried out without the administration of radioisotopes by measuring serum T4 before and two weeks following the ingestion of L-T3. Although total suppression of T4 secretion never occurs, even after prolonged treatment with L-T3, a reduction by at least 50% is normal. [477]

Variants of the test have been proposed to reduce the potential risks of L-T3 administration in elderly patients and in those with angina pectoris or congestive heart failure. With the availability of sensitive TSH determinations and the TRH test, which are less dangerous, thyroid suppression tests are no longer indicated.

Specialized Thyroid Tests

A number of specialized tests are available for the evaluation of specific aspects of thyroid hormone biosynthesis, secretion, turnover, distribution, and absorption. Their primary application is of investigative nature. They are only briefly mentioned here for the sake of completeness.

Iodotyrosine Deiodinase Activity

The test involves the intravenous administration of tracer MIT or DIT labeled with radioiodide. Urine, collected over a period of four hours, is analyzed by chromatography or resin column separation. Normally, only 4-8% of the radioactivity is excreted as such; the remainder appears in the urine in the form of iodide. [480] Excretion of larger amounts of the parent compound indicates inability to deiodinate iodotyrosine. The test is useful in the diagnosis of a dehalogenase defect (see Chapter 16).

Test for Defective Hormonogenesis

After administration of RAI, the isotopically labeled compounds synthesized in the thyroid gland and those secreted into the circulation can be analyzed by immunologic, chromatographic, electrophoretic, and density gradient centrifugation techniques. [481] Such tests serve to evaluate the synthesis and release of thyroid hormone, as well as to delineate the formation of abnormal iodoproteins.

Iodine Kinetic Studies

The iodine kinetic procedure is used to evaluate overall iodide metabolism and to elucidate the pathophysiology of thyroid diseases. The analysis involves follow-up of the fate of administered radioiodide tracer by measurement of thyroidal accumulation, secretion into blood, and excretion in the urine and feces. [482] Double tracer techniques and programs for computer-assisted analysis of data are available.

Absorption of Thyroid Hormone

Failure to achieve normal serum thyroid hormone concentration after administration of replacement doses of thyroid hormone is usually due to poor compliance, occasionally to the use of inactive preparations, and rarely, if ever, to malabsorption. The last can be evaluated by the simultaneous oral and intravenous administration of the hormone labeled with two different iodine isotope tracers. The

ratio of the two isotopes in blood is proportional to the net absorbed fraction of the orally administered hormone. [483,484] Under normal circumstances, approximately 80% of T4 and 95% of T3 administered orally are absorbed. Hypothyroidism and a variety of other unrelated conditions have little effect on the intestinal absorption of thyroid hormones. Absorption may be diminished in patients with steatorrhea, in some cases of hepatic failure, during treatment with cholestyramine, and with diets rich in soybeans. The absorption of thyroid hormone can also be evaluated by the administration of a single oral dose of 100 Âµg T3 or 1 mg T4, followed by their measurement in blood sampled at various intervals. [485,486]

Turnover Kinetics of T4 and T3

Turnover kinetic studies require the intravenous administration of isotope-labeled tracer T4 or T3. [487-491] The half-time (t1/2) of disappearance of the hormone is calculated from the rate of decrease in serum trichloroacetic acid precipitable, ethanol extractable, or antibody precipitable isotope counts. Compartmental analysis can be used for the calculation of the turnover parameters. [488,489] The calculated daily degradation (D) or production rate (PR) is the product of the fractional turnover rate (K), the extrathyroidal distribution space (DS), and the average concentration of the hormone in serum. Noncompartmental analysis may be used for the calculation of kinetic parameters. [488] The metabolic clearance rate (MCR) is defined as the dose of the injected labeled tracer divided by the area under its curve of disappearance. The PR is then calculated from the product of the MCR and the average concentration of the respective nonradioactive iodothyronine measured in serum over the period of the study. Simultaneous studies of the T4 and T3 turnover kinetics can be carried out by injection of both hormones, labeled with different iodine isotopes. [488 , 490,491]

Average normal values in adults for T4 and T3, respectively, are: t1/2 = 7.0 and 0.8 days; K = 10% and 90% per day; DS = 11 and 30 liters of serum equivalent; MCR = 1.1 and 25 liters/day; and PR = 90 and 25 Âµg/day.

The hormonal PR is accelerated in thyrotoxicosis and diminished in hypothyroidism. In euthyroid patients with TBG abnormalities, the PR remains normal, since changes in the serum hormone concentration are accompanied by compensatory changes in the fractional turnover rate and the extrathyroidal hormonal pool. [492] A variety of nonthyroidal illnesses may alter hormone kinetics [491 , 493] (see Chapter 5).

Metabolic Kinetics of Thyroid Hormones and Their Metabolites

The kinetics of production of various metabolites of T4 and T3 in peripheral tissues and their further metabolism can be studied. Most methods use radiolabeled iodothyronine tracers injected intravenously. [489-491] Their disappearance is followed in serum samples obtained at various intervals of time after injection of the tracers by means of chromatographic and immunologic techniques of separation. Kinetic parameters can be calculated by noncompartmental analysis or by two or multiple compartment analysis. Estimates have been made by the differential measurement in urine of the isotopes derived from the precursor and its metabolite. They are in agreement with measurements carried out in serum. [494] Conversion rates (CR) of iodothyronines, principally generated in peripheral tissues, can be calculated from the ratio of their PR, and that of their respective precursors. Some

iodothyronines, such as T3, are secreted by the thyroid gland as well as generated in peripheral tissues. Studies to calculate the CR require administration of thyroid hormone to block thyroidal secretion. [493]

On the average 35% and 45% of T4 are converted to T3 and rT3, respectively, in peripheral tissues. The conversion of T4 to T3 is greatly diminished in a variety of illnesses (see Chapter 5) of nonthyroidal origin and in response to many drugs (Table 5-3). Degradation and monodeiodination of iodothyronines can be estimated without the administration of isotopes. They are, however, less accurate. The conversion of T4 to T3 can be estimated semiquantitatively by the measurement of serum TT3 concentration after treatment with replacement doses of T4. [493]

Measurement of the Production Rate and Metabolic Kinetics of Other Compounds

The metabolism and PRs of a variety of compounds related to thyroid physiology can be studied using their radiolabeled congeners and application of the general principles of turnover kinetics. Studies of TSH have demonstrated changes related not only to thyroid dysfunction but also associated with age, kidney, and liver disease. [495,496] Studies of the turnover kinetics of TBG have shown that the slight increases and decreases of serum TBG concentration associated with hypothyroidism and thyrotoxicosis, respectively, are due to changes in the degradation rate of TBG rather than synthesis. [492]

Transfer of Thyroid Hormone from Blood to Tissues

Transfer of hormone from blood to tissues can be estimated in vivo by two techniques. A direct method follows the accumulation of the administered labeled hormone tracer by surface counting over the organ of interest. [497] An indirect method follows the early disappearance from plasma of the simultaneously administered hormone and albumin, labeled with different radioisotope tracers. [498] The difference between the rates of disappearance of the hormone and albumin represents the fraction of hormone that has left the vascular (albumin) space and presumably has entered the tissues.

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