Thyroid Endocrinology

Lecture Goal(s): To introduce normal thyroid function and to describe the normal effects of thyroid hormones on the body. Pathologic conditions of the thyroid and other abnormalities influencing thyroid hormone levels will be discussed. Correlation of laboratory data with disease will be emphasized.

Lecture Objectives: Upon completion of this class material each student will be able to do the following:

1. Cog/I Note the anatomical location of the thyroid.

2. Cog/II List and explain the four primary functions of the thyroid.

3. Cog/II Relate cretinism to a lack of thyroid hormone and explain the importance of early evaluation of thyroid function.

4. Cog/II Differentiate follicular cells from parafollicular cells of the thyroid on a basis hormones produced.

5. Cog/II Describe the synthesis and release of tri-iodothyronine (T_3) and thyroxine (T_4) by the thyroid including the following steps. Note the effect of thyroid stimulating hormone (TSH) on these steps.

   a. formation of thyroglobulin
   b. iodine trapping, including factors that influence this process
   c. incorporation of iodine into thyroid hormone
   d. release of thyroid hormone from thyroglobulin
   e. conservation of iodine and amino acids.

6. Cog/I Recognize the chemical structure of the following.

   a. monoiodotyrosine (MIT)
   b. diiodotyrosine (DIT)
   c. T_3
   d. T_4
   e. rT_3

7. Cog/II Compare T_3 and T_4 on the following characteristics.

   a. amount of normal daily secretion
   b. potency
   c. modifications once released into the blood stream

8. Cog/II Describe the production of reversed T_3 and note situations that are associated with increased rT_3 production.

9. Cog/I List the three most important transport proteins for thyroid hormones in the blood stream. Give the percentage of T_3 and T_4 transported by each and the percentage remaining free in the blood stream.

10. Cog/II Explain the feedback mechanism that controls thyroid hormone production.

11. Cog/II Explain the following nomenclature used in describing disease states associated with thyroid hormones.

a. primary hyper- and hypo-thyroidism
b. secondary hyper- and hypo-thyroidism
c. tertiary hyper- and hypo-thyroidism

12. Cog/II Explain each of the following test procedures that may be used in the laboratory analysis of thyroid function.

a. T_3Uptake
b. total T_4
c. free thyroxine index
d. total T_3
e. free T_3 index
f. free T_4
g. free T_3
h. TSH
i. TRH stimulation
j. thyroid antibodies

13. Cog/II Describe the general characteristics of the following disorders.

a. hyperthyroidism/subclinical hyperthyroidism
b. hypothyroidism/subclinical hypothyroidism
c. euthyroid sick syndrome

14. Cog/III Correlate data from the above laboratory analyses with the above thyroid/euthyroid states.

I. Thyroid Gland - The thyroid gland exists in two lobes with one lobe on either side of the trachea.

A. Functions - The thyroid gland serves many functions in the body. The four primary functions of the thyroid include:

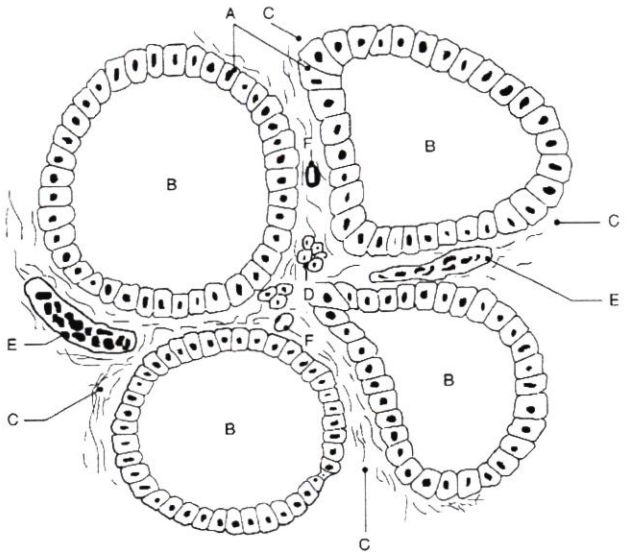
1. To conserve iodine for use in producing thyroid hormones.
2. To control all metabolic functions in all cells - If the level of the thyroid hormones increases, an acceleration of oxygen consumption and the production of heat occurs.
3. The thyroid gland is necessary for growth.
4. The thyroid gland is necessary for the differentiation of tissue.

a. **Cretinism** - An example of this is seen with the normal development of the brain. If the function of the thyroid gland was inadequate at birth, a disease state known as cretinism would develop. Cretinism is a congenital deficiency of thyroid hormone secretion and it is characterized by impaired physical and mental development. Cretinism must be detected within the first few months of life in order to prevent irreversible brain damage.

B. Anatomy of the thyroid gland - The thyroid gland contains primarily of two types of cells:

1 .**follicular cells (ascinar cells)** - Follicular cells are associated with the production of thyroid hormones. These are arranged spherically in a single layer which surrounds a lumen. Thyroglobulin is stored in the lumen.

2. **parafollicular cells** - Parafollicular cells are located in the interstitium between the follicular cells and they secrete the hormone calcitonin (secretion lowers both Ca and PO_4). The following illustrates the thyroid gland structure.



C. Diagram of thyroid

1. **A** - represents follicular cells
2. **B** - represents the lumen
3. **C** - represents parafollicular cells
4. **D** - represents the thyroid interstitium
5. **E** - represents venules
6. **F** - represents capillaries.

II. **Metabolism of thyroid hormones** - The main function of the thyroid gland is to produce and secrete metabolically active hormones that are essential for the regulation of various metabolic functions within the body.

A. **Hormones are produced within the follicular cells** - produced from carbohydrates, amino acids (most importantly, the amino acid tyrosine), and the element **iodine**. The two most important:

1. **tri-iodothyronine (T-3)** - contains 3 iodine atoms
2. **thyroxine (T-4)** - contains 4 iodine atoms

B. **Secretion of thyroid hormones -** released from the colloid and secreted into circulation.

1. **Thyroid Stimulating Hormone (TSH, thyrotropin)** - pituitary hormone that stimulates thyroid gland to secrete thyroid hormones.

D. **Iodine metabolism -**

1. **Reduced to iodide (I^-)** - in the gastrointestinal tract
2. Absorbed in the small bowel
3. Then enters either the excretory or metabolic pathways **(see diagram)**
4. **60-80% ingested iodine secreted in kidneys**
    a. **some is excreted in feces**
    b. **remainder is distributed into**
        1) **intracellular compartment -** intrathyroid iodine compartment contains about 90% of total body iodine (~6000-12,000µg)
        2) **extracellular compartment -**contains most other iodine, except fo a small but important amount that is found in cells.

C. **Steps in hormone production**

1. **Iodine is trapped by the thyroid cells** - (this is trapped as iodide at physiologic pH). This trapping process is an active transport process and therefore requires the expenditure of energy. Thyroid cells have the ability concentrate iodide against high chemical and electrical gradients because of this active transport process.
    a. **Iodine** - natural component of may foods (provided in adequate amounts in most US diets) - usually 250-700 µg/day, may vary throughout the world.
2. **Organification (iodination)** - process by which iodine is incorporated into thyroid homone (normally ~75µg/day) (iodide comes from both trapping and from the intrathyroid diodination of stored thyroid hormone precursors.)
    a. iodine oxidized in presence of thyroid peroxidase (TPO) into a reactive form
    b. **reactive form of iodine combines with the protein thyroglobulin** - a glycoprotein MW 660,000 D. This forms
        1) Monoiodotyrosine (MIT)

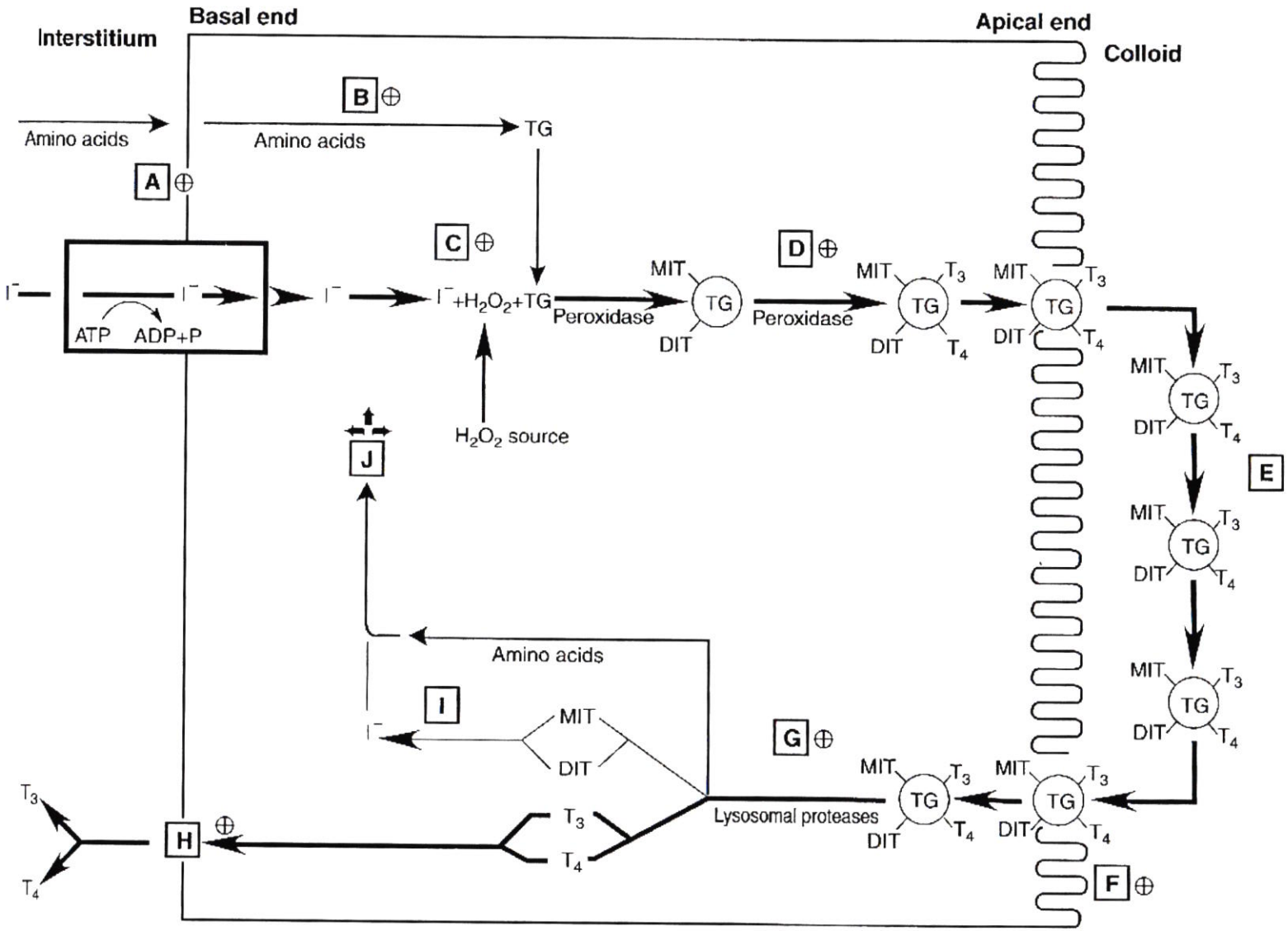
2) Diiodotyrosine (DIT)

3. **Coupling** - Enzymatic coupling of MIT and DIT takes place to form intrathyroglobulin
   a. triiodothyronine (T3)
   b. thyroxine (T4)

4. **Storage** - Iodinated thyroglobulin serves as storage pool of thyroid hormones.
5. **Secretion** - Thyroglobulin from these stores is degraded, forming the thyroid hormones, which are finally released into the circulation. Secretion mediated by Thyroid Stimulating Hormone (TSH)
   a. Droplets of thyroglobulin-containing colloid are engulfed by the follicular cells of the thyroid gland and then digested by lysosomal proteases. This degradation releases MIT, DIT, T3 and T4.
      1) T3 and T4 immediately secreted (resistant to intrathyroid deiodination)
      2) MIT and DIT are immediately deiodinated and their iodine is reused in subsequent thyroid hormone synthesis
   b. 80-100μg T4 and ~7μg T3 secreted per day
   c. Small amounts of reverse T3 (rT3) are also secreted.

D. **Low dietary intake of iodine** - results in a depletion of intrathyroidal deposits of iodine.
1. Decrease of thyroid hormone secretion
2. Increase in TSH secretion
   a. results in thyroid growth (hyperplasia & hypertrophy) thus iodine deficiency is temporarily compensated for with normal levels of hormone produced.
   b. persistent low ingestion - goiter and hyperthyroidism

D. **Diagram of hormone production - Next page**
   a. **A** - -represents iodine transport
   b. B - represents thyroglobulin (TG) synthesis
   c. C - represents iodide organification
   d. D - represents intrathyroglobulin oxidative coupling
   e. E - represents TG storage in the lumen (or colloid)
   f. F - represents endocytosis
   g. G - represents hydrolysis
   h. H - represents hormone secretion
   i. I - represents intrathyroidal deiodination
   j. J - represents recycling.
   k. the symbol ⊕ - Steps influenced by the thyroid stimulating hormone (TSH)


Interstitium
Basal end
Apical end
Colloid
Amino acids
Amino acids
TG
A
B
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ATP
ADP+P
I^-
$I^-+H_2O_2+TG$
Peroxidase
Peroxidase
H_2O_2 source
MIT
DIT
T_3
T_4
TG
Amino acids
Lysosomal proteases

III. **Thyroid hormone transport** - once released into bloodstream transported in two forms.

A. **Protein bound** - metabolically inactive; a reservoir providing a constant supply of hormone for tissues.

1. **Bound to three plasma proteins**
   - **a) Thyroxine-binding globulin (TBG)** Most important
   - **b) Thyroxine-binding prealbumin (TBPA)** also called Transthyretin; 2nd most important
   - **c) albumin**
2. **Role of binding proteins** - related to their relative affinities for each and on their relative concentrations in plasma
   - **a) T-4** - is secreted in the largest amount by the thyroid gland with approximately 99.95 percent of it being bound to a transport protein.
     1) TBG carries 70-75 percent
     2) TBPA carries 15-20 percent
     3) Albumin carries approximately 10 percent
   - **b) T-3** - is secreted from the thyroid gland in a lesser amount than T-4, but T-3 is more potent than T-4. Of the T-3 secreted, 99.7 percent is bound to transport proteins.
     1) TBG carries 60 percent
     2) TBPA does not carry T-3
     3) Albumin carries the rest
3. **Abnormalities of the binding proteins** - may result in abnormal total (bound) hormone concentrations in the blood even when normal amounts of free hormone.
   - **a.** Changes in TBG - Affect both T3 and T4 concentration since both bind TBG
   - **b.** ↑TBG = ↑bound thyroid hormone = ↓free hormone = ↑TSH = ↑thyroid hormone production = ↑hormone secretion.
   - **c. ↓TBG = opposite of increase**

B. **Free hormone** - physiologically active and readily available for cellular uptake

1. <1% of total plasma thyroid content

IV. **Metabolism of Thyroid Hormones** - Circulating T3 and T4 are either incorporated into the intralcellular pool, where they undergo partial transformations and exert their metabolic effects , or are degraded and eliminated by excretory organs.

A. **T3** - More metabolically important than T4.

1. **Thyroid secretion of T3** - Only about 20 percent of the T-3 present in the blood stream is a result of direct thyroid secretion.
2. It is estimated that 80 percent comes from the removal of an iodide from T-4 while in the peripheral circulation and in several organs. The two most important areas where this conversion is seen:
   a. kidney
   b. liver.
3. **Peripheral deiodination (monodiodination) of T4** - occurs on the outer ring at the 5' position to produce T-3.
4. **Reverse T-3 (rT-3)** - produced by the removal of one iodide from the inner ring of

T4, is metabolically inactive and is an end product of T4 metabolism. The chemical structure of rT-3 is illustrated in Figure 44-2, page 830 in Kaplan.

B. **T4** - Does possibly have some direct biological activity, but much less than T3. May be considered as a prohormone in some circles.

1. Peripheral deiodination is a rapidly responsive mechanism of control for thyroid hormone balance. Acute or chronic stress or illness causes a shift in the direction of this deiodination, favoring the formation of rT-3 rather that T-3, whereas the level of T-4 remains essentially unchanged. Various medications also shift peripheral deiodination toward the inactive product rT-3.

C. **Metabolic steps of thyroid hormones**

1. **Both T3 and T4 exert their biological effects by binding to specific intracellular receptors and are subsequently degraded through successive deiodinations.**
2. **Deiodination accounts for 80-85% of T3 & T4 metabolism**
3. **~35-50% of the T4 undergoing diodination is converted T3**
4. **~50-65% of the deiodinated T4 is converted into rT3**
5. **Most of the T3, T4 & rT3 are metabolized through a chain of successive deiodinations, resulting in the formation of iodinated intermediary metabolites and ultimately thyronine.**
6. **Both T3 & T4 undergo oxidative deamination and decarboxylation of the alanine side chains to form the acetic acid analogs tetrac and triac.**
7. **Small amounts of free T4 are eliminated in the bile and urine**
8. **Small amount of T3, rT3 and indirectly T4 are metabolized by being conjugated with glucuronic acid and sulfate and excreted in the bile.**

V Regulation of the Thyroid Gland

A. **Hypothalamic-pituitary-thyroid axis (HPTA)** - The regulation of the thyroid gland

1. **Process begins with the hypothalamus detecting a need for T-3 and T-4 control.** This may come from higher brain centers, drugs, or simply from the physiologic sensors in the hypothalamus to conditions such as stress, temperature, etc.
2. As a result of this, the hypothalamus produces and secretes a hormone known as thyrotropin-releasing hormone (TRH).
3. TRH passes down the venous system that connects the hypothalamus to the pituitary and acts on receptors in the adenohypophysis (i.e. anterior lobe of the pituitary).
4. This leads to the production of the thyroid-stimulating hormone (TSH).
    a. TSH is a glycoprotein that has a structure similar to some other tropic hormones. In particular LH (leutenizing hormone), FSH (follicle stimulating hormone), and HCG (human chorionic gonadotropin). All of these (TSH, LH, FSH, HCG) have both α and β subunits. The α-subunit of all of these hormones is very similar, while the β-subunit is immunologically distinct and provides biological activity.

5. The adenohypophysis will release TSH into the blood stream.

6. Once TSH reaches the thyroid it will stimulate the production and release of thyroid hormones. Recall the actions of TSH were noted on the diagram above with a ⊕ symbol. T-3 and T-4 will be produced and released into the blood stream where they move to the appropriate tissues and exert their control.

7. As levels of T-3 and T-4 increase, the levels of these hormones in the blood will inhibit the secretion of TSH by the adenohypophysis by a negative feedback action. It is the free form of the hormone that is responsible for exerting this negative feedback action.

8. It is also believed that T-3 and T-4 may have some direct action on the hypothalamus. This is believed to be the case because, in situations where there are low blood levels of T-3 and T-4, both TRH and TSH levels are found increased above their base level.

B. **Abnormalities associated with thyroid function** - are classified on the basis of thyroid regulation. Abnormalities may be associated with the thyroid gland itself, or, with one of the other glands that produces hormones that regulate the thyroid. The classification of thyroid abnormalities is as follows:

1. **Primary** hyper- or hypothyroidism refers to a problem with the thyroid gland itself.

2. **Secondary** hyper- or hypothyroidism refers to a problem with the adenohypophysis (anterior pituitary), and in particular, the production of TSH.

3. **Tertiary** hyper- or hypothyroidism refers to a problem with the hypothalamus specifically associated with the production of TRH.

VI. Thyroid Disease

When considering any hormonal disturbance, patient presentation and test results help delineate the disorder as hypo- or hyper-function. Additional testing localizes the defect as primary, secondary or tertiary. The table below summarizes the pattern of change in TSH and thyroid hormone levels seen in common disorders of thyroid hormone production.

TSH Level	Thyroid Hormone Level		
	Low	Normal	High
High	Primary Hypothyroidism	Subclinical Hypothyroidism	Secondary Hyperthyroidism*
Normal		Euthyroid	
Low	Secondary Hypothyroidism*	Subclinical Hyperthyroidism	Primary Hyperthyroidism

* In rare tertiary disorders, the pattern will be similar to that with secondary disease.

A. **Hyperthyroidism** - defined as excess levels of thyroid hormones in the blood stream. Hyperthyroidism is sometimes referred to as thyrotoxicosis.

1. **Symptoms of hyperthyroidism** - include nervousness, sweating, heat intolerance, moist and warm skin, tremor, angina, tachycardia, and menstrual irregularities.

2. **Typical laboratory results** - In overt (i.e., clinical) primary hyperthyroidism include
   a. **suppressed TSH** - The suppressed TSH results from the negative feedback effect of T-3 and T-4.
   b. **increased T-3**
   c. **increased T-4**
   d. **increased free T-4 (FT4)**
   e. **increased T-3 Uptake**
   f. **increased FTI.**
3. **Continued overproduction of thyroid hormone** - thyroid hormone production shifts toward T-3. This results from inadequate time to insert iodine into all four positions on the tyrosine backbone or from a lack of iodine because of the continual production of these hormones. As a result of this, approximately 10 - 15% of patients with hyperthyroidism have an increased T-3 with a normal T-4. This situation is known as **T-3 toxicosis (also know as T-3 thyrotoxicosis)**. Thus, in patients with suppressed TSH and normal T-4, analysis of T-3 is performed.

4. **Causes of Hyperthyroidism**

   a. **Graves' disease** - is the major cause of hyperthyroidism, constituting about 80% of all hyperthyroid cases. Graves' disease is an autoimmune disorder in which antibodies bind to and stimulate the TSH receptor.
      1) Approximately five times more common in females than in males
      2) most commonly occurs in younger individuals, although it can occur at any age.
      3)The diagnosis is usually suspected when the patient demonstrates the typical symptoms and laboratory results described above and has a diffusely enlarged thyroid gland.
      4) These patients also show a high radioactive iodine uptake.
      5) In questionable cases, antibody testing can be used. It has been found that 90% of the diagnosed patients have a positive TSI (which is considered to be a highly specific marker for Graves' disease) and 75% have anti-TPO (anti-thyroid peroxidase).

   b. **Thyroiditis** - Less commonly, hyperthyroidism is due to thyroiditis. In such cases, inflammatory damage to the thyroid gland can release thyroid hormone and cause transient hyperthyroidism. Patients with thyroiditis typically have
      1) a normal-sized or mildly enlarged thyroid gland
      2) low radioactive iodine uptake
      3) positive TPO.

c. **Nodular goiters -** a common cause of hyperthyroidism, especially in older individuals. In contrast to other forms of hyperthyroidism, the thyroid gland is not diffusely enlarged, but is characterized by the presence or more nodules. Autoimmune markers are typically absent in this form of hyperthyroidism.

5. **Subclinical hyperthyroidism -** With the use of TSH as the major diagnostic test for hyperthyroidism, a number of patients have been found to have suppressed TSH with normal levels of thyroid hormones. This has been termed subclinical hyperthyroidism. Patients that fall into this category typically have none of the classic signs and symptoms of thyroid disease. However, it has been found that patients with suppressed TSH have a significantly increased risk of developing cardiac dysrhythmias. Those individuals who develop dysrhythmias typically respond to drugs used to treat hyperthyroidism. The risk of those individuals classified as subclinical hyperthyroidism progressing to overt hyperthyroidism is considered to be low.

B. Hypothyroidism - is associated with diminished levels of thyroid hormones in the blood stream.

1. **Symptoms and signs include** - lethargy, slow speech and thought, weakness, fatigue, dry and cold skin, cold intolerance, hypercholesterolemia, constipation, weight gain, hoarseness and bradycardia.
2. **Laboratory results** - -in overt hypothyroidism include
   a. decreased levels of T-4, FT4, T-3 Uptake and FTI,
   b. increased levels of TSH,
   c. usually the presence of thyroid autoantibodies.
   d. The analysis of T-3 is not useful in cases of suspected hypothyroidism since T-3 may be normal due to increased T-3 production by the thyroid gland in response to increased TSH stimulation.

3. **Causes of Hypothyroidism**
   a. is usually due to autoimmune damage to the thyroid gland
      1) **most commonly Hashimoto's thyroiditis.** Demographics showthat this autoimmune disease is much more common in females than in males. Although it can present at any age, Hashimoto's thyroiditis is most common in young women.
   b. Transient hypothyroidism may occur in other forms of thyroiditis, including post-partum thyroiditis (seen in up to one-third of women after delivery).
   c. common after surgical removal of most of the thyroid gland
   d. may occur many years after treatment of Graves' disease.
   e. In neonates, hypothyroidism occurs in about 1:3000 - 1:4000 births, usually due to congenital absence of the thyroid gland. The prevalence of hypothyroidism increases significantly after age 50 - 60 with as many as 3 - 5% of older individuals having hypothyroidism (again, more commonly seen in females than males).

4. **Subclinical hypothyroidism** - T-4 and FT4 are typically found along the lower end of the normal reference range while TSH is increased. Although typically said to be asymptomatic, many patients have non-specific symptoms such as weakness, loss of energy, and trouble concentrating when carefully questioned. Up to 50% of persons with subclinical hypothyroidism develop overt hypothyroidism if untreated. Laboratory testing significantly assists the medical staff in predicting the course of the this disease. Patients with a TSH greater than 20 mU/L or a high anti-TPO titer generally develop clinical hypothyroidism rapidly. Absence of theTPO antibodies or lesser TSH elevations suggests a chronic subclinical state in which therapy may not be required.

5. **Monitoring therapy.** Treatment for hypothyroidism involves replacement therapy with synthetic thyroid hormones. Monitoring with TSH levels is recommended with the goal of treatment being to maintain TSH levels within the normal reference range. Since the half-life of T-4 is long (typically seven days), assessment of treatment should be delayed for 6 - 8 weeks.

C. **Euthyroid Sick Syndrome** (also known as Non-Thyroidal Illness) - is a situation seen in severely ill patients with normal thyroid function but abnormal thyroid laboratory test results. Typically the illness results in

1. **a decline in free T-3 (FT3) and total T-3.** - This decrease is caused by a decrease in the peripheral conversion of T-4 to T-3 and the increased peripheral conversion of T-4 to rT-3. TSH levels fail to increase in response to the decreased levels of free T-3 (i.e, TSH remains normal). There is controversy as to whether this failure of TSH to increase represents an adaptive response or an inappropriate failure of the pituitary gland to respond to the decreased T-3. During recovery from the underlying illness, thyroid hormone levels normalize with a typical transient elevation in TSH. Because of these hormonal changes in the severely ill, the most reliable indicator of thyroid status is FT4. FT4 remains normal throughout the entire course of the illness. The differential diagnosis between hypothyroidism and euthyroid sick syndrome is presented in the table below.

Laboratory Test	Hypothyroidism	Euthyroid Sick Syndrome
T-3	normal or decreased	decreased
FT4	decreased	normal
rT-3	normal	increased
TSH	increased (1°)/decreased (2°)	normal

The table below provides an overview of laboratory results in various thyroid disorders.

	TSH	Total T-4	FT-4	FTI	Total T-3	Antibodies
Overt primary hyperthyroidism[a]	↓↓	↑	↑	↑	→↑	Often
Overt primary hypothyroidism	↑↑	↓	↓	↓	NA[b]	Often
Subclinical hypothyroidism	↑	→	→	→	NA[b]	+/-
Euthyroid Sick Syndrome	→	→↓	→	→	↓	-
Subclinical hyperthyroidism	↓	→	→	→	→	+/-

[a] In cases of T-3 toxicosis, T-4 is normal while T-3 is increased.
[b] Not useful in hypothyroidism; results are typically normal.

D. **Screening for neonatal hypothyroidism**. **Early detection and treatment of hypothyroidism in the neonatal period is critical to eliminate the severe mental retardation associated with thyroid hormone deficience. Measurement of T-4 or TSH or a combination of these is used for screening which is performed on dry blood spots or cord serum. The detection rate depends on the test used and timing of specimen collection. Measurement of only T-4 leads to a high false positive rate, necessitating recall of a large number of infants for retesting. Causes of false positive results include low T-4 levels, which occur in both premature infants and those with congenital deficiency of TBG. Screening with only T-4 may miss infants with compensated or partial thyroid insufficiency. About 15% of infants with primary thyroid disorders have compensated hypothyroidism, that is, they have a serum T-4 within the normal reference range and an elevated TSH. Some programs screen with T-4 levels complemented with TSH measurements on specimens with the lowest 3% to 6% of T-4 results. Elevated TSH is the most sensitive test for the diagnosis of congenital hypothyroidism; however, false positive results are occasionally seen, in premature or severely stressed infants. In addition, by screening with TSH alone, those infants with congenital hypothyroidism caused by hypothalamic or pituitary disease will be missed. It also has been recommended that very-low-birth-weight infants should have additional screening at two and four to six weeks to detect late-onset, transient hypothyroidism.**

VII. Thyroid Testing

The first three laboratory assays being discussed make up the traditional thyroid screen. While improvements in assays, e.g. TSH, have led to a re-defining of the thyroid screen, reference is still frequently made to the older procedures resulting in their presentation here. The traditional thyroid screen consisted of a total T-4, T-3 Uptake, and a free thyroxine index. Also, total cholesterol levels were typically included based on the inverse relationship between thyroid hormone and the cholesterol level in the blood stream.

A. T-3 Uptake - The T-3 uptake test is not an assay for T-3. This test was unfortunately named for one of the test reagents, the radiolabeled T-3 reagent. T-3 uptake tests are designed to assess the unsaturated binding capacity of serum proteins for thyroid hormones, primarily thyroid (thyroxine) binding globulin (TBG). In the T-3 uptake test, the sample is mixed with radioisotopically-labeled T-3. The T-3-^{125}I reagent is added in excess to occupy all unsaturated binding sites on the TBG in the sample. T-3-^{125}I not bound to TBG is removed from the reaction mixture by the addition of a secondary binder, a silicate tablet. The T-3 uptake is the percent of the total radioactivity added which is taken up by the secondary binder. This assay principle is illustrated in Fig. 44-8, page 844 in Kaplan.

Referring to Fig. 44-8, D represents drugs occupying binding sites on TBG.

Considering Fig. 44-8, if the endogenous thyroxine (T-4) level is increased, as in hyperthyroidism, the patient's TBG is relatively saturated. More ^{125}I-labeled T-3 remains in a free state to be adsorbed by a secondary binder. Thus, in hyperthyroidism, the T-3 uptake will be increased. Conversely, when T-4 output is low, as in hypothyroidism, more labeled T-3 will bind to the TBG, yielding a comparatively low percentage of the radioactivity to be adsorbed by the secondary binder. Thus, in hypothyroidism, the T-3 uptake will be decreased.

A normal T-3 uptake range is 35 - 45%.

B. Total Serum T-4 - Total Serum T-4 is the second component in the traditional thyroid screen. This is a direct measure of T-4 in serum by a competitive immunoassay technique, such as radioimmunoassay. Methodologically, thyroxine is first released from endogenous thyroxine-binding proteins present in serum by preparing a protein free filtrate. T-4 is then released to the reaction mixture and is available for assay.

Normal total T-4 values are in the range of 5.5 - 11.5 μg/dL.

C. Free Thyroxine Index - The last component of the thyroid screen is the free thyroxine index. The free thyroxine index has been abbreviated in several different ways: FTI, FT4I, T-7. The free thyroxine index is determined by the T-3 uptake and total T-4 values as follows:

$$\text{FTI} = \frac{\text{T-3 uptake (\%)}}{100} \times \text{total T-4}$$

The T-3 uptake test is an assessment of unsaturated protein-binding capacity while the T-4 assay is a quantitative measurement of total serum thyroxine. It might be assumed that either of these two tests might be a suitable screening test for suspected thyroid disease. It is now clearly recognized, however, that in many circumstances the total T-4 level and/or the T-3 uptake may often be abnormal in euthyroid patients (patients that have normal thyroid function but abnormal thyroid laboratory test results) and may be normal in patients with abnormal thyroid function. Interpretation of such results cannot be accomplished without assessing both the total T-4 and the binding capacity of the TBG. Most of the circulating T-4 is bound to serum proteins, primarily TBG. Metabolism is regulated by a very small fraction of T-4, approximately 0.04 - 0.05 percent, which is not bound to proteins and can more readily diffuse into tissues. Thus, it is the free T-4 which actually establishes the patient as euthyroid, hypothyroid, or hyperthyroid. This free T-4 fraction, however, in the past was very difficult to quantitate

accurately. With the advent of new technologies, free T-4 is easier to measure and this is one of the changes that has led to a re-defining of the thyroid screen.

The level of free T-4 in serum is directly related to two parameters, the total T-4 and T-3 uptake test. Since free T-4 concentration is dependent upon the concentration of total T-4 and the unsaturated TBG, close correlations exists between free T-4 and the product of the total T-4 and the T-3 uptake. This product was named the "Free Thyroxine Index (FTI)," and, at the time of the traditional thyroid screen, it was the most widely accepted parameter of true thyroid status. Thus, even in states in which the TBG capacity is abnormal, thyroid status can be accurately assessed.

Normal FTI reference range is 2.2 - 4.7.

The utility of the FTI can be illustrated with the following examples.

> In pregnancy, estrogens increase the TBG level causing a low T-3 uptake. The T-3 uptake will be low because the increased TBG will take up most of the radiolabeled T-3, leaving very little to be picked up by the secondary binder. The increased TBG concentration in the blood stream will initially decrease the free T-4 in the blood stream. The pituitary gland will respond with increased TSH and increased T-4 production in order to maintain a normal free T-4 level. Thus, with the increased protein binding of the increased T-4 in the blood stream, total T-4 levels will be increased. The FTI will, however, be normal. The normal FTI indicates that the abnormal thyroid function tests are not caused by an abnormally functioning thyroid. In this case, the abnormal thyroid function tests resulted from the action of increased estrogens.
>
> In hypoproteinemia, TBG is often decreased. With a decrease in TBG, not as much radiolabeled T-3 will be picked up by TBG leaving a considerable amount to be picked up by the secondary binder. Thus, the T-3 uptake will appear elevated. With the low blood level of TBG, more free T-4 will remain in the blood stream causing negative feedback to decrease TSH and T-4 production. Thus, total T-4 will be decreased. Once again, the normal FTI would indicate that the thyroid is functioning normally and the abnormal thyroid function tests are the result of the hypoproteinemia.

The following is a tabulation of the T-3 uptake, total T-4, and FTI results which can be anticipated in various conditions. It should be noted that combinations of conditions are often encountered. Thus, even when abnormal thyroid status is present, abnormal TBG levels may give rise to normal T-4 or T-3 uptake results. Extreme abnormalities in TBG levels, such as what may be seen in hereditary TBG deficiency, may not be completely corrected by the FTI.

	total T-4	T-3Uptake	FTI
Euthyroidism, Age-related			
Cord Blood	↑	↓	N
Newborns	↑	↑	↑
Adult	N	N	N
Hyperthyroidism	↑	↑	↑
T-3 Thyrotoxicosis	N	N	N
Hypothyroidism	↓	↓	↓
Primary			
Secondary			
Tertiary			
Euthyroidism - Increased Thyrobinding Proteins	↑	↓	N
Pregnancy			
Estrogens (contraceptive agents, natural or synthetic estrogens, feminizing tumors)			
Hyperproteinemia			
Acute intermittent porphyria			
Hereditary TBG icnrease			
Acute hepatitis			
Euthyroidism - Decreased Thyrobinding Proteins	↓	↑	N
Androgens(glucocorticoids, natural or synthetic androgens, acromegaly)			
Hypoproteinemia (nephrotic renal disease, chronic debilitating diseases, liver disease)			
In Vivo Inhibition of T-4 Binding	↓	↑	N
Hydantoins (Dilantin)			
salicylates (large doses)			
Thyroid-Related Drugs			
Hyperthyroidism, adequately controlled	N	N	N
Hypothyroidism, adequately controlled			
L-thyroxine	N-↑	N-↑	N-↑
Liothyronine	↓	N-↓	↓
Liotrix (physiologic T-4:T-3 ratio)	N	N	N

D. **Total T-3** - T-3 is sometimes referred to as the neglected hormone. Since it is present in such a low quantity in the blood stream (typically in the ng/dL range), it could never really be measured accurately until immunoassay procedures came about, such as radioimmunoassay

Both T-3 and T-4 are considered to be physiologically important in that either hormone alone can maintain the euthyroid state. The total serum concentration of T-3 is about 1/70 that of T-4, but metabolic potency of T-3 is about four times greater on the basis of weight. Therefore, it appears that 2/3 of thyroid hormone activity is due to T-3.

The ability to measure total T-3 in serum depends upon the availability of an antibody to T-3 which does not have significant cross-reactivity with T-4. With the development of such an antibody, T-3 values have been found to be clinically significant in the diagnosis of thyroid disease in the detection of T-3 thyrotoxicosis. This is a hyperthyroid situation where total T-4 and Free T-4 are normal, but T-3 levels are elevated producing the hyperthyroid situation. This may be an abnormality in itself, or it may be part of some other hormonal disease. With T-3 thyrotoxicosis, any assay of increased serum T-3 levels provides the only laboratory test to confirm this diagnosis since these patients exhibit border line high or normal serum T-4 levels. Also, elevated T-3 levels may signal the onset of hyperthyroidism.

E. **Free Triiodothyronine Index (FT3I)** - Just as total T-4 levels are affected by varying concentrations of the thyroid hormone transport proteins, so are T-3 levels. Therefore there may be an occasion to calculate the free T-3 index. This would be calculated as follows:

$$FT3I = \text{T-3 (ng/dL)} \times \frac{\text{T-3 uptake (\%)}}{100}$$

F. **Free Thyroid Hormones** - Technology is available to measure free T-4 (FT4) and free T-3 (FT3) levels. These direct measures of free hormone must be able to measure the small amount of thyroid hormone that is free in the presence of large amounts of protein bound hormone.

The reference method for measuring free hormone is equilibrium dialysis. This is a very difficult and time consuming assay. A newer method that is catching on rapidly is called immune extraction. This involves incubating serum for a short period of time with an antibody to protein - thyroid hormone complexes. This is followed by a separation step which leaves free hormone in the supernatant fluid which can be separated and quantitated. The results of immune extraction generally correlate well with equilibrium dialysis.

G. Thyroid Stimulating Hormone - TSH - Thyrotropin - TSH is produced by the adenohypophysis. TSH regulates the release of T-3 and T-4. The production of TSH is inversely related to circulating thyroid hormone levels.

Each individual has a particular "set point" at which TSH will attempt to maintain thyroid hormone levels. When thyroid hormone production changes from this level, there is a logarithmic change in the production of TSH. **This makes TSH the most sensitive indicator for thyroid disease**.

TSH assays are a bit confusing in that they are often classified by "generations," which is based on the sensitivity of the test. Each generation represents a one log increment decrease in detection limit, i.e. making the test more sensitive.

	Detection limit
1st Generation TSH Tests	1 - 2 mU/mL
2nd Generation TSH Tests	0.1 - 0.2 mU/mL

1st generation normal reference range for TSH is <10 mU/mL. This is an acceptable level of sensitivity for hypothyroidism as TSH may rise to 30-500 mU/mL. But, for hyperthyroidism, the decrease in TSH may not be seen. With 2nd generation tests, one can begin to distinguish hyperthyroidism from normal. Today, 3rd and 4th generation tests are available and these tests work very well. These assays typically utilize chemiluminescent methodologies. The normal reference range for 3rd generation TSH is 0.4 - 4.2 μU/L.

Perhaps the most common components of a thyroid screen today include a TSH and free T-4, although there is some variation between hospitals.

H. Thyrotropin Releasing Hormone Stimulation Test

With improvements in TSH assays, the TRH stimulation test has limited clinical utility. This test involves the IV administration of synthetic TRH and the TSH response to the TRH administration is monitored in the lab. In hyperthyroidism, the response is flat. In primary hypothyroidism, there is an exaggerated increase in TSH. However, this level would have already been increased, and a diagnosis could have been made without TRH administration.

The major utility of this test is in differentiating secondary and tertiary hypothyroidism. In secondary hypothyroidism, a flat response should be seen as in hyperthyroidism. In tertiary hypothyroidism, a delayed and prolonged rise in TSH should occur.

Certain medications will interfere with this test.

I. Thyroid Antibodies - Thyroid diseases often result from autoimmune disorders and are associated with thyroid specific antibodies. The laboratory identification of the presence of these antibodies helps the medical staff pinpoint the underlying problem associated with thyroid function.

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1. Graves Disease

a. Thyroid stimulating immunoglobulins (TSI) - ↑ (Antibody to TSH receptor)

b. Antithyroid peroxidase (TPO) - ↑ (antibody to thyroid microsomes)

2. Hashimoto's Thyroiditis

a. Antithyroid peroxidase (TPO) - ↑ (antibody to thyroid microsomes)