JOURNAL OF NUCLEAR MEDICINE 6:853-901, 1965

Principles of, and Pitfalls in, Thyroid Function Tests

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INTRODUCTION

Thyroid function tests are now readily available to, and widely used by, practitioners of the medical arts. However, proper interpretation of the results of such tests requires an understanding of the physiological processes being evaluated, and how this evaluation is accomplished.

It is the purpose of this communication to describe the principles of, and some pitfalls in, the uses of certain well established clinical tests with an emphasis on the vagaries encountered. Not all thyroid function procedures will be reviewed, but only those which are in common usage, and which, by apparent inconsistencies, may puzzle the practitioner.

Thyroid diseases will not be discussed in any detail. The various clinical entities of thyroid dysfunction will be discussed only as they affect the function tests, and hyper- and hypothyroidism will be included only as points of reference for the laboratory procedures.

Hyperthyroidism may be defined as the state of response of the body tissues to too much thyroid hormone, and, conversely, hypothyroidism occurs when the body tissues function in the presence of too little thyroid hormone. No laboratory procedure now available specifically indicates the existence of hyper- or hypothyroidism. Of the clinical tests in current usage, the basal metabolic rate (BMR) best reflects the action of thyroid hormones on the body cells. However, many factors other than thyroid hormones are involved in the rate of body metabolism, and the BMR is perforce a nonspecific and frequently an imprecise diagnostic aid in thyroid dysfunction.

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Fortunately, laboratory tests which measure thyroid hormone production, secretion, and serum concentration have a high degree of correlation with the clinical status of the patient as related to thyroid hormones. These tests, because they do not directly reflect thyroid hormone effect on tissues, are occasionally altered by factors other than hyper- and hypothyroidism.

I. Serum Protein Bound Iodine (PBI).

A. General Considerations.

Nearly all of the circulating thyroid hormones, thyroxine (T_4) and triiodothyronine (T^3), are bound to serum proteins. The precipitation of serum proteins followed by careful washes, and the determination of the iodine content of the precipitate constitutes the basic procedure for the determination of the protein bound iodine (1, 2).

Normal values for the PBI determination are usually in the range of 3.5-8.0 μg/100 ml serum (3, 4), with all but 0.5-1.0 μg/100 ml representing hormonal iodine. At the University of Michigan Medical Center (UMMC), the normal range [by the method of Acland (5)], is 3.5-7.5 μg/100 ml serum.

The iodine concentration in the supernatant after the serum protein precipitation indicates the level of circulating inorganic iodine (iodide). The serum inorganic iodine may also be determined by subtracting the PBI from the total serum iodine concentration. The normal range for the serum inorganic iodine[1] by the former method (UMMC) is up to 5.0 μg/100 ml serum, but usually less than 3.0 μg/100 ml, and by the latter procedure (Bio-Science Laboratories, Los Angeles, California) is usually less than 10 per cent, but occasionally as much as 25 per cent of the total iodine (6).

The PBI determination should be accurate enough so that the difference in values of duplicate samples is less than 0.6 μg/100 ml and in different sera from the same individual less than 1.0 μg/100 ml (8). There is no difference in PBI values between normal men and women (9), and no change with age in males beyond adolescence (10), but a slight and significant decrease in PBI was noted in a group of patients of both sexes over age 50 (10a). There is a mild diphasic seasonal variation with PBI values being lower in midsummer and midwinter in populations living in a temperature climate (9).

B. Factors Affecting the PBI Determination.

1. Thyroid Diseases.

a. Hyper- and hypothyroidism will not be discussed here except to say that the PBI may be more accurate in the diagnosis of hypothyroidism than of hyperthyroidism (10a).

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[1]These normal values of inorganic iodine are erroneously high (7) because of the technique of determination, but they serve as a helpful reference point in estimating increased quantities of iodide in a patient.

b. Thyroiditis. Subacute (or granulomatous) thyroiditis, when in a severe form, will pass through several stages. Initially the serum PBI may be elevated from the release of stored hormone by the inflammation, and this may produce a true temporary hyperthyroid state (11-13). Some of the increase in PBI may be from a noncalorigenic iodinated protein which is also released from the diseased gland (14) (see under Butanol-Extractable Iodine). With subsidence of the disease the PBI returns to normal, but occasionally becomes temporarily subnormal until complete recovery takes place (13). Rarely permanent myxedema (and a low PBI) ensues (15). Mild cases of subacute thyroiditis may show but few of the above PBI changes.

Chronic lymphocytic (Hashimoto's) thyroiditis also may have associated a variety of PBI values. Occasionally a circulating noncalorigenic iodinated protein is released from the thyroid gland. This may result in a high PBI with eumetabolism, or a normal PBI with hypometabolism (16, 17). The late stage of chronic thyroiditis may be associated with frank myxedema and and a low PBI (16).

c. Intrathyroidal biochemical disurbances. The biochemical reactions involved in thyroid hormonogenesis may be disrupted at various sites (18). Again the secretion of a noncalorigenic iodinated protein may result in a PBI level which is higher than expected from the clinical picture. Hypothryoidism may occur from a severe biochemical blockade in hormonogenesis.

In rare cases the PBI may be very low in the presence of eumetabolism due to the secretion of triiodothyronine as the only circulating thyriod hormone (19, 20) (see below for the effect of administered triiodothyronine on the PBI).

2. Changes in the binding capacity of serum proteins for thyroid hormones. This subject, including the effect of estrogens and androgens on the PBI, will be discussed in the section on the Triiodothyronine Red Cell (or Resin) Uptake Test.

3. Administration of drugs.

a. Thyroid hormones. The administration of thyroid hormones to euthyroid individuals with an intact thyroid-hypothalamus-pituitary axis will result in a suppression of hormonogenesis equivalent to the biological activity of the exogenous hormone given. At full suppressive doses of thyroid hormone, the serum PBI will reflect only the exogenous hormone. The PBI measurement of a myxedematous individual receiving replacement

therapy will be a manifestation of only exogenous hormone at any dosage level.

The alterations of the PBI from administered thyroid hormone in a subject where the thyroid-hypothalamus-pituitary axis is no longer functioning because of hyperthyroidism will be discussed under the section on Suppression Tests.

In myxedematous and normal subjects, the maximum responses in the PBI may be expected after about four weeks of thyroid hormone administration in suppressive or nearly suppressive amounts (see Table I). After withdrawal of the hormone treatment, the effect on the PBI disappears in about the same period of time (24).

b. Iodine.[1]

1) Iodides (or inorganic iodine), when administered to a patient, may interfere with a valid PBI determination by either or both of the following mechanisms:

a) Simple contamination of the PBI determination may occur from the overwhelming presence of iodine in the patient's serum. If the washes of serum protein precipitate remove 97 per cent of the serum supernatant

TABLE I

EFFECT OF THYROID HORMONES ON THE PBI
Euthyroid or Myxedematous Subjects Receiving Maintenance Dosage

Hormone	*Daily Dosage (mg)*	*PBI (μg/100 ml)*	*References*
Desiccated Thyroid (Armour)	120–180	Normal (4.0–7.2) or Low Normal (2.9–6.2)	(21) (22)
Desiccated Thyroid (Warner-Chilcott Special Prep.)	120–180	Low (3.3–3.7)	(23)
Purified Thyroglobulin (Proloid of Warner-Chilcott)	120–180	Low to Low Normal (1.6–4.8)	(22)
Thyroxine (Synthyroid of Flint)	0.2–0.3	High Normal to High (5.6–11.2)	(21, 22)
Triiodothyronine (Cytomel of Smith, Kline and French)	0.05–0.1	Low (0.4–1.4)	(22)

[1]Lists of substances, including those used in external applications, may be obtained from laboratories performing PBI determinations and from companies supplying radioactive iodine.

(containing the circulating iodide), the remaining three per cent of the supernatant with a normal inorganic iodine, 3.0 μg/100 ml, adds little, 0.03 μg/100 ml, to the precipitate iodine (PBI). However, if the serum inorganic iodine level should be 100 μg/100 ml or more, than the contamination of the precipitate and PBI by supernatant iodide is appreciable. This type of interference with a valid PBI determination should be easily recognized by an elevated serum inorganic iodine value.

b) The production of a nonhormonal iodinated protein, or protein-like substance, by some body tissue(s) (25) in the presence of high levels of iodide may lead to artifactual rises in the PBI. This is probably a more troublesome type of alteration in the PBI concentration by administered iodides. The mechanism is poorly understood, and the PBI changes are "capricious, of variable magnitude, and sometimes large" (4). Although an elevated serum inorganic iodine concentration (especially greater than 10 μg/100 ml) is usually suggestive of iodide contamination, it is not always well correlated with the magnitude of PBI aberration.

It is said that the ingestion of up to 125 mg of iodide per day will not appreciably alter the PBI levels of an individual (26). However, this may be true for only relatively brief periods of administration (10 days in the study of Friend (26) and four to seven weeks in the work of Danowski, et al (27)), and lesser amounts of daily iodide intake over weeks or months may have more definite effects on the PBI test.

The elevation of the PBI concentration by iodides may persist for one half to two and one half months after moderate dosage, 200-600 mg/d, and as long as four months after massive amounts, 3-6 g/d, of iodides (27, 28).

2) Iodine compounds (organic iodine) are, when in the serum, bound by proteins, and thereby distort the protein bound iodine determination. Iodine compounds are removed from the body by metabolic degradation with the release of iodide, and/or by excretion of the intact, or partially degraded, compound. Arbitrarily, these iodinated substances may be divided into three types by the time required for biological removal from the human body:

a) Short-lived compounds, which artifactually elevate the PBI concentrations for 3-8 days, include most of the intravenous aqueous radiocontrast media, as iodopyracet[1] and sodium diatrizoate[2] (29, 30).

b) Intermediate-lived drugs, affecting the PBI values for 6 to 12 weeks, include, among others, most of the common gallbladder contrast media (iodopanoic acid,[3] iodoalphionic acid[4] (29, 30), and iodopamide methylaglucamine[5] (31).)

c) Long-lived agents, producing elevated PBI levels for years, have as typical examples:
iodized poppy seed oil (Lipiodal) (32, 33), ethyl iodophenylundecylate[6] (29), and the unusual gallbladder contrast medium, iophenoxic acid.[7] This last compound will persist at least 33 years, and will cross the placenta (35).

Because of its common usage, providone-iodine[8] deserves comment. This agent is absorbed after topical application and elevates the PBI level, but the length of effect is not yet recorded (36).

In general, the more rapid the degradation of an iodine compound, the higher will be the inorganic iodine level. However, the elevation of the serum inorganic iodine concentration from the intermediate-lived iodinated compounds may disappear before the PBI value returns to normal, and thus eliminate the inorganic iodide determination as an index of contamination in the PBI (37). The long-lived compounds produce only a modest rise in inorganic iodine concentration which may persist for months (29, 33), but in the case of iophenoxic acid, there is no increase in the serum iodide value (38).

d. Antithyroid drugs. There are many compounds which will inhibit thyroid hormonogenesis to variable degrees. It requires many months to alter the PBI concentration in euthyroid individuals by such agents. However, hyperthyroid patients may have a prompt reduction in the PBI values, even to hypothyroid levels, in response to therapy by antithyroid drugs (39).

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[1]Diodrast, Hypaque.
[2]Telepaque.
[3]Winthrop Laboratories, N.Y., N.Y.
[4]Priodax, Schering Corp., Bloomfield, N.J.
[5]Cholografin, Squibb & Sons, N.Y., N.Y.
[6]Pantopaque, Lafayette Pharmaceuticals.
[7]Teridax Schering Corp., Bloomfield, N.J.
[8]Betadine, Physicians' Products Co., Inc., Petersburg, Va.

e. Miscellaneous drugs.

1) Adrenal corticosteroids (in doses equivalent to 100 mg of cortisone or more) have been shown to produce a mild to moderate depression in PBI values (40, 41), but inconsistently (41, 42). The mechanism of this action has not been fully elucidated (43).

2) Although in the chloric acid method of Zak (2) the PBI values are falsely low for a few days following the administration of a mercurial diuretic to a patient, this is not true when the alkaline dry ash method of Barker is used (44). Other clinical diuretics do not affect the PBI concentration (44).

4. Extra-thyroidal diseases.

a. Choriocarcinoma and embryonal testicular carcinoma may elevate the PBI values as well as other thyroid function parameters, probably from secretion of a thyrotropin-like substance from the tumors (45-47).

b. Acute intermittent porphyria may increase the PBI concentration, possibly by increasing the total extra-thyroidal thyroxine pool (48, 49).

c. Collagen diseases may be associated with an elevation in PBI values of unknown etiology (50).

d. Disturbances in PBI concentrations by liver disease, nephrosis will be considered under the Triiodothyronine Red Cell Uptake Test.

Addendum: Recently, therapy with Au salts has been found to result in low PBI levels for an unknown length of time due to artifactual changes in the laboratory technique induced by the gold (206).

II. Serum Butanol-Extractable Iodine (BEI) and Thyroxine-by-Column Chromatography (T_4 by Column) and Other Tests of Serum Hormonal Iodine.

A. General Considerations.

Serum extracted with acidified n-butyl alcohol and subsequently treated with alkali will result in a solvent residue of thyroxine and triiodothyronine. This hormonal extraction is expressed in terms of iodine concentration: Butanol-Extractable Iodine. A similar result is achieved by column chromatography using Dowex-1, x-2 resin and the iodine concentration is termed: Thyroxine-by-Column (52). The values obtained by both methods are similar with a normal range of 3.2-6.4 μg/100 ml of serum (52, 53). The BEI concentrations generally average 0.6 μg/100 ml below the PBI values, but differences of 2.0 μg/100 ml may not be unusual (17, 51). Both the BEI and T_4-by-Column are difficult to perform and are likely to be less precise than the PBI determination.

TABLE IIA

EFFECT OF IODOTYROSINES AND IODIDE ON RELIABILITY OF PBI, BEI AND COLUMN METHODS*

Additions to serum	Serum level (μg. iodine/100 ml.)				
	PBI method	BEI method	Column method (fraction)		
			1	2	3
None	4.8	3.9	3.5	0.6	0.1
MIT+DIT†	10.8	4.0	3.3	0.6	0.2
KI‡	7.1–9.9	3.8	3.1	0.9	0.1

* PBI and BEI values are averages of 4 determinations; column values are averages of duplicate analyses.

† Monoiodotyrosine and diiodotyrosine present at a concentration of 3.3 μg. per 100 ml. (as iodine) each.

‡ KI present at a concentration of 1000 μg. per 100 ml. (as iodine); precipitate washed four times with distilled water.

Table IIA. These data are reproduced from the article by Pileggi *et al* in *J. Clin. Endocr.* 21:1272, 1961 by permission of the authors and publishers. The column method results are expressed as a sum of fractions 1 and 2.

The BEI, and presumably T_4-by-Column, measurements under physiologic circumstances will fluctuate in the same direction as the PBI values (see above). The BEI concentration may be in the low normal range in adolescent males (54).

B. Factors affecting the BIE and T_4-by-Column Determination. The principal value of these techniques is to circumvent the artifactual changes which invalidate the PBI as a thyroid function test.
   1. The major indication for use is the presence of iodine contamination of sera.
      a. The administration of iodides does not alter the values of BEI and T_4-by-Column (Table IIA).
      b. In general, most iodine compounds distort the results of both of these specialized methods of estimating thyroid hormone levels in serum. However, there are exceptions such as iodipamide methylglucamine (Cholografin) and iodoalphionic acid (Priodax) which elevate the PBI and BEI but not the T_4-by-Column values. Table IIB lists a number of iodine compounds in common medical use and their effects on these tests.
   2. The release of noncalorigenic iodinated proteins by the thyroid gland has been mentioned under the section on the PBI. In general, these unusual substances are insoluable in the butanol extraction, and possibly do not show up in the T_4-by-Column. A variety of thyroid diseases appear to produce the syndrome of an unusually large difference between the PBI and BEI values: goitrous cretins, goitrous euthyroid adults, colloid goiter, subacute thyroiditis (14), chronic thyroiditis, Hashimoto's disease, (14, 17), follicular adenoma (14),

autonomous adenomas (55), cancer (14), hyperthyroidism (56), and following ^{131}I therapy (57). In these cases the BEI is thought to reflect more accurately the clinical state of the patient.

3. A summary of the BEI changes as related to the respective PBI values is given in Table IIC.

C. Two newer methods of serum thyroid hormone assessment apparently eliminate any influence of iodine compounds:

1. The use of Blau's reagent and countercurrent distribution (58).
2. The use of serum thyroxine binding proteins and Sephadex column separation (59).

It is to be hoped that one of these methods may become a routine laboratory procedure to aid in the diagnosis of thyroid dysfunction.

III. Thyroidal Radioactive Iodine Uptake (RAIU) Test.

A. General Considerations.

The concept of thyroidal uptake of radioiodine may be best illustrated as a body iodide pool (somewhat ill-defined) from which the thyroid gland extracts its requirement of iodide. If this iodide pool is labeled uniformly with an administered tracer of radioidide, the per

TABLE IIB

EFFECT OF *in-vitro* ADDITIONS OF ORGANIC IODINE COMPOUNDS ON RELIABILITY OF PBI, BEI AND COLUMN METHODS*

Iodine compound added to serum		Serum level (μg. iodine/100 ml.)				
Chemical name	Common	PBI method	BEI method	Column method (fraction)		
				1	2	3
None	None	4.8	3.9	3.4	0.5	0.0
N,N'-adipyl-bis-3-amino-2,4,6-triiodobenzoic acid	Cholografin	>25.0	7.5; 20.0	3.4	0.6	0.0
3,5-Diiodo-4-pyridone-N-acetic acid, n-propyl ester	Dionosil	>25.0	>25.0	5.7	2.6	>25.0
o-Iodohippuric acid	Hippuran	>25.0	>25.0	>25.0	0.6	0.0
5-Iodo-2-thiouracil	Itrumil	22.0	17.0	>25.0	4.7	2.0
3,5-Dipropionylamino-2,4,6-triiodobenzoic acid	Miokon	7.0; 12.9†	4.3	9.1	12.8	13.1
1-Methyl-3,5-diiodo-4-pyridone-2,6-dicarboxylic acid	Neo-Iopax	23.0	4.4	3.0	0.6	0.0
3-(3-Butylamino-2,4,6-triiodophenyl)-2-ethyl acrylic acid	Orabilex	>25.0†	>25.0	>25.0	15.2	2.3
Iodopropylidene glycerol	Organidin	8.7†	>25.0	3.4	0.4	0.0
3-(4-Hydroxy-3,5-diiodophenyl)-2 phenylpropionic acid	Priodax	>25.0	12.5;>25.0	3.5	4.5	6.2
3-Acetylamino-2,4,6-triiodobenzoic acid + polyvinyl pyrrolidone	Salpix	>25.0	4.7; 7.1	3.3	0.8	1.6
Iodomethane sulfonic acid	Skiodan	>25.0	4.1	3.3	0.5	0.0
3-(3-Amino-2,4,6-triiodophenyl)-2-ethyl propionic acid	Telepaque	>25.0	>25.0	>25.0	13.3	8.9
3-(3-Hydroxy-2,4,6-triiodophenyl)-2-ethyl propionic acid	Teridax	>25.0	>25.0	>25.0	>25.0	>25.0
3-Acetylamino-2,4,6-triiodobenzoic acid	Urokon	>25.0	5.5; 6.4	3.5	0.6	0.2

* All values are averages of 2 analyses on separately prepared samples (PBI and BEI in duplicate; column analyses, single determinations).
† Elevated inorganic iodine levels in screening test (25–500 μg. per 100 ml.).

Table IIB. These lists are reproduced from the *J. Clin. Endocr. 21*:1272, 1961 (Pileggi *et al*) by permission of the authors and publishers. The column method results are expressed as a sum of fractions 1 and 2.

cent of total ^{131}I given to the subject that is accumulated in the thyroid gland will also represent the percentage of the iodide pool (in μg) that has entered the gland.

Normal values of RAIU (in per cent of dose) may be established at any time after the administration of the tracer ^{131}I, but useful and convenient times have been 2, 4, 6 and 24 hours. Because of variation in early iodide absorption, uptakes should not be performed until at least two hours after ingestion of ^{131}I.

The normal limits vary from laboratory to laboratory depending upon the details of technique, and upon the geographical area which determines, to a considerable degree, the average iodide intake and subsequent iodide pool in a member of the residing population (58). Problems in technique

TABLE IIC

Summary of PBI and Associated BEI Changes[1]

Elevated PBI	*BEI Values with Reference to PBI*
Hyperthyroidism	Concordant
Chronic thyroiditis	Normal and discordant
Subacute thyroiditis	Normal and discordant
Increased hormone binding by serum proteins (T3 Test)	Concordant
Thyroxine administration	Concordant
Iodine administration	
Iodides	Normal and discordant
Iodine compounds	Usually concordant, but may be normal
Extrathyroidal disease	
Choriocarcinoma	Concordant
Pheochromocytoma	Concordant
Acute intermittent porphyria	Concordant
Acute liver disease (binding protein change)	Concordant
Depressed PBI	
Hypothyroidism	Concordant
Chronic thyroiditis	Concordant
Subacute thyroiditis	Concordant
Decreased hormone binding by serum proteins (see T3-Test)	Concordant
Triiodothyronine secretion or administration	Concordant
Extrathyroidal disease	
Nephrosis (binding protein change)	Concordant
Cirrhosis (binding protein change)	Concordant

[1]Normally the BEI varies concordantly with the PBI, but averages 0.6 μg/100 ml less (51).

have recently been analyzed in an international meeting (59), and the procedure used at the UMMC Nuclear Medicine Unit follows the suggestions of this conference.

The reproducibility of the RAIU, for unknown reasons, is less precise than one would expect from the experimental technique (60). The variability of the 24 hour RAIU in the same individual has been recorded as ± 7 per cent, of the uptake value, but on occasion is as high as 10 per cent (61). Earlier times of uptake estimation after the tracer administration probably have similar reproducibilities.

There is a slight decrease in percentage of radioiodine accumulated by the thyroid of males at two and six hours with increasing age (62), but no statistically significant decline was seen in the 24 hour RAIU values with aging (62, 63). No definite influence of sex or season on the RAIU measurements was found in one study (63). More recently it has been suggested that the warmer summer months may be associated with a decrease in RAIU values, perhaps because of a smaller distribution space for ^{131}I (64).

Normal values are summarized in Table IIIA.

B. Factors Affecting the Thyroidal Radioactive Iodine Uptake.

1. Thyroid Diseases.

a. Hyper- and hypothyroidism. Uptakes determined a few hours after the administration of the tracer ^{131}I have been found to be more accurate than the 24 hour interval in the diagnosis of hyperthyroidism. The change from the 8 to the 48 hour values was

TABLE IIIA

Normal Values of Thyroidal Radioactive Iodine

	Hours After Dose	*Normal Value (% of dose as a range or mean ± SD)*
Oral Administration of ^{131}I		
At UMMC	2	1.4 – 13.4
	6	2.0 – 25.9
	24	10.5 – 38.9
In New England (65)	24	20 – 50
Intravenous Administration of ^{131}I		
131 Males Aged 41–94 (62)	2	12.2 ± 3.6
	6	20.3 ± 6.4
	24	35.9 ± 9.9

shown to be the most accurate of the uptake tests in differentiating hyperthyroidism (−46 to +7% change) from euthyroidism (0 to +23% change) (66).

After treatment of hyperthyroidism the RAIU tends to be less reliable as an index of the clinical state than the PBI determination. The former may be elevated in the presence of euthyroidism (67)

The 24 hour RAIU is probably more reliable than earlier uptake values in the diagnosis of hypothyroidism.

b. Thyroiditis. As with the PBI, the RAIU measurements change during the various stages of subacute thyroiditis. Initially, there is often a very low uptake which may be associated with an elevated PBI level (11, 13), a picture that is to be distinguished on clinical grounds from iodine administration. With recovery from the disease, the RAIU returns to normal, and, on occasion, rises temporarily to even supernormal values. Again, permanent myxedema, with low thyroid uptakes, may rarely complicate the course of subacute thyroiditis (15).

Chronic thyroiditis of the Hashimoto type exhibits, as with the PBI, a broad range of RAIU values. The RAIU levels are occasionally elevated beyond normal (16, 68), and are frequently higher than expected from the clinical, BEI, and BMR findings. Inefficient use of the accumulated iodine by the thyroid gland, as in the production of nonhormonal iodinated compounds, may account for the descrepencies between the RAIU and other function tests. Hypothyroidism and very low RAIU measurements may be recorded late in chronic thyroiditis.

c. Intrathyroidal biochemical disturbances. This wide spectrum of disorders may be associated with the full range of RAIU values: very low when an iodide trapping defect is present, normal with some mild disorders of various types, high with an early plateau and subsequent fall at 24 hours in defects involving iodine organification, and persistently high, even in the presence of hypothyroidism, when a deficiency in the deiodinase enzyme or an inability to couple iodotyrosines exists (18).

2. Administration of Drugs.

a. Thyroid hormones. The difference in responses of suppression in the RAIU between normal individuals and those with hyperthyroidism will be discussed under the section on Suppression Tests.

The normal thyroid gland usually recovers after cessation of suppression of function by triiodothyronine treatment with supernormal RAIU values in 6-12 days, and normal uptake measurements in 16-21 days (24). The administration of desiccated thy-

roid to normal subjects for varying periods of up to several years resulted in RAIU suppression that persisted in most cases for about two weeks after withdrawal of the drug, but occasionally low uptakes were present for 6-12 weeks (69). In some patients, withdrawal of desiccated thyroid medication is also followed by a brief high rebound phase (69, 69a). A crude thyroxine preparation reduced RAIU values to low levels which gradually returned to normal over one to two months following the therapy (70).

b. Iodine.

1) Iodides (inorganic iodine)

Iodides, in the vast majority of cases, affect the RAIU tests by altering the body iodide pool from which the thyroid gland extracts its needs for hormonogenesis.

A chronic deficiency in daily iodide ingestion will result in a low iodide pool, and the percentage of this pool required for normal thyroid hormonogenesis is higher than normal (Table IIIB). Individuals with low iodide pools may not be rare in the United States, (71) although such instances are generally considered characteristic of endemic goiter areas.

Normally, man ingests 100-300 μg of iodide with food and water each day, but iodides of unusual quantity may enter the body through the skin, vagina, respiratory mucosa, and by parenteral injection as well as via the gastrointestinal tract. The normal human iodide pool may be pictured as containing 280 μg of iodide[1] from which the thyroid gland accumulates 70 μg (25%) in 24 hours (7). The relationships of different iodide pools to the RAIU are demonstrated in Table IIIB.

Since in normal individuals hormonogenesis continues in the presence of excessive iodide pools, the thyroidal requirement for iodide, 70 μg/d, does not change.[2]

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[1]This value is too high if the iodide pool is calculated from the clearance of serum iodide (7). However, this pool must turn over rapidly, and the use of 280 μg is convenient for purposes of illustration.

[2]Actually the thyroid gland is slow to recognize increased serum levels of iodides. Thus, with iodide ingestion of up to 1000 μg/d, the thyroid gland for a few days takes up more iodide so that the RAIU (the percentage of the enlarged iodide pool) changes very little (7,72,82). Gradually, the thyroid gland adapts to the high iodide environment, and the RAIU falls as the total iodide uptake by the gland returns toward normal. It should be emphasized that the failure of changing pool size to alter the RAIU occurs only over a brief period of time and a relatively narrow range of iodide ingestion. The vast majority of cases of excessive iodide intake are beyond this range and affect the RAIU by dilution of the pool.

This sluggish adaptation in the accumulation of iodide by the thyroid gland in response to varying serum iodide concentrations is fortunate for the clinician, since moderate fluctuations in daily iodide ingestion are not reflected in the RAIU. A diet of measured iodide content as a preparation for a RAIU is obviated by this thyroid phenomenon.

TABLE IIIB

Variable Iodide Pools and the RAIU

	Iodide Pool (μg)	*Thyroid Uptake at 24 Hours* (μg)	% ^{131}I (*RAIU*)
Normal	280	70	25
Iodide Deficiency	100	70	70
Iodide Excess	2800	70	2.5
Iodide Excess	28000	70	0.25
TSH in Iodide Excess	28000	105	0.38

Iodide pools exceeding 28,000 μg are not unusual since Lugol's iodine and saturated solution of potassium iodide contain respectively, 8,300 and 50,000 μg per drop.

In a single administration 2000 μg of iodide will lower the RAIU to a modest extent in euthyroid individuals although all of this reduction may not be due to dilution of the iodide pool in such an acute experiment (72). Chronic excessive iodide administration will result in an expanded iodide pool and a low RAIU roughly proportional to the level of iodide intake.

The length of the depressive effect of prolonged and excessive iodides on the RAIU after withdrawal of the medication is usually 3 to 14 days (73). Occasionally a prolonged suppression up to one year may occur (73), presumably from a chronically expanded iodide pool which, for unknown reasons, is not reduced to normal by renal iodide excretion. Also, a *rebound* of RAIU is sometimes seen within five days of withdrawal of iodide therapy when the uptake of ^{131}I rises to supernormal levels and persists in this range for as long as 49 days (73).

Knowledge of the concentration of serum inorganic iodine is frequently helpful, but this is only a rough and insensitive index of body iodide pool size.

Although the administration of thyroid stimulating hormone (TSH) will increase the function of the normal thyroid gland during the RAIU depression by excessive iodides, this increased function cannot be detected in the presence of a large iodide pool because of the technical inability to measure the change in the very small RAIU values (Table IIIB).

There appears to be no significant loss or retention of iodide through defects in excretion (74).

Another possible but much less frequent influence of excessive iodides on the thyroidal uptake of ^{131}I is through pharmacologic effects on the gland. Such effects are not well understood but appear to involve an inhibition of thyroid hormone release and a block of organification of iodine (75, 76). This latter action of

iodide on the thyroid gland is comparable to the Wolff-Chaikoff effect in animals (77).

The pharmacologic effect in the normal human thyroid is induced by quantities of iodide greater than 2 mg (72), but this must be temporary since hormonogenesis eventually proceeds without abnormality (76).

A permanent pharmacologic action of iodides is seen in four types of thyroid disorders:

a) Iodide goiter (78, 79)
b) Hashimoto's disease (80)
c) Autonomous nodules (80)
d) Graves' disease (81, 82) (Here the permanent inhibition of function may be more on thyroid hormone release than at the site of iodine organification (75). However, the thyroid in Graves' disease recovers rapidly from the effects of iodides, and by 72 hours following ingestion of 100 mg of potassium iodide, the RAIU is near pretreatment levels (83).)

It is possible that some instances of iodide goiter may have Hashimoto's (84) or Graves' disease (85) as underlying disorders.

2) Iodine compounds (except iothiouacil sodium[1]) affect the RAIU solely by their degradation to iodide and alteration occurs principally by dilution of the body iodide pool.

In general the duration of RAIU depression (33, 86) corresponds to the elevation of the PBI by the respective compounds (see PBI section above). However, the time required for return to normal for the RAIU is less predictable than that of the PBI (30), as might be expected from the variable RAIU recovery periods after the administration of iodides. In cases of hyperthyroidism, there is a rapid return to basal RAIU levels, and following the intermediate-lived contrast media, as iodoalphionic acid (Priodax) and iodopanoic acid (Telepaque), such subjects manifest elevated RAIU values within one week (87).

In the case of iophenoxic acid (Teridax) the degradation is so slow, permitting a biological life of this drug of 33 years, that no significant iodide is released, and the RAIU is unaffected by this gallbladder contrast medium (38).

The influence of Betadine on the RAIU has not yet been reported.

c. Antithyroid drugs. Thiourylene type of drugs inhibit organification of iodine in the thyroid gland, and the RAIU will thereby be reduced at 24 hours after administration of the tracer, but

[1]Itrumil, Ciba Pharmaceutical Co., Summit, N.J.

since the trapping of iodide is unaffected, iodide accumulation and the early RAIU values may be increased following an augmentation of thyrotropin secretion due to decreased thyroid hormonogenesis. A rebound of the RAIU to high levels may occur following the cessation of these drugs (88).

Perchlorate and thiocyanate treatments inhibit the trapping of iodide by the thyroid gland, and the resulting RAIU values are low at all intervals following a tracer dose.

d. Miscellaneous drugs. Adrenal corticosteroids in relatively large doses (greater than 100 mg/d of cortisone) lower RAIU values (40, 41) simultaneously with reduction of the PBI concentrations. The mechanism is not entirely understood (43), Phenylbutazone therapy also depresses RAIU values by an undetermined action (89).

3. Extrathyroidal diseases.
   a. As was noted above, all thyroid function parameters, including the RAIU, may be increased in cases of choriocarcinoma and embryonal cell carcinoma of the testis probably because of secretion of thyrotropin-like substances from the tumors (45-47).
   b. Gastrointestinal malabsorption states ordinarily do not influence the RAIU significantly (74).
   c. Impaired liver function may be associated with increased RAIU values with normal PBI measurements (90, 91). It is possible that poor dietary habits have led to diminished iodide pools in these individuals.
   d. Renal diseases usually have little or no effect on the RAIU values (74, 92), but occasionally the urinary hormonal loss in nephrosis may lead to elevated levels of thyroid uptake (93).
   e. Congestive heart failure, for unknown reasons, is occasionally associated with low RAIU measurements (94).

A summary of the factors affecting the RAIU and the corresponding PBI changes is seen in Table IIIC.

IV. Stimulation Tests.

A. General Considerations.

When the diagnosis of hypothyroidism is established, the important question of pathogenesis requires attention. Differentiation of primary thyroid failure from the hypothyroidism associated with pituitary hyposecretion of thyrotropin may be accomplished by the clinical history and physical examination, but occasionally this problem requires special laboratory aids for solution.

The diagnosis of the type of myxedema, primary or pituitary, that is present in an individual is not readily verified by the usual laboratory tests of endocrine function. Thyroid function tests give low values which are usually compatible with either disorder. Evaluation of other endocrine organs may be misleading. A selective loss of thyrotropin in pituitary disease may produce little in the way

of changes in other endocrine organs that might provide clues as to the principal disease process (95, 96). Also, thyroid gland failure itself frequently leads to depression of adrenal and gonadal function tests (97, 98), although insufficiency of clinical significance may not exist in these latter endocrine organs. Serum levels of thyrotropin are ordinarily high in early primary thyroid failure, but prolonged myxedema may be associated with low or absent serum thyrotropin values (99-101).

Estimation of the thyroid functional integrity through stimulation tests offers a laboratory method of resolving the dilemma.

TABLE IIIC

Summary of Thyroidal Radioactive Iodine Uptake Changes

Type of RAIU Disturbance	*Concomitant PBI*
ELEVATED VALUES	
Hyperthyroidism	High
Hyperthyroidism Adequately Treated	Normal
Low Iodide Pool	Normal
Thyroid Diseases	
Hashimoto's (Early)	Normal or High
Subacute Thyroiditis (Recovery)	Low or Normal
Intrathyroidal Biochemical Disturbances	Low or Normal
Rebound from Inhibition of Hormonogenesis	
After Iodide Therapy	Normal
After Antithyroid Drug Therapy	Low or Normal
Extrathyroidal Diseases	
Cirrhosis	Low or Normal
Nephrosis	Low
Choriocarcinoma	High
DEPRESSED VALUES	
Hypothyroidism	Low
Large Iodide Pool	
Iodide Administration	Normal or High
Organic Iodine Administration	High
Thyroid Diseases	
Hashimoto's (Late)	Low
Subacute Thyroiditis (Early)	Normal or High
Intrathyroidal Biochemical Disturbances	Low or Normal
Thyroid Hormones	Depends on Type
Antithyroid Drugs	Low or Normal
Adrenal Corticosteroids	Low
Extrathyroidal Diseases	
Congestive Heart Failure	Normal

The normal human thyroid gland responds to the parenteral administration of commercially prepared thyrotropin with a release of thyroid hormone in minutes, and an increase in radioiodine uptake in 8-15 hours (102, 103). These responses serve as a basis for evaluating the competency of the thyroid gland, and aid in distinguishing primary thyroid disease from pituitary insufficiency.

B. Problems in Thyroid Stimulation Tests.

Unfortunately, reports of thyroid stimulation tests have been varied as to technique and response, and only a few have described more than 10 subjects. Not only is there a lack of general agreement upon the magnitude and number of doses of TSH, but the criteria for the responses in normal subjects, and those with primary and secondary myxedema are not clearly defined. It is important to know not only how stimulation test values in disease vary from normal, but also how the results found in primary hypothyroidism differ from those of pituitary failure. Comparisons of the latter type are relatively rare in the medical literature.

1. Types of tests.

Tabulated in Tables IVA and IVB are a few thyroid stimulation tests which are representative of reports found in the literature. These are arbitrarily divided into a. those employing multiple injections, and b. those using a single dose of thyrotropin.

2. Merits of Individual Protocol Features.

a. Dosage of Thyrotropin.

A dosage of thyrotropin based upon body weight appears to give a more uniform RAIU response than would be expected from a fixed quantity of TSH for all individuals. Einhorn (102, 103) has demonstrated that the normal RAIU response to TSH is maximal following 0.025 U (USP) per kg of body weight in a single injection. However, it was emphasized that a plateau in the RAIU dose-response curve following TSH was true of subjects with normal thyropituitary function, and it is possible that larger quantities of hormone may be required to elicit a maximal change when thyroid and/or pituitary dysfunction exists.

The rise in PBI concentration following the administration of TSH was not maximal below a dosage of 0.3 U/kg, and is not very large when the quantity of hormone administered is less than 0.1 U/kg (103).

Four (110) and five (104, 109, 111) units of TSH produced, in the ensuing 48 hours, little or no increase in the PBI concentration of patients receiving thyroid hormone therapy, although a response was elicited in the RAIU measurements during this time. Subjects with pituitary insufficiency were found to respond to injected TSH in a manner similar to those treated with thyroid hormones, *i.e.* an increase in the RAIU which was slightly less than normal (111, 112), but no definite rise in PBI (111). The experience with hypopituitary patients has not been uniform, and TSH may elicit a normal augmentation in the RAIU (106, 107), and, with multiple doses, nearly

normal PBI increases (104, 105). Three daily injections of thyrotropin produce a cumulative rise in the RAIU (113).

Because of the high frequency of untoward symptoms following receipt of TSH in large quantities,[1] individual doses of any practical test must be limited to about 0.1 U/kg (5-10 U in most subjects), although multiple injections of 5U do not seem to induce added symptoms (113).

b. Time of Testing

The maximal increase in the RAIU values of normal subjects appeared between 18 and 24 hours following a TSH injection (102, 103).

The augmentation of the PBI concentration in normal individuals appeared to be greatest between 24 and 48 hours subsequent to the administration of thyrotropin (108, 109). Although a peak PBI level may be achieved as early as 15 hours post-TSH, (114), PB ^{131}I data also point to the 24-28 hour period for the maximal PBI response to thyrotropin (102). The administration of thyroid hormone to subjects, who then presumably have subnormal endogenous thyrotropin secretion, may result in a peak PBI response that is delayed to 72 hours following a TSH injection (109).

c. Type of RAIU test.

Jefferies notes that 10 per cent of individuals will have a natural variation between two 3-hour RAIU tests of a magnitude to obscure any TSH effect (115). This variability may be no less between two 24-hour RAIU tests, (60), although this longer period for uptake tests is more commonly used.

3. Recommendations for a Thyroid Stimulation Test.

Using the knowledge gained from the literature as noted above, it is possible to recommend a thyroid stimulation test protocol for the average clinical laboratory.

a. A baseline 24 hour RAIU and PBI are determined.
b. Thyrotropin, 5 units, is given intramuscularly (the remaining hormone in a commercial vial may be stored for a few days in a refrigerator).
c. Twenty-four hours after the thyrotropin injection, and following a count of residual activity in the thyroid gland, a second tracer of ^{131}I is administered, and a 24 hour RAIU is completed the following day (48 hours post-TSH).
d. A second PBI is obtained 48 hours after the TSH injection.
e. Normal values may be estimated from the data of Taunton, *et al*, Tables IV A and IV B, but are best determined for each laboratory.

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[1]Symptoms (principally hyperthyroidism, nausea and vomiting, fever, and precordial pain) occurred in 7% of males and 15% of females following 0.1 U/kg of TSH (102); and 8% and 17% of all subjects tested after 5U on 3 days and 10U on 1 day, respectively (104).

f. If there is a subnormal response in the results of either the RAIU or the PBI, the stimulation test should be repeated using thyrotropin 5U injections on each of three days instead of one. Again, normal values may be seen in Tables IV A-B.

4. Pitfalls in Stimulations Tests.

a. It should be recognized that the thyroid stimulation test as described above is an indirect assessment of pituitary hyposecretion of thyrotropin as a cause of hypothyroidism.[1] Since some normal subjects will have RAIU and PBI values that, through natural biological variation, fall outside an accepted range of normal, a true state of hypothyroidism should be proven to exist before accepting adequate thyroid gland responses to TSH as indicative of pituitary dysfunction.

b. Neutralizing antibodies to commercial thyrotropin have been demonstrated in human subjects after multiple injections of this hormone (116). Although such antibodies usually do not result in a refractoriness to TSH until after many doses have been given, some patients will be encountered who have had multiple tests of thyropituitary function using commercial thyrotropin. Caution should be exercised in the interpretation of poor thyroidal responses to TSH in these individuals.

c. Occasionally pituitary failure will be associated with irreversible thyroid atrophy (105, 114, 117, 118), and possibly quite frequently in Sheehan's syndrome (104). Thus, stimulation tests, as with all laboratory data, should comprise only a part of a complete clinical evaluation. Prolonged thyroid hormone therapy may also result in thyroid gland suppression that is unresponsive to the usual stimulation test (105, 118). In a few instances of the latter category, the thyroid may resume normal function after cessation of hormone treatment, and the gland may later be responsive to TSH (118).

d. Untoward reactions, and possibly death (104), may result from stimulation tests, especially with the larger thyrotropin doses. The test should be used with clear indications. Athough probably rare, adrenal crisis may be precipitated by the increased metabolism from the thyroid hormone released by TSH in a patient with pituitary insufficiency (119). This would be more likely to follow multiple injections than a single dose of thyrotropin. Again, a complete clinical evaluation will alert the physician to the possibility of hypoadrenocorticism which may be worsened by thyroid stimulation. The latter catastrophe can be obviated by prior adrenocortical hormone therapy.

[1] A test which assesses endogenous thyrotropin secretion by evaluating its effect on the thyroid gland has been described (95).

e. It is possible that a myxedematous patient who retains a remnant of thyroid tissue may respond with both a rise in PBI and RAIU values after many doses of thyrotropin (117).

C. Uses of the Thyroid Stimulation Test.

1. Because thyrotropin hyposecretion may be an isolated pituitary defect (96), and because the underlying hypophyseal disease may be of serious nature, probably all cases of spontaneous hypothyroidism should receive a thyroid stimulation test with the possible exception of patients with myxedema associated with a goiter and serum thyroid autoantibodies.
2. The functional status of the thyroid gland in patients taking thyroid hormone may, in part, be assessed by a stimulation test while continuing the therapy (109-112).
3. In patients who have received iodides, and possibly iodine compounds, a stimulation test may be performed to assess thyroid gland responsiveness to thyrotropin if the change in PBI is used as the criterion (120). It should be emphasized that the PBI must not be far outside normal limits when evaluating a rise after TSH, since the normal technical error in PBI determination is high when values above 15 μg/100 ml are obtained. The RAIU is not ordinarily responsive to TSH after iodine compounds (112) or iodides (120).
4. Stimulation tests have been helpful in establishing the existence of autonomy within the thyroid gland. If a nodule in the thyroid gland becomes independent of thyrotropin for function, the thyroid hormone produced by this nodule is frequently sufficient to suppress pituitary secretion of TSH, and the remaining thyroid parenchyma, in which function remains dependent upon thyrotropin, atrophies. A responsed of this suppressed tissue to exogenous TSH, as noted on RAIU testing and scintiscan is presumptive evidence for autonomy in the functioning nodule (121, 122). To be more precise, the diagnosis of an autonomous thyroid nodule should include a suppression test (see Suppression Tests).
5. Diffuse disease of the thyroid gland may be associated with an inability of the gland to respond to thyrotropin. When this occurs in the euthyroid patient, the clinical state has been described as *low thyroid reserve* (109, 111). Thyroid stimulation tests may be of clinical aid in assessing the extent of the disease process in the many diffuse thyroid disorders which include: Graves' disease after treatment with ^{131}I, subtotal thyroidectomy, or propylthiouracil (109, 111, 122a), subacute thyroiditis (109, 111, 118), and Hashimoto's thyroiditis (16, 114, 118).

   The above diseases should be differentiated from active Graves' disease where there is an adequate PBI response, but little or no rise in the RAIU values following TSH (123). The resistance of the RAIU to the influence of thyrotropin in Graves' disease may be more

TABLE IVA

STIMULATION TESTS (RAIU RESPONSE)

References	Subjects and No.	TSH* Dose (U)	TSH* Freq of Inject	Radioiodine Uptake: Baseline % Dose	After TSH: Time: Tracer (hrs after TSH)	After TSH: Time: Count (hrs after Tracer)	After TSH: Values: Difference (After-Before) Mean ± SD or Range	After TSH: Values: % difference $\frac{\text{After-Before}}{\text{Before}} \times 100$
a. Multiple injections of TSH								
1. Schneeberg *et al* (107)	Normals (6)	10	qd x 3	<15	?	24	+29 +15 to +37	
	Normals (18)	10	qd x 3	>15		24	+18 +6 to +35	
	Pituit Insuff (12)	10	qd x 3			24	+34 +20 to +49	
	Prim Hypothyr							
	Thyroidectomy (5)	10	qd x 3			24	−6 to +11	
	Spontaneous or Cretin (17)	10	qd x 3			24	−8 to +5	
2. Taunton *et al* (104)	Normals (10)	5	qd x 3	17	18	24	±18	
	Pituit Insuff							
	Sheehan's (3)	5	qd x 3	1	18	24	±1	
	Other (5)	5	qd x 3	5	18	24	±11	
	Prim Hypothyr (15)	10	qd x 3	4	18	24	±3	
b. Single injection of TSH								
1. Fletcher & Bosford (106)	Normals (14)	10		<30**	8	24		>49
	Pitiut Insuff (12)	10			8	24		>49
	Prim Hypothyr (5)	10			8	24		0
2. Jefferies *et al* (109)	Normals (12)	5			21	3	+20.4 ±7.8	
3. Taunton *et al* (104)	Normals (11)	5		28	18	24	+25 ±11	
	Pituit Insuff							
	Sheehan's (3)	5		1	18	24	+2 ±2	
	Other (5)	5		4	18	24	+10 ±6	

*Thytropar, Armour Pharmaceutical Co.
**>30% baseline resulted in smaller responses to TSH.

TABLE IVB

STIMULATION TESTS (PBI RESPONSE)

References	*Subjects and No.*	*TSH**		*Protein Bound Iodine after TSH*		
		Dose (U)	*Freq of Inject*	*Time after last TSH (hours)*	*Difference (After-Before) µg/100 ml Mean ± SD or Range*	
a. Multiple injections of TSH						
1. Skanse (105)	Normals (51)	10	qd x 3	24	5.5	+2.1 to +8.4
	Pituit Insuff (25	10	qd x 3	24	4.2	+0.2 to +9.9
	Prim Hypothyr (30)	10	qd x 3	24	0.3	−1.0 to +1.5
2. Taunton *et al* (104)	Normals (7)	5	qd x 3	42	4.7	±30
	Pituit Insuff					
	Sheehan's (2)	5	qd x 3	42	−0.1	±1.3
	Other (4)	5	qd x 3	42	3.3	±3.4
	Prim Hypothyr (7)	10	qd x 3	42	−0.1	±0.6
b. Single injection of TSH						
1. Jefferies *et al* (109)	Normals (12)	5		24	3.1	±1.1
2. Taunton *et al* (104)	Normals (9)	5		42	1.5	±1.6
	Pituit Insuff					
	Sheehan's (3)	5		42	0.5	±0.0
	Other (4)	5		42	0.3	±1.3

*Thytropar, Armour Pharmaceutical Co. except in Skanse's work where Actyron (Ferring A.B.) was used.

apparent than real since the thyroidal clearance of iodide is increased by TSH (124). However, the thyroid gland of Graves' disease may still exhibit some qualitative abnormality in response to the exogenous trophic hormone (75).

V. Suppression Tests.

A. General Considerations.

Normally, a fine homeostatic balance, mediated through the hypothalamus and pituitary gland, exists between the rate of thyroid hormonogenesis and the amount of thyroid hormone acting on the tissues in man. The addition of exogenous hormone to a normal human subject results, within a few days, in a slowing of thyroid function to maintain an eumetabolic state.

In hyperthyroidism, this feedback system is no longer operative. Here, excessive endogenous thyroid hormone does not depress thyroid function. It can then be predicted that, in hyperthyroid patients, the administration of thyroid hormone in quantities which suppress hormonogenesis in the normal individual will not alter thyroid function. This resistance to the suppressive action of thyroid hormone in the hyperthyroid gland provides the basis for additional tests to differentiate hyperthyroidism from euthyroidism.

In the hyperthyroidism of Graves' disease the mechanism(s) which permit thyroid function to be unaffected by exogenous thyroid hormones are unknown. It does not appear to be a change in the sensitivity of the hypothalamus or pituitary gland to thyroid hormones, as occurs to adrenocortical hormones in the analogous disease, bilateral hyperplasia of Cushing's syndrome. Whereas, adrenal function in the latter disorder is inhibited by large quantities of administered adrenocortical steroids (125), analogous doses (and more) of thyroid hormones have failed to suppress hormonogenesis in Graves' disease (126) (Table VA). The production of an abnormal humoral substance, long acting thyroid stimulator (LATS), has been suggested as a pathogenetic mechanism

TABLE VA

COMPARISON OF SUPPRESSION TESTS IN THYROID AND ADRENAL GLANDS

	Thyroid Gland and l-Triiodothyronine (μg)	*Adrenal Gland and Dexamethasone (mg)*
Daily replacement dosage in patients with hormonal insufficiency	50–100 (127)	0.75 (130)
Daily dosage that will regularly suppress normal function	50–150 (128, 129)	2.0 (125)
Daily dosage that will suppress hyperplasia of Graves' disease or Cushing's syndrome	None known (2000 μg failed) (126)	8.0 (125)

in Graves' disease (131, 132), and it is possible that this factor is responsible for the unsuppressible thyroid function found in this disorder.

The failure of thyroid function to be suppressed by ingested thyroid hormones is almost invariably present in hyperthyroidism (75). It should be noted, however, that a lack of suppression after hormonal treatment does not necessarily indicate that hyperthyroidism is present. Cases of Graves' disease with euthyroidism (eye signs only) (133), and patients with autonomous thyroid nodules (121, 122) even when euthyroid, demonstrate this phenomenon of unsuppressibility in thyroid hormonogenesis.

If one accepts the concept that all, or nearly all, patients suffering from hyperthyroidism demonstrate a failure of functional suppression by administered thyroid hormones, then a clinical test should be devised so as to place as many euthyroid subjects as possible in the *suppressible* category. Complete separation of hyperthyroid and euthyroid subjects by a thyroid suppression test has been mitigated by the need for a practical clinical method.

B. Problems in Thyroid Suppression Tests.

1. Types of tests.

Suppression tests may be divided into two groups according to the phase of thyroid hormonogenesis evaluated: a. thyroid uptake of radioactive iodine, and b. thyroid release of ^{131}I.

a. Suppression Tests Using the RAIU.

Many variations of protocol have been described, but the three examples shown in Table VB are representative of thyroid suppression tests using the RAIU. Differences in protocols appear to be of two major types: the type of thyroid hormone used, and the quantity of daily hormone in relation to the duration of treatment.

1. Type of Hormone.

Although desiccated thyroid preparations contain some triiodothyronine, pure triiodothyronine has a more rapid maximal suppressive activity, and can be administered for shorter periods of time for the same effect. Careful assessment of the minimum time required for good thyroid suppression by desiccated thyroid has not been reported, but probably two to three weeks will prove to be an optimum time. The use of thyroxine as the suppressive agent will probably require several weeks to be effective (136, 137). Triiodothyronine given for seven days appeared to produce a more uniform lack of suppression in hyperthyroid patients under treatment with antithyroid drugs than did thyroxine over a period of 21 days (137).

2. Quantity of daily hormone in relation to duration of treatment.

Dresner and Schneeberg (134) described a good statistical separation of hyperthyroid from euthyroid patients after giving 300 μg of triiodothyronine for two days, and Bakke, *et al* (138) suggest that human pituitary is depleted of thyrotropin by the ad-

TABLE VB

TYPES OF THYROID SUPPRESSION TESTS USING THE RAIU

Reference	*Subjects and No.*	*Method*			*Results (24 hr RAIU)*		
		Hormone	*Daily Dose (mg)*	*Days Given*	*% of Dose*		*RAIU after ÷ RAIU before*
					Before Hormone	*After Hormone*	
1. Werner & Spooner (128)	Euthyroid (41)	T_3	0.075	8	37 ± 2.2*	12 ± 1.4**	
	Hyperthyroidism (20)	T_3	0.075	8	56 ± 3.4	62 ± 3.4	
	(28)	T_3	0.150	8	63 ± 9.0	63 ± 11.1**	
2. Dresner & Schneeberg (134)	Euthyroid (35)	T_3	0.300	2	48.8	26.2	0.533± 0.088+
	Hyperthyroid (18)	T_3	0.300	2	81.1	75.3	0.930 ± 0.086+
3. Cassidy & Jagiello (135)	Euthyroid (56)	Des. Thyroid	180	21		<20 in all except 1 <10 in all except 4	<0.65 in all except 1
	Hyperthyroid (26)	Des. Thyroid	180	21		>20 in all except 1	>0.66 in all++

*Mean ± SE.

**All but 1 euthyroid < 20% and all euthyroid < 20% at 150 μg/d. All hyperthyroid > 35%.

+0.073 will separate hyper- and euthyroid groups so there is no overlap of means ± 2SE.

++0.65 gives good separation of hyper- and euthyroid groups.

ministration of T_3 over the same period of time. However, there is evidence that an number of euthyroid subjects will require a longer duration of treatment for adequate RAIU suppression. Even with T_3 in daily doses of 450 μg, suppression did not occur after four days of treatment in some patients, although smaller quantities of hormone were effective in reducing the RAIU in seven days (137). It would seem that, within practical limits, time is more important than hormone dosage in suppression tests.

b. Suppression Test using Thyroidal Release of ^{131}I.

In this test the thyroid gland is permitted to accumulate a tracer quantity of ^{131}I, then further uptake is blocked by methimazole. The release rate of thyroidal ^{131}I, of which the principal component is probably thyroid hormone, is measured by daily neck counts. Ecklund and Ryan (139) have shown that 75 μg of T_3 per day inhibit the release of ^{131}I from the normal thyroid, but not from hyperthyroid glands. A good separation of the two groups of subjects was achieved.

Advantages suggested for this type of test over the procedures using the RAIU were that a shorter time period, less than one week, is required, and that antithyroid therapy may be instituted during the diagnostic procedure. Whether or not this test is safer in the severely ill patient remains to be proved. Confirmation of this procedure as an accurate clinical tool is needed before it will receive widespread acceptance.

2. Recommendations for a Suppression Test.

The need for a practical clinical test, especially for outpatients, makes the test of Dresner and Schneeberg (134) attractive. Unfortunately, the precise time when the tracer dose of ^{131}I is given for the RAIU after T_3 treatment was omitted from the communication by the authors. However, since it may be necessary to continue hormonal treatment longer to achieve suppression, this uncertainty of time for the tracer dose may be overcome in a compromise protocol.

a. The following protocol is suggested for the routine clinical laboratory.
   1) A baseline 24-hour RAIU is obtained.
   2) Triiodothyronine 75 μg *q.i.d.* (300 μg/d) is administered for two days and then followed by 25 μg *q.i.d.* (100 μg/d) on days three and four.
   3) After a measurement of residual radioactivity, a repeat 24 hour RAIU is performed between days three and four.
   4) If inadequate suppression is induced, the T3 (100 μg/d) is continued through day eight.
   5) After once again determining the ^{131}I remaining in the thyroid gland, a third 24-hour RAIU is determined between days eight and nine.
   6) Values obtained may be compared with those of Dresner and Schneeberg (134) for the suppression test of days three to

four, and with the results of Werner (128) for the longer test. It should be recognized that the above described protocol is neither that of Dresner and Schneeberg nor of Werner, but rather an attempt at compromise for the sake of a practical and useful clinical method.

More recently, a modified protocol has been suggested by Werner whereby T_3 is given 50 μg/d for eight days, and if inadequate suppression is achieved, the test is repeated with a daily dose of 100 μg of the hormone (129). From the original data, this revision would seem to be reasonable, and it would avert many of the untoward reactions obtained with 150 μg/d of T_3 (128). No new data were given in support of this modified protocol, although the criteria of the previous test for suppression were used, and, in addition, it was suggested that the uptake after hormonal treatment should, in normal subjects, be less than one-half of the baseline value (129). However, other investigation support the efficacy of the lower dosage of T_3 (140).

For best results, the criteria for normal and abnormal suppressibility should be developed in the individual laboratory using the test.

b. For careful investigative purposes, probably the suppression test of Werner (128), which has received many years of trial, should be used.

c. Occasionally, patients in whom there is a question of unsuppressible thyroid function have been receiving desiccated thyroid in doses of 180 mg/d for prolonged periods of time (as for eye signs of Graves' disease). While continuing the hormone, a RAIU may be performed in these subjects to achieve a suppression test. Again, although this procedure is not identical to that of Cassidy and Jagiello (135), the criteria for suppression suggested by these investigators will serve as a guideline for the results obtained.

A few words should be said about the PBI determination during suppression tests. The administration of desiccated thyroid (Armour and some preparations of Warner-Chilcott) in doses of 120-200 mg/d to euthyroid subjects results in normal PBI levels after two to four weeks (23, 69a, 141). It would seem reasonable that, if thyroid function is unsuppressible by administered thyroid hormones, the exogenous and endogenous hormones should be additive and reflected by an augmentation of the serum PBI. A rise in PBI in hyperthyroid patients, who had received desiccated thyroid, was found after one week of treatment, but for reasons that were unclear, this increase in PBI was temporary (142). However, during desiccated thyroid therapy, a prolonged increase in PBI values has been seen at University of Michigan Medical Center in a few individuals who had

unsuppressible thyroid function associated with normal or borderline-high basal PBI levels. Thus, it is possible that PBI changes may be a useful adjunct in suppression tests. Unfortunately, no definitive criteria for normal and abnormal results in PBI values during suppression tests have been established. Treatment with triiodothyronine for the seven to ten days of the usual suppression test (126, 143) is too brief to achieve, in normal subjects, the low PBI levels commonly associated with the administration of this hormone. Therefore, the PBI determination provides no helpful information in suppression tests using T_3 over the customary time period.

3. Pitfalls in the use of Suppression Tests.
   a. The difficulties in establishing reliable criteria for a suppression test in an individual laboratory, and the vicissitudes of the RAIU procedure are obvious.
   b. A careful assessment of the reliability of an outpatient should be made before prescribing a hormone for self-administration.
   c. It is conceivable that the quantity of iodide released from administration desiccated thyroid may be sufficient to affect the RAIU and thus, give the appearance of suppression in hyperthyroidism (129).
   d. For unknown reasons, some euthyroid patients with nodular (144) and diffuse goiters (145) may require prolonged periods, 20 days to several months, of thyroid hormone administration to achieve good suppression of the RAIU.
   e. Patients recovering from iodine-induced hypothyroidism (iodide goiter) may manifest unsuppressible thyroid function under treatment with triiodothyronine (146). It is uncertain if these cases represent autonomous function due to intrathyroidal metabolic degrangements induced by iodide, or if they are examples of Graves' disease in which iodides have brought about an unusual therapeutic response.
   f. In patients exhibiting unsuppressible thyroid function, the activities of exogenous and endogenous hormones are additive, and will result in an accentuation of body metabolism. Usually, this is not great, and most individuals tolerate suppression tests well. However, patients with severe illness, especially those with heart disease, may be unable to withstand an added metabolic load, and in these individuals diagnosis should be obtained by methods other than a suppression test.

C. Uses of Suppression Tests.
   1. Suppression tests have greatest utility in differentiating cases of hyperthyroidism from normal subjects when it is difficult or impossible to do so by the more simple methods of assessing thyroid function. In clinical practice, only a few individuals in whom thyroid disease is suspected will require a suppression test to establish a diagnosis.

2. The ophthalmopathy of Graves' disease is occasionally manifest without hyperthyroidism being present or having occurred in the past (133). The clinical picture is then perplexing, since there is no pathognomonic sign in the eye disease. However, some, but not all, of the patients who develop the ophthalmopathy of Graves' disease while remaining euthyroid demonstrate thyroid function that is unsuppressible by thyroid hormones (133, 147, 148). Suppression tests, if positive in such patients, will enhance an understanding of what may initially appear as obscure eye disorders.
3. Following successful management of hyperthyroidism by any of the therapeutic programs in general use (prolonged antithyroid drugs (148-150), subtotal thyroidectomy (148, 150), or radioiodine (148, 150)) thyroid function quite regularly becomes normally suppressible. Persistence of unsuppressible function following hyperthyroid therapy is often associated with continuing or recurrent disease, while normal suppressibility has a good correlation with permanent cure of hyperthyroidism (149, 150, 150a). After treatment of hyperthyroidism with antithyroid drugs for one year the medication was withheld, and a suppression test (desiccated thyroid 180 mg for 21 days) resulted in the following 24 hour RAIU values (mean $\pm$ SD): 27 subjects who remained euthyroid: 23 $\pm$ 20 per cent; 26 individuals who subsequently developed recurrent hyperthyroidism: 58 $\pm$ 23 per cent (150a). Therefore, suppression tests may be of aid in assessing the adequacy of therapy in hyperthyroidism, especially in cases when, after prolonged administration of antithyroid drugs, it is desired to discontinue therapy (149, 150a).

   If it is assumed that Graves' disease and the associated unsuppressibility of thyroid function are mediated by some humoral thyroid stimulating factor(s), it is difficult to understand why therapy directed against only the thyroid gland should result in a return to a state of normally suppressible function. Perhaps there is either some intrinsic change within the thyroid gland, or some critical level of hormone production which facilitates, in Graves' disease, the phenomenon of unsuppressibility, and which is interrupted by a therapeutic attack on the gland.
4. The diagnosis of autonomous thyroid nodules, in which metabolism is independent of thyrotropin, is greatly aided by a suppression test in conjunction with a thyroid scintiscan (121, 122). The function, whether in the hyperthyroid or euthyroid range, of such autonomous tissues is unsuppressible by administered thyroid hormones.

   Since the thyroid gland is diffusely involved in Graves' disease, the differentiation of an autonomous nodule from the former disorder usually presents no problem. However, it is possible that Graves' disease may develop in a thyroid gland where only a remnant of tissue remains, and, under these circumstances, the clinical picture may be confusing, especially if eye signs are lacking. Thus, evidence that

there is perinodular tissue which is normally suppressible is essential to the diagnosis of an autonomous nodule. A stimulation test, also accompanied by a scintiscan, will usually delineate thyroid tissue which has been suppressed by the hormone secreted by the autonomous nodule (see Stimulation Tests).

VI. Radioactive Triiodothyronine Red Blood Cell (or Resin) Uptake Test (T3-Test).

A. General.

1. Thyroid Hormone Binding Proteins in Plasma or Serum.

Some information regarding the binding of thyroid hormones by plasma or serum proteins is essential to an understanding of the T3-Test. A brief résumé of this subject follows.

Three serum proteins which have distinct affinities for binding thyroid hormones have been identified. These are listed as to relative affinity for thyroxine and triiodothyronine at physiologic concentrations of the hormones in Table VIA.

In addition to characteristic affinities for the thyroid hormones, thyroid binding globulin, (TBG), and thyroid binding prealbumin, (TBPA), exhibit maximal capacities for thyroxine in a given volume of serum (151, 152). It is this latter quality which enables a measurement to be made of serum TBG and TBPA. More direct analyses of these proteins are limited by the minute quantities involved. By a technique of paper electrophoresis at pH 8.6, the capacities of TBG and TBPA are, respectively, about 25 and 245 μg of thyroxine per 100 ml of serum (153). Thus, in discussing changes in these serum proteins, one is referring to alterations in capacity to bind hormone, usually thyroxine. It is not known if the differences encountered in the binding capacities of TBG and TBPA in patients are quantitative (concentration of protein) or qualitative (intrinsic affinity for hormone) in nature. There is a suggestion that alterations seen in TBPA may be quantitative (154), but the character of those in TBG must await the development of a technique of quantitative purification for clarification.

Thyroxine binding albumin (TBA) has such a weak affinity for thyroid hormone that it seems to have little or no clinical importance in the protein binding of thyroid hormones. It will not be discussed further.

TABLE VIA

AFFINITIES OF SERUM PROTEINS FOR THYROID HORMONES

	Affinity for Thyroxine[1]	*Affinity for Triiodothyronine*[1]
Thyroid binding globulin (TBG) located between $\gamma 1$ and $\gamma 2$ globulins (151)	4+	1+ − 2+
Thyroid binding prealbumin (TBPA) (152)	1+ − 2+	0
Thyroid binding albumin (TBA) (151)	Trace	Trace

[1]Graded 0 to 4+

The complex of thyroid hormones and plasma proteins is thought to follow the law of chemical mass action (151), and it may be put into an equation:

[T4] [TBG] = k [T4-TBG] where

k is an intrinsic association or affinity constant;

[T4] is the concentration of free thyroxine;

[T4-TBG] is the concentration of thyroxine bound by TBG, or the concentration of occupied binding sites on TBG, and may be represented by the plasma or serum PBI (when this test is valid),[1] and

[TBG] is the concentration of unoccupied binding sites, or unsaturated portion, of the binding protein.

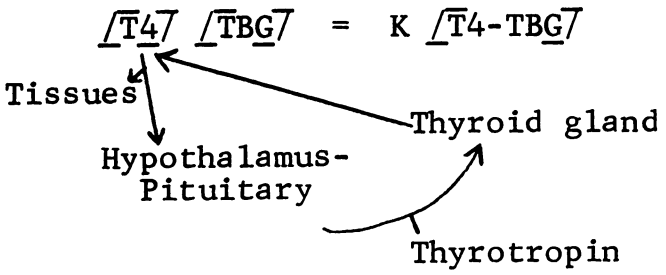
The other plasma or serum binding proteins, TBPA and TBA, follow the same law for association with thyroxine. However, the affinities of TBPA and TBA for thyroxine are much less than that of TBG, and under physiologic conditions the bulk of plasma hormone is carried in the TBG. An integration of the equations established for each binding protein can be used to illustrate the complex *in vivo* system, but for a simplified explanation of the hormone-protein associations, the reactions involving TBG alone will suffice.

The normal serum (and probably plasma) free thyroxine concentration is very low, about 1/1000 of the total serum thyroxine level (155), but this unbound hormone is the active fraction which regulates thyrotropin secretion[2] and the metabolism of body tissues (Fig. VIA).

As long as the regulating system of hypothalamus, pituitary, and thyroid gland is intact, the serum free thyroxine will remain at normal levels, and the individual will be euthyroid. This homeostatic process will prevail in the presence

FIGURE VIA

The Feed-Back Control of Serum Free Thyroxine and Binding Protein Relationship



Legend: See text for explanation of equation

[1]Actually, the serum protein bound thyroxine iodine concentration is usually 0.5 - 1.0 μg/100 ml less than the PBI, and the thyroxine iodine must be multiplied by 1.53 to convert this value to μg of thyroxine.

[2]The precise mechanism of this "feedback" system involving hypothalamus, pituitary, and thyroid glands is not known.

of altered capacities of serum binding proteins, such as TBG, and the associated change in the bound hormone. It is then understandable that the protein bound thyroxine may be pictured as a serum reservoir for the serum free thyroxine as shown in Fig. VIB-1.

Figure VI B

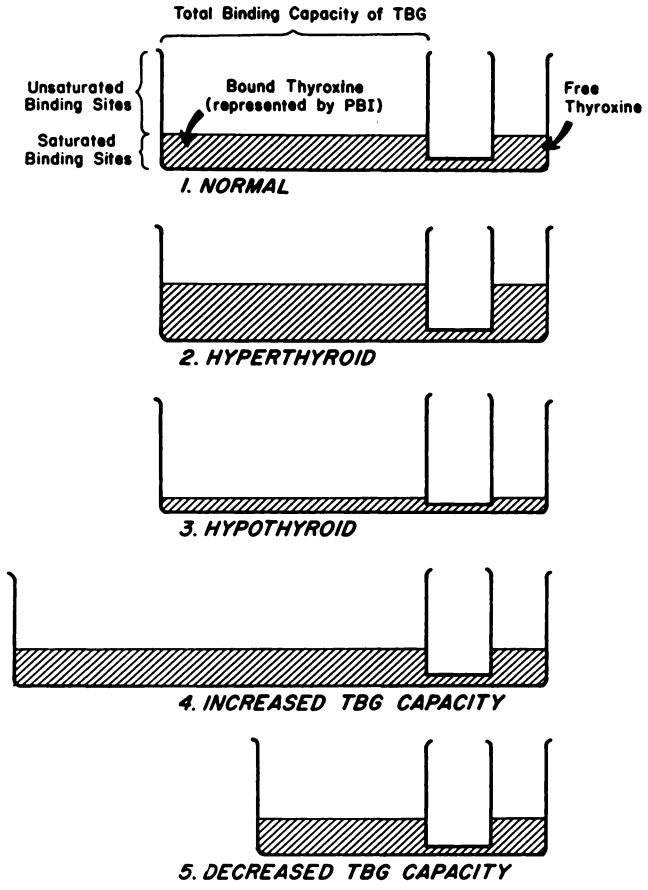


Figure VIB

The serum TBG molecules are illustrated as a vessel, which holds a liquid thyroxine (shaded), and which is connected by a pipe to a second vessel (not drawn to scale) containing free thyroxine. The thyroxine in the TBG vessel may be represented by the serum PBI concentration, and is in equilibrium with the free thyroxine.

1. **Normal euthyroid state with normal concentration of PBI and free thyroxine.**
2. **Hyperthyroid state with the high PBI and high free thyroxine.**
3. **Hypothyroid state with low PBI and low free thyroxine.**
4. **Increased TBG capacity with high PBI, but normal free thyroxine.**
5. **Decreased TBG capacity with low PBI, but normal free thyroxine.**

When abnormal TBG capacities, whether high or low, become stable, thyroid hormone production and RAIU values will be normal. It would appear logical that thyroid hormonogenesis, and the RAIU as an index of glandular activity, must be temporarily altered when rapid changes in TBG capacity occur. With an increasing TBG capacity, the serum protein bound thyroxine must be expanded to maintain a normal free thyroxine concentration and euthyroidism, and a falling TBG capacity will induce opposite changes (see Fig. VIB-4, B-5). However, the required alterations in hormonogenesis may be very slight since, in a clinical situation, they are not reflected in the RAIU test (156).

2. Basic Principles in the T3-Test.

If a substance which binds thyroid hormones, such as red blood cells (RBC), is added to human plasma, an equilibrium will be established, whereby the native plasma thyroxine is distributed between the RBC and the serum binding proteins, the minute quantity of free hormone present being ignored in these assessments. The proportion of thyroxine bound to the plasma proteins and to the RBC will depend upon the relative total affinity of each binding substance for the hormone. The affinity of the proteins is so great for thyroxine that only one to two per cent of this hormone will be found associated with the RBC (157). Therefore, assessment of differences in an equilibrium established for thyroxine in a plasma-RBC system cannot be easily measured, even when the thyroxine is labeled by an added trace quantity of thyroxine-^{131}I.

Fortunately, triiodothyronine is bound to the same sites on TBG as is thyroxine, but with a lesser affinity. If triiodothyronine in the form of T_3-^{131}I, is added to the system, an equilibrium of distribution between the plasma proteins and RBC is established for this hormone. A larger and more accurately measured proportion, commonly 11-19 per cent after two hours, (158), of triiodothyronine is found with the RBC than was true of thyroxine. This RBC fraction of T_3-^{131}I is easily assayed by radioisotope counting after the plasma proteins have been carefully washed away. The test has been classically performed by comparing the radioactivity (T_3-^{131}I) in the washed RBC with the original or total activity in the system. However, the radioactivity in an aliquot of the supernatant plasma may be measured, expressed as a fraction of the total T_3-^{131}I in the system, and then the RBC portion determined by extrapolation.

The T3-Test as outlined above is illustrated in Fig. VIC-1. If the quantities of RBC and T_3-^{131}I are held relatively constant, it is apparent that the only variable in the T3-Test is the unsaturated portion, or the unbound sites, of the native plasma proteins, principally of TBG.[1]

Subsequent to the development by Hamolsky (157) of the T3-Test using RBC, it has been found that resins in various forms (Amberlite IRA-400 in granular form (161), or as polyurethane foam sponge (162, 163); CG50, Type II (164); and CG400 (165) may be substituted for the RBC in the test. The several advantages of resins over red blood cells on the T3-Test may be listed as follows:

[1]Free thyroxine is not measured by any tests of this type. Special methods must be employed for evaluation of this quantitatively small hormonal fraction (155, 159, 160).

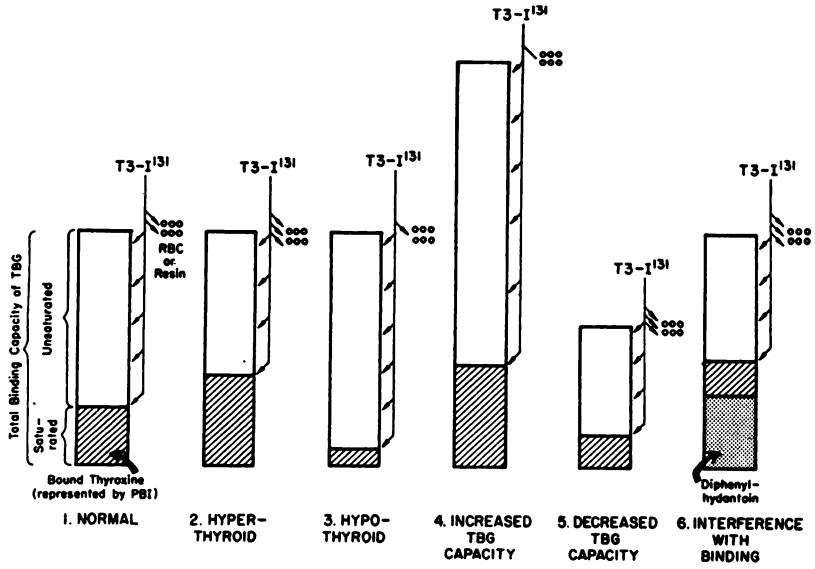


Figure XI C

Figure VIC

The T3-Test results are illustrated by showing the TBG capacities as bars, and the respective protein bound thyroxine levels (corresponding to the serum PBI's) are the shaded areas on the TBG bars. The free thyroxine concentrations are not shown here.

a. Serum may be frozen for use at convenient time with the resins.
b. Pooled serum aliquots may also be frozen and used as reference standards for each test run with the resins, thus eliminating the somewhat unpredictable day-to-day variations in the test (163).
c. The influence of intrinsic abnormalities in the erythrocytes on the test (166, 167) is avoided with procedures using the resins.
d. No hematocrit correction is necessary in the resin tests, although a hematocrit correction has improved the methods using RBC, (168).
e. Hypercapnia in the patient, which elevates the results obtained with the RBC T3-Test (169), does not affect the results obtained when the resin is employed (161).

Commercially prepared kits with resins are available to enable the T3-Test to be performed very simply and with reasonable accuracy.[1]

Labeled thyroxine has been employed in the place of triiodothyronine-^{131}I in a test using resin as a competitive hormone acceptor (162, 170, 171). It remains to be seen whether or not the use of thyroxine-^{131}I will have any distinct advantages over the more established method.

[1]Triosorb, Abbott Laboratories, North Chicago, Illinois; Tresitope, E. R. Squibb & Sons, New York; TBI, Nuclear Consultants Corporation, St. Louis, Missouri.

The results obtained using a technique employing resins are commonly expressed as a percent of the control or pooled serum. Values of a T3-(resin)-Test in 67 euthyroid subjects were 99.1 (SD ± 4.3) with a range of 91 - 106 per cent of the control (165). The standard error in duplicate samples should be about ±3 when expressed as a percent of the control serum (172).

B. Factors Affecting the T3-Test.

1. Alterations in Metabolic States Related to Thyroid Hormones.

Changes in the serum concentrations of thyroid hormone, bound and free, as related to the serum binding proteins in hyperthyroidism and hypothyroidism may be seen in Figs. VIB-2, and VIB-3.

Demonstrated in Figs. VIC-2 and VIC-3 are, respectively, the high T3-Test results in hyperthyroidism, and the low values in hypothyroidism. The accuracy of the T3-Test may not be as good for the diagnosis of myxedema as for thyrotoxicosis, at least when using the resin sponge method (163).

2. Alterations in Serum Thyroid Hormone Binding Protein Capacities.

a. Increased and decreased serum TBG capacities are portrayed with the associated serum thyroxine changes in Figs. VIB-4 and VIB-5. With alterations in the serum TBG capacity, the serum bound thyroxine, represented by the PBI, must change in the same direction. However, if the hypothalamus-pituitary-thyroid axis is intact, the free thyroxine is maintained at a normal concentration, and the patient is euthyroid.

The results of T3-Tests in cases of increased and decreased TBG capacities are seen in Figs. VIC-4 and VIC-5. It may be seen from Figs. VIB-4 and VIB-5 that the concentration of unsaturated TBG is directly related to the total TBG capacity. Since the T3-Test values are inversely related to the concentration of unsaturated TBG, then it follows that the T3-Test results will change in a direction opposite to that of the TBG capacity. Further, in the euthyroid individual, alterations in TBG capaciy result in changes in PBI values that are reciprocal in direction, but roughly of the same magnitude, as the deviations in the T-3 Test.

Examples of changes in TBG capacities encountered in clinical practice are seen in Table VIB. The effects of estrogens, ovulatory suppressants, androgens, and anabolic hormones on the PBI and the T3-Test are manifest after 7 to 21 days of therapy, and disappear in about the same period of time (173-175, 181, 182).

The augmentation of the PBI and the depression of the T3-Test in pregnancy is seen by three to four weeks postovulation (158, 189), and, although the TBG capacity is not quite normal by the fifth week postpartum (189), the T3-Test returns to nongravid levels by one to two weeks after delivery (158).

b. The capacity of TBPA to bind thyroxine is frequently diminished in severe or chronic illness, but no abnormal increase in capacity of this hormone binding protein has been reported (151).

Although TBPA does not bind triiodothyronine in any significant quantity, reduction in the TBPA capacity for thyroxine causes a change in the plasma equilibrium for the binding of endogenous thyroxine, and a greater than normal

proportion of this hormone is then bound to TBG. A reduced TBPA capacity affects the T3-Test by this shift of thyroxine to the TBG binding sites.

However, because, under normal conditions, the thyroxine bound by TBPA is less than that bound by TBG, diminution in the capacity of the former binding protein, produces only a small, but occasionally significant, fall (mean 0.8 μg/100 ml) in the PBI (190). An abnormally low TBPA capacity, as seen in acute illness, may result in an augmentation of T3-Test values (191).

3. Changes in the Interaction of Thyroid Hormones and the Binding Proteins.

a. Certain drugs have been found to bind to TBG on a competitive basis with thyroxine and triiodothyronine. The overall effect is a reduction in the total binding capacity of the serum TBG which is available for thyroid hormones, and this results in PBI and T3-Test values which suggest a low TBG capacity. However, when the TBG capacity is determined directly, the techniques employed wash out the offending drugs, but not the thyroid hormones, and the value obtained is normal (192).

Diphenylhydantoin[1] is a classic example of a drug which, in pharmacologic doses, competes with thyroxine for the serum TBG binding sites (192) and, thereby, affects both PBI and T3-Test values (193) (Fig. VIC-6).

TABLE VIB

EXAMPLES OF CHANGES IN TBG CAPACITIES AND T3-TEST RESULTS

	Reference
Increased TBG Capacities and Decreased T3-Test Results	
Pregnancy	(151)
Estrogen administration (including the common ovulatory suppressants—oral contraceptives—as norethynodrel[1])	(151, 173-175)
Perphenazine[2] administration (prolonged)	(176, 177)
Acute liver disease and cirrhosis	(151)
Familial or hereditary	(178, 179)
Idiopathic	(180)
Decreased TBG Capacities and Increased T3-Test Results	
Androgen Administration	(151, 174, 175)
Anabolic hormone administration	(181)
Nephrosis	(151)
Advanced cirrhosis	(183)
Familial or hereditary	(184, 185)
Idiopathic	(186-188)

[1]Enovid, G. D. Searle and Company, Chicago.
[2]Trilafon, Schering Corporation, Bloomfield, New Jersey.

[1]Dilantin Sodium, Parke Davis & Co., Detroit, Michigan.

In addition, certain low potency thyroid hormone analogues, as d,l-tetraiodothyronine, have an affinity for the TBG molecule and, when administered to a patient, enter into competition with thyroxine for the serum protein binding loci (192).

b. In a mechanism analogous to that described for TBG, salicylates and dinitrophenol compete with thyroid hormone for the binding sites on TBPA (192). Because, as noted above, the latter protein binds quantitatively less serum thyroxine, displacement of the hormone from TBPA by drugs produces smaller changes in the PBI and T3-Test values than when there is competitive interference with TBG binding.

However, dinitrophenol (194) and salicylates (190) in large doses may bring about an appreciable reduction in the PBI concentration, and presumably there will be associated a small, but definite, increase in the T3-Test result.

4. Treatment with Thyroid Hormones.

The effects of therapy with various thyroid hormone preparations in the T3-(red cell)-Test have been investigated and the results are noted below. Probably T3-Tests using resins will give comparable information under these circumstances.

a. In the section on PBI, it was pointed out that treatments of normal and myxedematous individuals with replacement doses of desiccated thyroid produce normal PBI levels (21, 22, 69a). As would be expected if such a situation were illustrated (Fig. VIC-1), this therapy is associated with normal T3-Test values (69a, 195).

b. Administration of comparable doses of l-triiodothyronine are associated with very low serum PBI concentrations (22). If this therapeutic state is figuratively depicted so as to demonstrate the T3-Test, the unsaturated portion of the TBG will be relatively large. It is not surprising then, that the T3-Test frequently (50 per cent of the cases) gave low results during the administration of triiodothyronine (195).

c. Although treatment with l-thyroxine usually produced abnormally high PBI levels while maintaining euthyroidism (22), these slightly augmented PBI concentrations were apparently insufficient to alter the T3-Test values, which, in such cases, remained in the normal range (69a, 195).

5. Miscellaneous Factors.

Pitfalls in technique have been extensively evaluated for T3-Test using red cells (158, 169), resin (165, 196), and resin sponge (197). However, one technical hazard deserves emphasis here. The finding of impurities, principally radiothyroxine and radioiodide, in the triiodothyronine-^{131}I obtained from commercial suppliers may be frequent, and may increase in quantity with time (198). Such impurities can, to a considerable extent, alter the results of the T3-Test. Assessments of the quality of triiodothyronine-^{131}I should be made whenever control serum or plasma gives unusual T3-Test values.

There appears to be no effect of age on the T3-Test except in the newborn, where conflicting results have been reported: low results (199), possibly from a

TBG capacity augmented by maternal estrogens (189); and elevated values (200) which may reflect an unusually high free thyroxine concentrations in the neonatal state (200a).

Premenopausal, nonpregnant females, possibly from their natural estrogens, have a lower normal range for the T3-(red cell)-Test than do males (158), and this difference, although small, may be statistically significant (201). A similar small inequality in the results was found between the sexes in tests using the resin (172, 196).

No seasonal variation in the T3-Tests results has been noted (157).

Although the administration of anticoagulants, such as bishydroxycoumarin[1] and ethyl biscoumacetate[2] may elevate the T3-Test values when red cells are used (158), it is not known how such drugs act, or if they affect the test when performed with resin.

C. Uses of the T3-Test.

1. The T3-Test provides an additional laboratory assessment of thyroid function. The test has the distinct advantage of requiring: a) only slightly more than two hours to perform, and for the patients, b) no special preparation, and c) no radiation exposure.

2. Estimates of changes in serum thyroid hormone binding protein capacities, especially in TBG, can be made by combining the T3-Test with the PBI determination (Figs. VIC-1—VIC-6). The direct determination of TBG binding capacity is a laborious technique and cannot be done on a routine basis in most clinical laboratories.

Thus, in cases of unsuspected binding protein changes, the peculiar results in either the PBI or the T3-Test may be clarified by correlating the two laboratory procedures. In fact, it was found that multiplying the PBI result by the T3-Test value gave an arbitrary index of free thyroxine, which correlated better with the clinical state of the patient than did either the PBI or T3-Test alone (202).

3. The diagnosis of thyroid dysfunction in pregnant subjects has been limited by a reluctance to use radioisotopes, and by a lack of well-defined ranges for the PBI and the BMR in the gravid state.

When compared with the nonpregnant normal female values, the augmentation of the PBI should be approximately of the same magnitude as the depression of the T3-Test level, and the combination of the two tests is a valuable adjunct in assessing thyroid function during pregnancy (197).

When accompanied by a distinctly low T3-Test, the failure of the PBI to rise in pregnancy is suggestive of hypothyroidism. Normal nonpregnant T3-Test levels in the face of PBI values greater than 8.0—9.0 μg/100 ml are compatible with pregnancy complicated by hyperthyroidism (203).

Failure of the normal increase in TBG capacity to occur in pregnancy may be associated with early abortion (189, 204). Such abnormal events may be predicted by the T3-Test. When the values of this laboratory procedure do not fall in the first few weeks after conception, abortion may be imminent (158).

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[1]Dicumarol, Abbott Laboratories, North Chicago, Ill.

[2]Tromexan, Geigy Pharmaceuticals, Ardsley, N.Y.

4. Since neither inorganic iodides nor iodine compounds interfere in the binding of thyroid hormones by plasma or serum proteins (Fig. VIA), the T3-Test is of special importance in the diagnosis of thyroid dysfunction when the PBI and the RAIU are rendered unreliable in the patient who has received iodine containing drugs.

However, for reasons that are unclear, the administration of iodides, may produce slight elevations in the T3-Test results when red cells are used (158, 201). These aberrations were considered minor, and, as a general rule, high plasma iodide concentrations are thought not to significantly affect the value of the T3-Test.

There is a disquieting report of distinct elevations in the T3-(red cell)-Test values following the administration of the new oral cholecystographic contrast medium, sodium ipodate[1] (205). The precise mechanism involved in this alteration of the T3-Test is unknown, but presumably the compound competes with thyroxine for serum protein binding sites. If a competitive mode of action is found, this radiographic contrast agent will also affect the T3-Test in which resin is used, and will constitute a major pitfall in the laboratory assessment of thyroid function.

[1]Oragrafin sodium, E. R. Squibb & Sons, New York.

SUMMARY

We have reviewed the physiological principles involved in the assessment of thyroid function by the following methods: 1) protein-bound iodine, 2) butanol-extractable iodine and thyroxine by column chromatography, 3) thyroid radioactive iodine uptake, 4) thyroid stimulation tests, 5) thyroid suppression tests, and 6) triiodothyronine uptake by RBC or resin. Factors other than disturbances in thyroid function which affect these laboratory procedures are discussed with reference to the potential value of each test in certain clinical situations.

ACKNOWLEDGMENTS

The author is indebted to Drs. Beierwaltes and Matovinovic for helpful comments and criticisms.

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