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Large Grotesque Head:
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THYROID FUNCTION & DISEASE

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Preface

This book was written in an attempt to provide answers to practical clinical questions about thyroid function and disease and, at the same time, to provide a pathophysiologic basis for these answers. Of the involved medical specialties, there is perhaps a closer tie between the understanding of the physiologic mechanisms and clinical disease in the thyroid, in particular, and in endocrinology, in general. *Thyroid Function and Disease* attempts to highlight this relationship.

Although books in which each chapter is written by a different expert are comprehensive, they often suffer from fragmentation and lack of cohesiveness. The realization that the interests of these three coauthors spanned the major areas of the thyroid provided the possibility of cohesiveness as well as comprehensiveness.

This perception was reinforced by extensive discussions as to how material should be presented and where it should be placed in the book. All the illustrations were done by a single individual to reinforce commonality.

With the advent of the new biology, cellular and molecular, there is a revolution in our understanding of thyroid function and disease. No book can be completely up-to-date; however, we have attempted to give the reader a foundation that would allow these revolutionary scientific advances to fit into an overall picture of thyroid function and disease.

PART I
CLINICAL EVALUATION

1
Histogenesis, Histology, Gross Anatomy, Physical Examination, and Imaging of the Human Thyroid Gland

ROBERT VOLPÉ

DEVELOPMENT OF THE THYROID

The word *thyroid* derives etymologically from the Greek word *thyreos,* meaning oblong shield. The German word for the thyroid gland—*Schilddrüse*, similarly, means *shield gland.* Actually, this term is not quite appropriate, because the thyroid gland does not resemble an actual shield; indeed, it is a bilobar organ, but is so named because of its close proximity to the thyroid cartilage, which does have the appearance of a Greek shield.[6]

HISTOGENESIS OF THE THYROID

The thyroid gland begins to show colloid formation at about 7 weeks of gestation.[16] However, it is not until about 5 weeks later that follicles develop with increasing storage of colloid. Thyroid function, however, may be observed as early as the 29th day of gestation at a time when the thyroid gland is a simple cell mass attached to the buccal floor.[4] Immunoreactive thyroglobulin synthesis is well established by that time, long before the onset of colloid formation or development of mechanisms of trapping and organic binding of iodide, which occur at about the 10th or 11th week.[17] Colloid may be formed by iodination of thyroglobulin, which appears in intracellular canaliculi (part of the smooth endoplasmic reticulum), preceding colloid formation by many weeks, whereas colloid formation and protein iodination appear to occur concurrently. The stimulus to thyroglobulin synthesis does not depend on thyroid-stimulating hormone (TSH), which is not detected until about the 13th week.

The human fetal gland manifests a full spectrum of organically bound iodinated products, including thyroxine by about the 10th week of gestation.[18] By the time the thyroid follicle is able to accumulate colloid, the gland commences trapping iodide, binds it to tyrosine, and begins to form iodothyronines, thyroxine, and triiodothyronine. Thyroxine may first be demonstrated within the thyroid itself and shortly thereafter within the serum by the 11th week.[5]

HISTOLOGY OF THE THYROID

The thyroid gland contains two types of cells, namely, follicular cells and parafollicular cells (C cells or light cells). The parafollicular cells

constitute only a small proportion of the total cell population.

The apex of the follicular cell is directed toward the lumen of the follicle with the base directed toward a basement membrane. These cells have a somewhat variable height, and this height will differ further depending upon the activity of the gland and iodine intake.[8] The cytoplasm of the follicular cell contains a variety of inclusions, the most prominent of which are intracellular colloid droplets up to 2 microns in diameter.[9, 10] The colloid within these droplets appears similar to the colloid within the central lumen of the follicles. The nuclei are usually located in the basal region of the cell. The cells are arranged in follicular form with the central lumen's containing colloid. Follicles are in turn arranged in aggregates, each surrounded by an extensive network of blood vessels, connective tissue cells and fibers, plasma cells, mast cells, and some ganglion cells. Aggregates of follicles form lobules of various sizes.[8]

In the normal adult, mitoses in follicular cells are infrequent. In studies with the electron microscope, the apical surface of the follicular cell is observed to have microvilli that extend into the central lumen of the follicle. Invaginations of these microvilli entrap colloid within the cytoplasm, by a process of pinocytosis, to form colloid droplets (see subsequent discussion).[6] The follicular cells are separated from each other by a cell membrane with the usual junctional complexes. The basement membrane on the basal surface helps to separate the follicles from each other and in turn is separated from the cell membrane proper. The basement membrane consists of a relatively homogeneous material, which has not as yet been fully characterized. The cytoplasmic inclusions that can be observed clearly on electron microscopic studies consist of vesicles adjacent to the apex, which correspond to the colloid droplets. There is also a variety of electron-dense bodies of various sizes and shapes, which are presumably lysosomal in nature. These ultimately join with the colloid droplets to form a "derived lysosome," which then digests the ingested colloid so as to permit ultimate release of thyroid hormone at the base. The thyroid follicular cells also contain extensive rough endoplasmic reticulum in the cytoplasm. There are scattered mitochondria as well as a well-developed Golgi apparatus located proximally to the nucleus, consisting of saccules, coated vesicles, and some apical vesicles.[6]

EFFECTS OF TSH

In follicular cells, following the administration of TSH, intracellular droplets (phagosomes) increase sharply and represent ingested luminal colloid. Some of these fuse with lysosomes, forming structures called phagolysosomes.[20] The apical portion of the follicular cell develops cytoplasmic processes (streamers) following TSH injection, and these fuse around portions of the follicular colloid, thereby leading to their endocytotic uptake into the cell, where they form the aforementioned colloid droplets. TSH administration will also lead to a change in the shape of the follicular cell from a cuboidal to that of a tall columnar shape. Mitoses will become evident, and increased numbers of cells will infold into the lumen of the follicles. The luminal colloid will be absorbed, and there will be a great increase in stromal vascularity.[6]

THE PARAFOLLICULAR CELL

There is a second type of thyroid cell within the human thyroid, namely, the parafollicular cell (C or light cell) that produces calcitonin.[21] Through the basal cell of the follicle these oval or ellipsoid cells come into contact with the basal follicular membrane. Their apical poles are directed toward the follicular lumen but are separated from it by a thin sheet of follicular parenchymatous cells. On electron microscopic examination, mitochondria are seen to be more numerous in the light cells than in the adjacent follicular cells. Moreover, the administration of calcium results in degranulation of the cytoplasm. Finally, the light cell has a much higher activity of alpha-glycerophosphate dehydrogenase.[11, 12]

GROSS ANATOMY OF THE THYROID GLAND

The physician should understand the anatomy of the thyroid gland, because goiter is a common condition. (The embryonic development of the thyroid and its relationship to hypothalamic and pituitary development will be dealt with elsewhere in this text.) In the average North American adult, the normal thyroid gland weighs approximately 18 to 20 gm.[2]

Encapsulated in fibrous tissue, it is held around the anterior and lateral aspects of the trachea by loose connective tissue. The lateral lobes on each side of the trachea are connected by a thin band of thyroid tissue, the isthmus. The isthmus lies just below the cricoid cartilage, and a pyramidal lobe occasionally extends superiorly from the isthmus. The isthmus overlies the second and third cartilaginous rings of the trachea. The carotid sheath and sternocleidomastoid muscles lie bilaterally, and the infrahyoid muscles lie anteriorly. Generally, there are two pairs of parathyroid glands under the posterior surfaces of the lateral lobes. The recurrent laryngeal nerves lie in grooves between the trachea and the lateral lobes of the thyroid gland (Fig. 1–1).

Normal variations in shape, size, position, and configuration of the thyroid are numerous. A prominent isthmus or pyramidal lobe may be evident, one lobe may be absent, or one lobe may be considerably larger than the other. Lobulations of the gland may be evident, resembling nodules. A median ectopic thyroid may be encountered and mistaken for lymph nodes.

The arterial supply of the thyroid is primarily from the superior and inferior thyroid arteries (Fig. 1–2). The superior thyroid arteries derive from the external carotid arteries, and the inferior thyroid arteries are branches of the subclavian arteries. The right lobe of the thyroid gland has a richer blood supply than the left, is often larger, and more frequently develops nodules. There is collateral circulation from small perforating branches along the trachea.

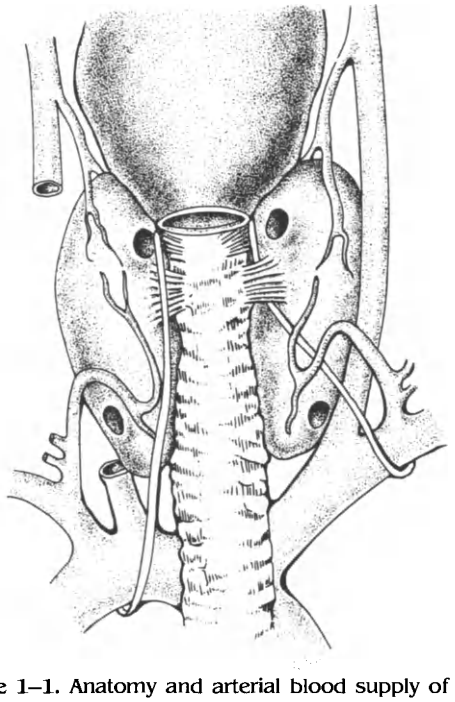

Figure 1–1. Anatomy and arterial blood supply of the thyroid gland.

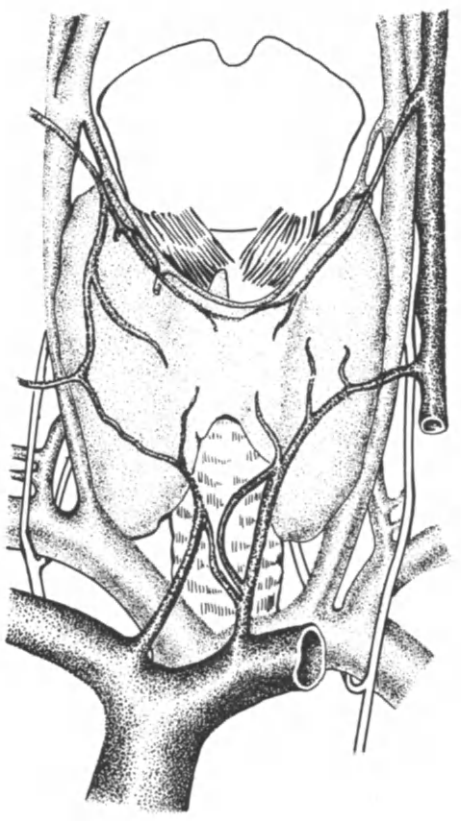

Figure 1–2. Anatomy of the venous supply of the thyroid gland.

The thyroid veins, which afford drainage from the thyroid gland, vary greatly in size, numbers, and location. These veins may be massively distended when there is occlusion of venous return at the thoracic inlet. The variation in pattern and location and the dilatation that may occur as a result of large intrathoracic goiters may create some difficulties during thyroidectomy.

The thyroid is rich in lymphatic capillaries that encircle the thyroid follicles and are adjacent to the parafollicular cells (Fig. 1–3). The collecting trunks are adjacent to the capsular veins and follow these along the lines of venous drainage. The regional nodes of the thyroid gland comprise the internal jugular vein from the subdigastric group; the pretracheal, paratracheal, prelaryngeal, and recur-

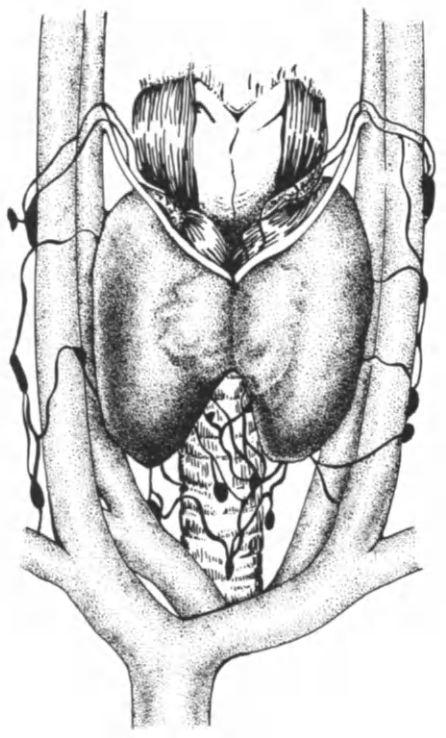

Figure 1–3. Lymphatic drainage of the thyroid gland.

rent laryngeal nerve chains; and the retropharyngeal and retroesophageal groups. The anterior superior mediastinal nodes may also become involved when retrograde or collateral lymphatic flow occurs.[2]

The thyroid gland receives its nerve supply from both adrenergic and cholinergic nervous systems, the former arising from the cervical ganglia and the latter from the vagus nerve. These nerves that control arterial and venous flow were thought to exert only neural influence on thyroid gland function. However, it has been shown that adrenergic influences can affect the thyroid parenchymal cells directly.

Thus, the nerves to be considered include the vagus nerve, the recurrent laryngeal nerve, the superior laryngeal nerve, the cervical sympathetic nerves, and the ansahypoglossal nerve.

The vagus nerve descends deep to the internal carotid artery, continuing between the common carotid artery and internal jugular vein, passing in front of the subclavian artery. The right vagus nerve gives rise to the right recurrent laryngeal nerve, which then passes behind the subclavian artery and reaches the region of the thyroid via the thoracic inlet. The left vagus nerve gives rise to the left recurrent laryngeal nerve at the aortic arch, and it then ascends along the trachea to the region of the thyroid.

The recurrent laryngeal nerves that are both motor and sensory supply the trachea and subglottic region of the larynx. Each recurrent laryngeal nerve passes deep to the respective thyroid lobe, passing in front or behind the inferior thyroid artery or its branches. They then give off sensory branches to the trachea and motor branches to the abductor and adductor muscles of the larynx.

The superior laryngeal nerve arises from the inferior ganglion of the vagus nerve, then running medially behind the internal carotid artery, where it divides into an external and an internal branch. The external branch continues in a caudad direction, medial to the superior thyroid artery, to provide terminal motor branches to the cricothyroid and internal constrictor muscles.[2]

The cervical sympathetic chain is situated deep to the common carotid artery. Occasionally, with metastatic thyroid carcinoma, this chain may be directly invaded by involved lymph nodes, resulting in Horner's syndrome.

THE PARATHYROID GLANDS

There are usually four parathyroid glands, although occasionally larger numbers may be found. These glands are small, light brown, soft, and measure approximately $5 \times 3 \times 3$ mm. The upper pair are usually located behind the upper and middle third of either lobe of the thyroid gland, often just lateral to the recurrent laryngeal nerve where it enters the larynx. The upper pair are usually supplied by a small branch of the inferior thyroid artery. The two lower parathyroid glands may be intrathyroidal, intracapsular, or behind the lower pole of the thyroid gland. They also may be situated elsewhere, such as in the anterior mediastinum or within the thymus gland.

ANOMALOUS ANATOMY

Development of the thyroid gland is considered elsewhere, and only brief mention is made here of anomalous anatomic variants that may be encountered. These include, most commonly, development of a pyramidal lobe or tract. More uncommonly, thyroglossal duct cysts, lingual thyroids, and lateral aberrant thyroid rests may be observed (Fig. 1–4).

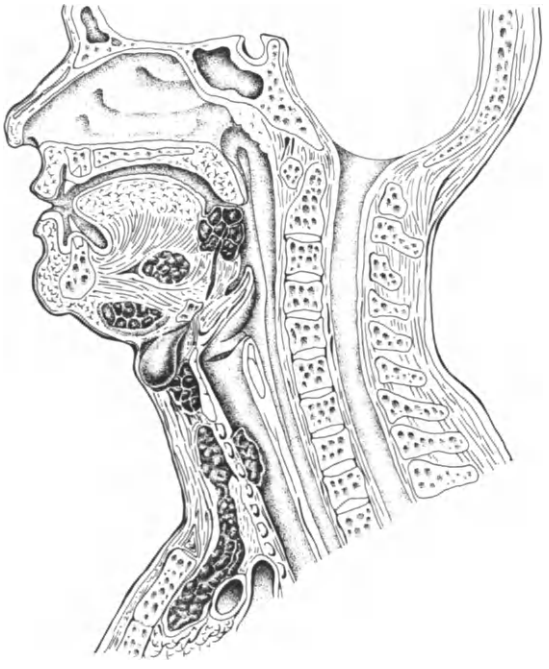

Figure 1–4. Aberrant and normal locations of thyroid tissue.

Lingual Thyroid (Figure 1–5)

A lingual thyroid occurs when there is no primary contact of the median thyroid, in its process of separating from its place of origin in the floor of the embryonic pharynx, with the endothelial tubes of the embryonic heart. Without this contact, the thyroid retains its primitive position and then differentiates as a lingual thyroid.[14]

The lingual thyroid is located in the area of the foramen cecum. It is in a superficial position and does not extend to involve the muscle of the tongue. A lingual thyroid may be a very small amount of functioning tissue and, when present as the sole representative of functioning thyroid tissue, often has the size and appearance of a raspberry.[2] These lingual thyroids may enlarge further and can cause local obstructive symptoms, including dysphagia, airway obstruction, and speech impairment. Neoplasia in lingual thyroids has been observed more commonly than chance alone would dictate, undoubtedly due to the ectopic nature of the tissue and to the increased TSH stimulation that often occurs because the thyroid tissue itself may be somewhat inadequate in amount.

Certainly removal of a lingual thyroid surgically in the absence of a normally situated thyroid will result in hypothyroidism. In comparison, if initiated early, thyroxine therapy will cause the lingual thyroid to regress.[2]

Lingual thyroids can be well demonstrated by means of thyroid scanning when the scanner is placed in an appropriate position to pick up the activity at the base of the tongue.

In a patient who also has a normal thyroid in addition to lingual thyroid tissue, removal of the normal thyroid tissue by surgery will result in some enhancement of the activity of the lingual thyroid, also demonstrable by scanning.

Median and Lateral Aberrant Thyroid Rests

The median thyroid rests are located along the course of the developing thyroid.[2] These rests of thyroid tissue may be solitary or multiple and may occur along with a normal thyroid in its usual position. Alternatively, they may represent the only functioning thyroid tissue present. In the last instance, the amount of thyroid tissue may be either sufficient to maintain a patient in a euthyroid state or inadequate for this purpose. As with the aforementioned lingual thyroid, there is a tendency for tumor development.

Thyroid rests may also be found closely related to the hyoid bone and are most often evident with thyroid scanning after surgical removal of a normally placed thyroid gland. These rests may be associated with a thyroglossal duct cyst or with a pyramidal lobe placed just inferiorly to the rest.

A thyroglossal duct cyst may occur in the presence or absence of a normal thyroid gland. Indeed, about 60% of patients with thyroglossal duct cysts have thyroid glands in the normal position. Thyroglossal duct cysts are positioned in the mid-line and may discharge cyst fluid intermittently.

The pyramidal lobe is the most common thyroid rest and actually occurs in 23 to 68% of patients. It is most often readily demonstrated following removal of the thyroid gland itself.

Cancer of the thyroid is much more common in these various rests than in normal thyroid tissue. Carcinoma of the thyroid is discussed in a separate chapter in this text, and the clinician should be aware that the demonstration of thyroid rests carries with it the possibility of thyroid malignancy.[2]

Lateral aberrant thyroid rests are rare. There has been some confusion between such

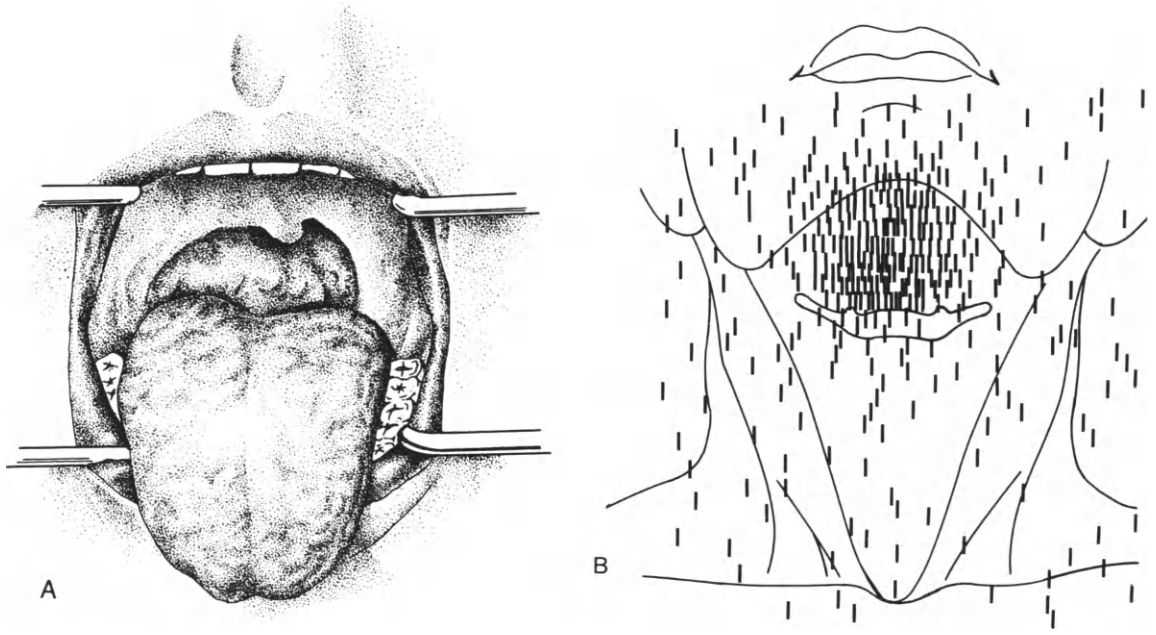

Figure 1–5. *A,* The position and appearance of the lingual thyroid as a fleshy mass at the base of the tongue, approximately the size of a raspberry. *B,* A scintiscan of the lingual thyroid is shown, in which all of the functioning tissue is situated in the same position as in *A.*

rests and the presence of lymph node metastases from follicular thyroid carcinoma. Thus, most so-called lateral aberrant thyroids are actually metastases from the thyroid gland proper. However, a few lateral aberrant thyroids have been demonstrated and reported in the literature, but because of their scarcity tissue of this type should be viewed with great suspicion.[2]

THORACIC AND MEDIASTINAL GOITERS

Most intrathoracic goiters result from the extension of a nontoxic goiter in the neck or may occur in a patient with severe kyphosis or a short neck. Since the thoracic inlet is a fixed bony aperture, thyroid enlargement within this region may cause compression of adjacent soft tissues. Thus, airway obstruction, blood vessel obstruction, and dysphagia may occur. Moreover, the thyroid tissue lying below the inlet may enlarge further so that the greatest mass of the goiter may lie in the thorax.

An anterior mediastinal goiter may be the result of the previously described process. However, there may also be anterior mediastinal rests that may become goitrous and even result in superior vena caval obstruction.

Posterior mediastinal goiters may also arise from rests that extend behind or beside the esophagus. These will then lie behind the great vessels of the heart, closely adjacent to the recurrent laryngeal nerve. A cardiac thyroid may also occur as a rest that results from the primary contact between cardiac endothelium and thyroid-forming cells remaining intimate.[14] Thus, a part of the gland is included between the endothelium and the enveloping myocardial mantle. Otherwise, posterior mediastinal goiters can be located on chest radiographs and may be large enough to compress lung tissue.

PHYSICAL EXAMINATION OF THE THYROID GLAND

Examination of the thyroid gland is an integral part of every complete history and physical examination.[19] The neighboring neck structures must also be carefully examined; first, because they are so frequently encroached upon whenever there is thyroid enlargement; second, because of the possibility of lymph node involvement in certain diseases of the thyroid; and third, because abnormalities of the neck region may not be related to thyroid disorders in any way and may have to be considered on their own merits or in respect

to a differential diagnosis when thyroid disease is a consideration.

It is obvious as well that a history and physical examination must include all systemic elements, since thyroid disease can have many systemic ramifications and, conversely such an inquiry may result in suspicion of the presence of a thyroid disorder.

However, this chapter will not deal with the overall systemic history and physical examination, although these aspects are discussed in the various parts of this text that relate to specific diseases.

LOCAL EXAMINATION OF THE THYROID GLAND

Classically, examination of the viscera utilizes most of the senses; inspection, palpation, auscultation, and percussion are the four hallmarks of any examination. In the case of the thyroid, the first two, inspection and palpation, generally provide most of the information.[19]

Inspection

The patient should be placed in a position that allows reasonably good lighting, sufficient to observe the structures within the neck but also with minimal shadowing that places the various structures in relief. The chin is elevated, and the area closely inspected for operative scars, drainage sites, and evidence of previous radiation in the form of telangiectasia. The observer then inspects the thyroid region at the base of the neck anteriorly.

The normal thyroid gland cannot generally be detected on inspection. In the absence of thyroid enlargement, evidence of tracheal deviation or venous dilatation may suggest the presence of a substernal goiter. When the patient swallows, such a substernal goiter may become evident by fullness in the suprasternal notch.

Large goiters can be observed readily on inspection. To determine whether a particular structure represents a goiter, the patient is asked to swallow. Since the thyroid gland attaches to the trachea, it will rise with it during deglutition. Generally, this diagnostically excludes other structures in the neck, since most structures either do not move at all or do not move significantly in response to swallowing.

Conversely, an abnormal thyroid gland may not move with swallowing, owing to fixation of the thyroid tissue to adjacent structures. Such a finding often may suggest the possibility of thyroid carcinoma although other lesions occasionally may produce similar findings.

Palpation

Palpation of the thyroid gland is possibly the most important maneuver to determine the size, shape, consistency, and tenderness of the gland. There are several means by which palpation can be performed, depending on the particular preference of the examiner. It is important to have the patient flex the neck minimally and turn the chin slightly toward the side to be examined so as to afford relaxation of the sternocleidomastoid muscles, an important prerequisite to the palpation of the thyroid.

The examiner may stand in front of the patient, may be above the patient, or may be on an equal level with the patient. Others prefer to stand behind the patient being examined. In any event, the first maneuver is to palpate the thyroid area with the forefinger in order to determine size, consistency, contour, and symmetry, as well as to determine whether tenderness is present. Thereafter, the larynx and the thyroid are displaced to one side, by pressure with the thumb or fingers of one hand upon the thyroid cartilage; it is then possible to palpate the displaced lobe of the thyroid more readily. Both sides are examined in a similar fashion, during which time the patient is allowed to swallow. It is possible to feel the normal-sized thyroid gland in a thin person, and it is certainly quite easy to feel any enlargement of the thyroid gland even in an obese patient. Careful notation should be made of the size, shape, and consistency of the thyroid gland. The size should be estimated either in approximate weight or in some other measurement. The firmness should be in a score of 1 to 5 where 1 is normal and 5 is extremely hard. When the gland is not palpable, abnormalities may still be demonstrable by imaging (see Ultrasonography, to follow).

When nodularity is encountered, it is important to attempt to separate this from lobularity as it may be seen in diffuse toxic goiter. Cysts may be suspected when a nodule is hemispheric in shape, rubbery in consistency, and smooth in outline. It is sometimes possible to transilluminate such cysts, although fluctuation is rarely demonstrable.

Tenderness of the gland is generally due to

inflammation or infection but may also occur with rapid expansion of the thyroid capsule, as with hemorrhage into a cyst, thyroid malignancy, or even hyperthyroidism.

At the same time, note should be taken of other palpable structures in the neck that may relate to thyroid disease. Examination also includes the determination of the position of the trachea (e.g., Is it deviated because of thyroid enlargement?). To determine the position of the larynx and trachea, the index finger is placed on the trachea in the suprasternal notch and then the same finger is placed on each side of the notch. A space available to the finger will be equal on both sides if the trachea proves to be in the mid-line. One should next examine the carotid arteries for the quality of pulsation and for bruits. Similarly, the veins should be inspected and venous pressure estimated. The cervical lymph nodes should then be palpated, with particular attention to the Delphian group of nodes that overlie the trachea, cricoid cartilage, or thyroid cartilage in the mid-line as well as the mastoid nodes situated at the angle of the jaw.

Percussion and Auscultation

Percussion is sometimes useful to determine the presence of a large substernal goiter which would widen the anterior mediastinum. Auscultation may be found useful, particularly in reference to the presence of bruits, which are found not uncommonly in toxic goiters. Murmurs that may be transmitted from the heart or carotid arteries may sometimes be heard over the thyroid and be misconstrued as bruits. Thus, the information to be gained from percussion and auscultation is relatively limited.

Other Aspects of the Examination

It is important for the physician to examine other structures in the neck, since they may become involved in thyroid disease. For example, dyspnea, stridor, cough, dysphagia, or choking sensations may occur as a result of tracheal or esophageal compression. Signs of venous obstruction may be observed due to local venous pressure, resulting from enlarged thyroid tissue or lymph node metastases.

Hoarseness or loss of the voice may be secondary to recurrent laryngeal nerve palsy, either as a result of a lesion in the region or of damage to the nerve during surgery. Sympathetic nerve damage may result in Horner's syndrome.

Lymph node enlargement in the region may result from thyroid malignancy. The upper pretracheal node (i.e., Delphian node) may be enlarged, not only with thyroid malignancy but with Hashimoto's thyroiditis as well. Since it lies just superior to the thyroid isthmus, it has received considerable attention in the past.

Physical examination may also elicit anomalies of thyroid development, including thyroglossal duct cysts and lateral aberrant thyroid tissue. More often than not, such findings usually represent metastatic lymph node metastases.

Examining the pharynx with a mirror will often be necessary to demonstrate lingual thyroids. Direct or indirect laryngoscopy will have to be employed when recurrent laryngeal nerve injury is suspected.

Finally, it must be emphasized that a complete general physical examination will be necessary to determine whether a particular patient has any clinical evidence of hyperthyroidism or hypothyroidism. Findings in either disorder are discussed separately in this text.

THYROID IMAGING

Roentgenography

Roentgenography of the thyroid region is useful in establishing the location, size, and character of a thyroid mass.[19] Substernal extension of a goiter is thus demonstrable, and one can determine whether the mass is in the anterior or posterior mediastinum. Compression of the trachea is also made evident. Calcification within the thyroid gland, as well as metastatic deposits within the chest, may be observed. A fluoroscopic study with barium may suffice to show abnormalities of the thoracic inlet. Computerized axial tomography (CAT) will also demonstrate these abnormalities very elegantly but is generally not necessary. Surgeons sometimes find a preoperative CAT scan useful to assess the extent of a malignant thyroid lesion or the substernal or retrosternal extension of a goiter. The technique is also useful in managing patients with postoperative thyroid malignancy when they have cryptic masses within the neck.

Thyroid Radionuclide Scanning

Methods for localization of thyroid tissue by external imaging using radionuclides would

involve (1) those in which the isotope is normally concentrated by thyroid tissues (e.g., isotopes of iodine and the ^{99}m pertechnetate ion); (2) the administration and following of any one of a number of pharmaceutical agents, which may be preferentially concentrated by abnormal thyroid tissue (e.g., ^{75}selenomethionine, ^{67}gallium citrate, ^{131}cesium chloride, and ^{32}phosphate); and (3) fluorescent scanning, which is a rarely utilized approach to thyroid scanning and in which no isotope is administered to the patient. The thyroid is radiated with gamma rays derived from an ^{241}americium source; this interacts with stable iodine within the thyroid, which in turn emits x-rays that are fluorescent, and these can be picked up by a specialized silicon detector.[13] The last two procedures mentioned are not routinely used in the investigation of thyroid disease. For scanning, ^{123}I and ^{99}m technetium (^{99}m Tc) are the radionuclides of choice, because of the low radiation exposure. The half-time of ^{99}mTc is 6 hours; 99% of the emissions are 140 KeV gamma rays, with no beta emissions. The energy deposited *in situ* is low, and when coupled with the short half-life provides a very low radiation dose to the subject.[1] Thyroid irradiation is about 0.1 rad/mCi as compared with about 1.0 rad/μCi for ^{131}I, a factor of 10^4 less than that for ^{131}I.

Although ^{99}mTc is actively trapped in the thyroid by the same mechanism as iodine, it does not undergo organification. This isotope achieves maximum accumulation within the thyroid in an average of 17.8 minutes with a range of 5 to 30 minutes. Since larger doses of radionuclide can be administered because of the short half-life and low level of irradiation, high resolution thyroid scans are obtainable within 20 minutes after injection. ^{131}I is still commonly used, however, for whole body scanning for the detection of functioning metastatic thyroid carcinoma.

Several imaging instruments are available but are not discussed here. Primarily, Anger-type gamma cameras are currently being employed widely.

The indications for scanning include the determination of the presence of solitary thyroid nodules (functioning or nonfunctioning), determination of the nature of abnormal neck or chest masses, determination of the size and shape of the thyroid, evaluation of thyroid remnants after surgery, detection of functioning thyroid metastases, detection of anatomic variants and surgical ectopic thyroid tissue, diagnosis of congenital athyreosis or lingual thyroid, and evaluation as to whether the thyroid is suppressible or not.

In clinical practice, scans are most often requested for the evaluation of the functional activity of solitary nodules suspected of being malignant. As stated elsewhere in this text, cold nodules are not predictably cancerous and warm nodules do not completely exclude that possibility.[13] The value of scanning in particular lesions is discussed elsewhere in the text, and the use of other isotopes or fluorescent scanning is beyond the scope of this chapter.

ULTRASONOGRAPHY

Ultrasonography or echography has become increasingly used to characterize the density of lesions within the thyroid gland. The technique is able to differentiate interfaces of different acoustic densities utilizing sound frequencies in the megahertz (MH_2) range (above the frequency of audible sound).[7, 15] A transducer is applied to the neck that transmits the signal and receives the echo reflections. Liquid transmits sound without deflection, whereas air-filled space will not transmit ultrasound. Generally, two-dimensional or B mode ultrasonography is employed, which uses a scanner that moves the transducer across the neck. A composite image of echoes is assembled electronically. Special gel or oil is used to make contact with the skin, and this is essential to avoid artefacts.

This type of equipment is able to differentiate between solid and cystic lesions, when they are larger than 1 cm in size. It is excellent for localization of lesions and has been used to guide the needle during fine-needle aspiration biopsy. B mode ultrasonography has also been proposed as a method for the estimation of thyroid gland weight. However, this technique cannot distinguish between benign and malignant lesions, and claims that it can detect parathyroid adenomas have been disputed.

High resolution ultrasound equipment is now generally available. These new instruments utilize a 10 MHz transducer, permitting images to be analyzed instantaneously and dynamically. High-resolution echograms can identify solid structures as small as 4 mm and cystic structures as small as 2 mm. Minute thyroid nodules can thus be demonstrated by this technique, which is far more sensitive than isotope scanning or palpation. The volume and

shape of the entire thyroid gland also can be readily determined.[1]

Other Techniques

Thyroid angiography or lymphography has been employed by a few workers, but neither technique has been found to be very useful. Thyroid thermography has been proposed to attempt to differentiate between benign and malignant lesions, which are both cold on isotopic scanning. Early evidence suggests thermography has no advantage over ultrasonography.[13]

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PART II
THYROID GLAND AND CONTROL

2
Thyroid Hormone Biosynthesis

GERARD N. BURROW

EVOLUTION

Thyroid hormone is synthesized as a prohormone, thyroglobulin, which is exported from the cell-like insulin or thyroid-stimulating hormone (TSH). However, unlike the pancreas or the pituitary, thyroid hormone is exported into the colloid, a storage area, and then to satisfy demand is brought back into the cell for further processing. The thyroid gland is also unique in that it is the only gland in the body to incorporate iodine in the synthetic process. Although iodine is concentrated in salivary glands, mammary glands, gastric mucosa, and choroid plexus, iodothyronines are not formed in these organs. Finally, the thyroid gland synthesizes the huge glycoprotein thyroglobulin, with a molecular weight of 660,000, to obtain approximately three thyroid hormone molecules with a combined molecular weight of 2700. The reasons for these unique aspects of thyroid hormone biosynthesis may be better understood in evolutionary terms although the explanations are far from clear.

Evolutionary Origins of the Thyroid

Iodine binding to proteins is widespread in nature, including algae, corals and mollusks, and many other invertebrates. Most of this binding is to structural proteins and has been thought not to include mono-iodotyrosine (MIT) and diiodotyrosine (DIT) synthesis.[130] However, in the jellyfish, iodine plays a role in strobilation that involves conversion from the sessile to the medusa or free-floating form with the concomitant formation of MIT and DIT. Whether this iodine binding in invertebrates represents a precursor form of thyroid hormone is not clear.

The thyroid gland as a distinct histologic entity is found only in vertebrates where it always develops from a primordium in the midventral floor of the pharynx.[15] The origin of vertebrates from lower forms is obscure. Their closest relatives are the protochordates, tunicates, and *Amphioxus*. However, even in the last two groups, evidence of thyroid evolution from prevertebrate ancestry is inconclusive. In forms below vertebrates, there is no significant formation of T_4 or T_3. Whether the iodotyrosines found play any functional role is unknown, although strobilation of the jellyfish suggests some form of control. Interest has centered therefore on the role of the thyroid

in the lower vertebrates[156] (Fig. 2–1). (See Chapter 5.)

Evolution of Thyroid Gland Morphology

The basic functional unit is the follicle, which is similar in all vertebrate thyroids. There is a single layer of epithelium that encloses the colloid and is surrounded by a blood and lymphatic capillary bed. Follicles in most vertebrates form a compact organ that is often divided into two symmetric lobes. However, in reptiles and elasmobranchs, it generally remains a single median organ. In cyclostomes, the follicles are loosely distributed in the area of the lower jaw, anterior to the heart, and in many teleosts and some Urodela amphibians, the capsule is markedly reduced. However, these morphologic variations have no apparent physiologic significance.

Since the thyroid develops from a primordium in the midventral floor of the pharynx, comparable regions of the protochordates have been objects of attention. In these areas, their pharynges filter minute organisms for feeding purposes. The endostyle, a groove in the floor of the pharynx, produces mucus which traps the food organisms (Fig. 2–2). The endostyle has been thought to be homologous with the thyroid. The larval form of the cyclostomes has retained the primitive feeding habit that is lost in the adult, although other cells of the

Figure 2–1. *Xenopus laevis* (South African clawed toad). *Left to right,* newly metamorphosed toad; metamorphosing tadpole; giant tadpole showing no metamorphosing signs with thyroidal hormone synthesis blocked by immersion in $KClO_2$. (Reproduced from Dodd, J. A. and Matty, A. J.: Comparative aspects of thyroid function. *In* Pitt-Rivers, R. and Trotter, W. R., eds.: The Thyroid Gland. London, Butterworth Publishing Co., Ltd., 1964, with permission.)

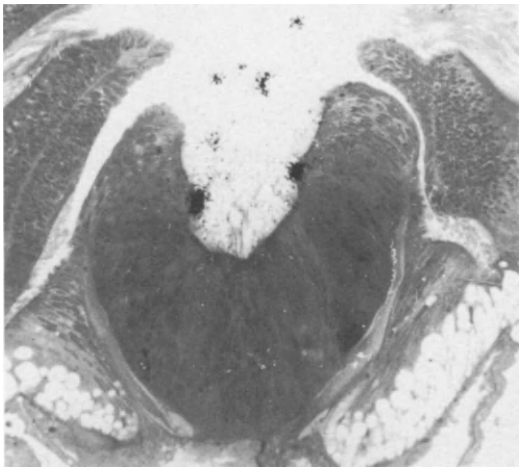

Figure 2–2. Light microscopic autoradiograph of $^{125}I^-$ of a transverse section of the endostyle of the adult amphioxus. (Reproduced from Ericson, L. E., Fredricksson, G., and Ofverholm, T.: Cell Tissue Res. 27:241–267, 1985, with permission.)

subpharyngeal gland transform into typical vertebrate thyroid follicles.

Studies with radioactive iodine have indicated the presence of T_4 and T_3 in the intermediate column of cells of the cyclostome larva's subpharyngeal gland.

Origin of Thyroid Hormones

Iodine atoms are large and bulky and stabilize the two phenyl groups of the iodothyronine, at right angles to one another. Interestingly, studies have indicated that iodine atoms are not necessary and nonhalogenated thyroid hormone analogues, such as dimethyl isopropylthyronine, have intrinsic thyromimetic activity. Why then did this very complicated system evolve to trap, store, and re-use iodine? Conceivably, iodotyrosine might have served a structural or enzymatic function in early chordates, and iodine itself may play some role. The suggestion has been made that with the filtering mode of feeding and widespread peripheral iodination in primitive chordates, iodothyronines would be available internally through intake and digestion. Natural selection then would have favored the development of the hormonal signals that regulate metabolism, utilizing these iodothyronines.

These hypotheses raise the question of the possible functional significance of the distinctive protein within which the coupling of tyrosines takes place. We have found 330,000 molecular weight material that was not reducible in the hagfish so that this large protein was present even in the most primitive vertebrate. With the huge size of the thyroglobulin molecule, one question to be answered is whether other active substances are present in the molecule in addition to thyroid hormone.

Significant sequence homology has been reported between the C-terminal portion of bovine thyroglobulin and acetylcholinesterase.[132, 157] The suggestion has been made that thyroglobulin evolved from condensation of a duplicated copy of the acetylcholinesterase gene with an archaic thyroglobulin gene, encoding the major hormonogenic domain.[157]

EMBRYOLOGY OF THE MAMMALIAN THYROID

Similar to all vertebrates, the mammalian thyroid forms from a mid-line outpouching of the entoderm in the floor of the primitive buccal cavity. At about the same time, lateral anlagen are derived from the ultimobranchial portion of the fourth pouches, which are destined to become the calcitonin-secreting parafollicular C cells. In the human embryo, the thyroid anlage is visible by 16 to 17 days' gestation and is in contact with the endothelium of the developing heart. At about 24 days, the wall of the distal portion of this vesicle increases in size and becomes multilayered through mitotic division to form a flask-like vesicle with a narrow neck that communicates with the buccal cavity. At this stage, the developing gland resembles the endostyle of the cyclostome. This hollow vesicle becomes bilobed. The stalk ruptures by 38 to 40 days, and the thyroid becomes a solid mass of laterally expanding tissue. The thyroid is pulled lower by the descent of the heart until by 45 to 50 days, the gland reaches its definitive location in the anterior lower neck. The ultimobranchial tissue that has grown down and forward becomes incorporated within the lateral lobes of the median anlage.

The caudal movement of the thyroid is accompanied by a rapid evolution of the stalk or thyroglossal duct. At some point, the thyroglossal duct fragments usually in the middle of the duct. Frequently, the fragmentation does not reach both ends. The persistent portion of the distal end of the duct differentiates into thyroid tissue, becoming the pyramidal lobe. Although the lobe cannot be identified in 25% of postnatal thyroids, prolonged TSH stimulation of the gland usually results in visualiza-

tion on radioactive scan. The pyramidal lobe is in the mid-line initially but may be displaced partially to the left. The anterior pituitary gland is also a derivative of the primitive buccal cavity—Rathke's pouch—and its embryologic development parallels that of the thyroid. Secretory granules can be identified in anterior pituitary cells at 70 to 84 days, and TSH can be identified by both assay and immunoassay.

Histology

Histologically, the human thyroid gland develops in three stages. From 47 to 72 days of gestation, there is a precolloid stage followed by a "beginning colloid" stage from 73 to 80 days. Before 72 days, there is no central colloid cavity or organic iodine present in the fetal thyroid. However, by 73 to 80 days of gestation, the fetal thyroid matures in terms of the ability to produce iodothyronine (Fig. 2–3). Central colloid cavities appear to form by the gradual enlargement and fusion of intercellular canaliculi, possibly activated and controlled by thyroglobulin. Further maturation beyond 80 days involves follicular growth.[136, 137]

Radioactive iodine has been given to women immediately prior to termination of pregnancy and uptake measured in the fetal thyroid.[63] The fetal thyroid is first able to concentrate radioactive iodine at the beginning colloid stage or 73 to 80 days of gestation. Incubation of fetal thyroid slices with ^{125}I corroborated that the iodine was organically bound at this stage.[136]

Iodide-concentrating ability and capacity to synthesize T_4 appear about the same time, which corresponds to the final stages of follicular lumen formation. Although TSH has been identified in cultured fetal pituitaries of 14 weeks' gestation, which coincides with the period during which the thyroid begins to function, growth and development of the fetal thyroid do not appear to be TSH dependent.[70] Furthermore, fetal serum TSH concentrations and serum-free thyronine concentrations are correlated.[72] These findings suggest that fetal serum TSH concentrations are responsive to serum-free thyroxine concentrations as early as 12 weeks of gestation. However, thyroglobulin synthesis and hormone storage occur in the absence of pituitary gland.[17] Optimal radioactive iodine concentration and efficient hormone synthesis seem to be TSH dependent.[82]

PROTHYROID HORMONE SYNTHESIS AND PROCESSING OF THYROGLOBULIN

Pioneering studies on insulin biosynthesis led to the concept that the polypeptide hormones

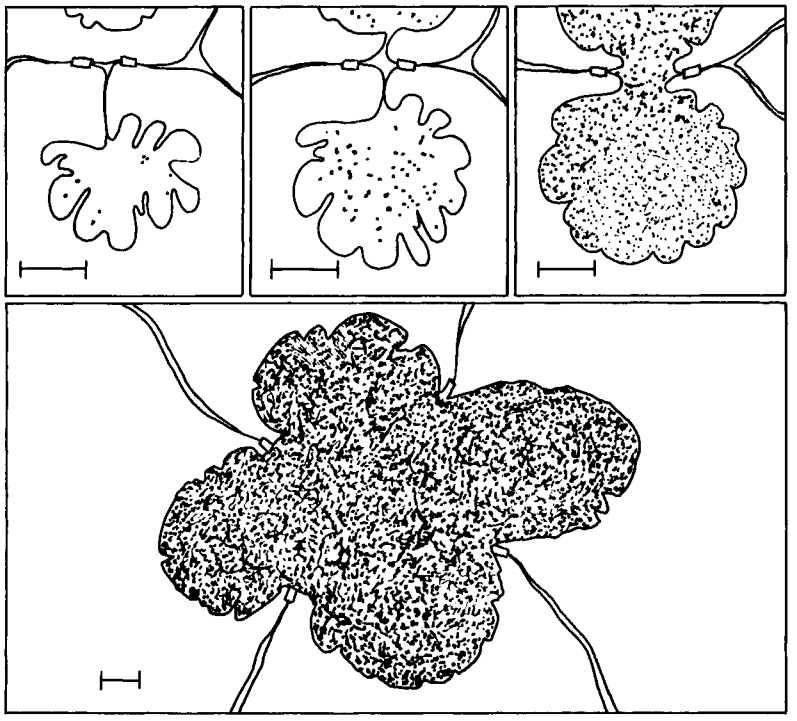

Figure 2–3. Diagrammatic representation of the development of central colloid spaces in the human fetal thyroid. Desmosomes, represented as collars, play an important role in final organization by limiting the spread of the disgorged contents of the canaliculi. In the bottom panel intracellular canaliculi are shown in the final phase of union, when the early colloid space resembles a cloverleaf in cross section. The marker represents 1 μ. (Redrawn after Shepard, T. H.: Onset of function in the human fetal thyroid: Biochemical and radioautographic studies from organ culture. J. Clin. Endocrinol. Metab. 27:945–958, 1967, with permission.)

were synthesized as larger molecules that underwent a series of processing steps before the active hormone was produced. Thyroid hormone undergoes extremely complicated processing before the active hormone is eventually released from the gland (Fig. 2–4).[51] Proteins destined for export from the cell are synthesized with a "signal" peptide that attaches to the rough endoplasmic reticulum and thyroglobulin is no exception. The signal peptide is cleaved from the free prothyroid hormone. Prothyroid hormone then undergoes a series of processing steps, including glycosylation, iodination, phosphorylation, degradation, and deiodination to form the active hormone, triiodothyronine.

Preprothyroid Hormone Synthesis

Thyroglobulin is synthesized with a signal peptide that allows the thyroglobulin to be inserted into the rough endoplasmic reticulum.[52] Cotranslationally, the signal peptide is cleaved and mannose and N-acetylglucosamine are added to initiate formation of glycoprotein. The large size of the thyroglobulin molecule precludes the separation of the signal peptide by molecular weight and requires indirect methods. Nevertheless, the data are consistent with the signal hypothesis.[22] Following the synthesis of the polypeptide chain, thyroglobulin undergoes a series of processing steps.

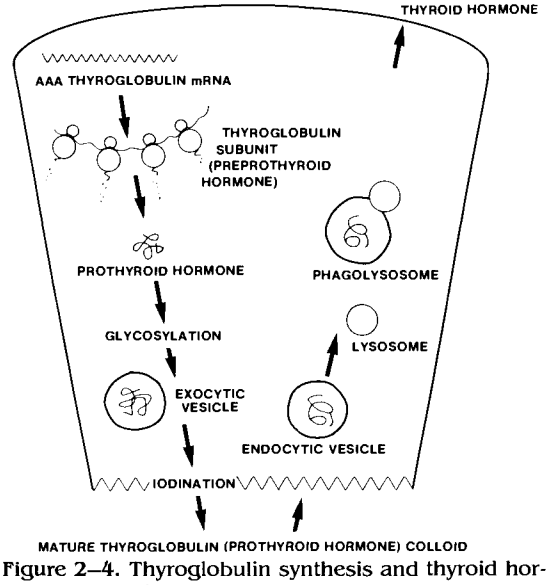

Figure 2–4. Thyroglobulin synthesis and thyroid hormone production.

Glycosylation

Thyroglobulin ultimately contains 8 to 10% carbohydrates[150] distributed in two types of glycopeptide units. Prothyroid hormone is transported through the smooth endoplasmic reticulum to the Golgi appartus where the terminal carbohydrates are attached.[106]

Two chains of oligosaccharides have been identified, which are linked to the polypeptide backbone by an asparagine chain (Fig. 2–5). Chain A (simple) has a molecular weight of 1800 and contains mannose and *N*-acetylglucosamine.[9, 10] Chain B (complex) has a molecular weight of about 3000 and is a branched structure that contains sialic acid, fucose, and galactose in addition to mannose and *N*-acetylglucosamine. The number of chains varies with the species, but in humans there are seven to eight A chains and 22 B chains. Two other carbohydrate chains have been identified in humans that contain galactosamine.[147] Assembly of the carbohydrate chains begins cotranslationally with the removal of the signal peptides in the rough endoplasmic reticulum[52] and is completed in the Golgi.

Carbohydrate is preformed as a dolichyl pyrophosphate–linked oligosaccharide containing *N*-acetylglucosamine, glucose, and mannose.[78, 151] This complex is transferred intact to newly formed peptide, containing an asparagine-×-serine (threonine) sequence in the turnover of the rough endoplasmic reticulum. Removal of glucose and a variable amount of mannose results in formation of the mature chain A, whereas excision of glucose and mannose residues is required both in the rough endoplasmic reticulum as well as in the Golgi before the peripheral oligosaccharide chains can be added. A number of transferases have been isolated that catalyze this reaction.[148, 149] Glucose plays an imperfectly understood role in increasing the affinity of the transferase for the peptide substrates.

Studies with an inhibitor of core glycosylation, tunicamycin, have indicated that glycosylation is necessary for the secretion of thyroglobulin into the colloid.[53] In addition, glycosylation appears to prevent intracellular breakdown of the prothyroid hormone. In thyroid cells incubated with tunicamycin, the intracellular thyroglobulin had a molecular weight of 64,000 compared with 330,000 in control cells. Finally, inhibition of glycosylation also resulted in decreased iodination of the thyroglobulin molecule perhaps due to

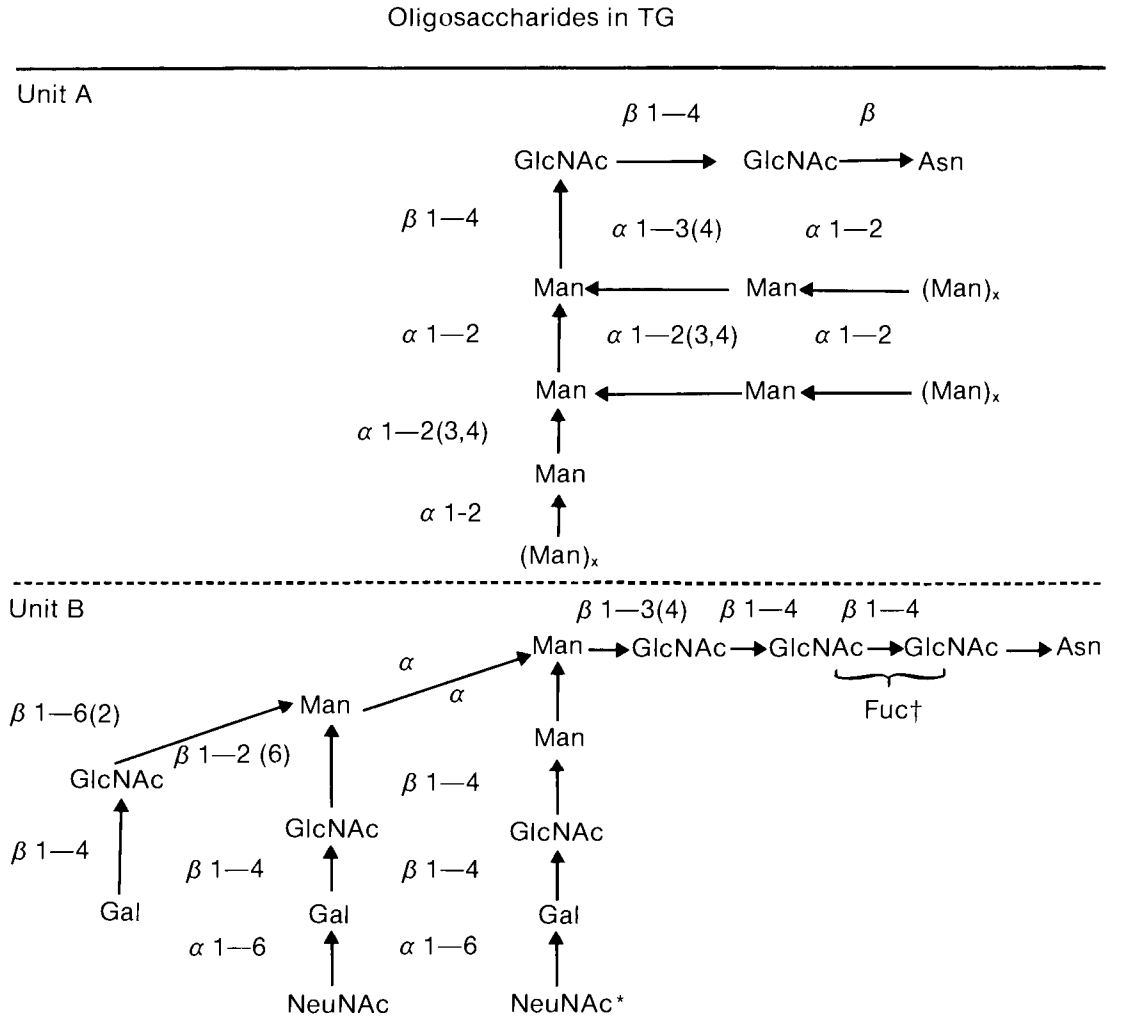

Figure 2–5. Structure of the oligosaccharide chains in thyroglobulin. (Asn = Asparagine; Gal = galactose; Man = mannose.) (Reproduced from Edelhoch, H. and Robbins, J.: Thyroglobulin: Chemistry and biosynthesis. *In* Werner, S. C. and Ingbar, S. H., eds.: The Thyroid, 4th edition. New York, Harper & Row, 1978, with permission.)

*This sialic acid residue might be N-glycollyl neuraminic acid (GlcNAc) rather than N-acetyl neuraminic acid (NeuNAc).

†Location of fucose is uncertain.

conformational changes. In an experimental rat thyroid tumor, which lacks membrane-bound sialyltransferase, defective thyroglobulin synthesis occurs.[105]

Exocytosis

The terminal sialic acid residues are added in the Golgi complex, and thyroglobulin is processed in exocytotic vesicles for transport to the apical membrane (Fig. 2–6).[6] The membranes of these exocytotic vesicles fuse with the thyroid apical plasma membrane, and the thyroglobulin is discharged into the colloid. There may also be a signal sequence in the thyroglobulin peptide, which directs it toward the apical membrane. TSH induces a rapid depletion of exocytotic vesicles and translocation of labelled thyroglobulin from the apical region of the cell to the periphery of the follicle lumen. The rate of thyroglobulin exocytosis appears to be dependent on the concentration of TSH.

Suppression or elimination of TSH by pretreatment with thyroid hormones or by hypophysectomy results in a significant decrease in thyroid hormone secretion. This inhibition of thyroid hormone secretion is due to a marked

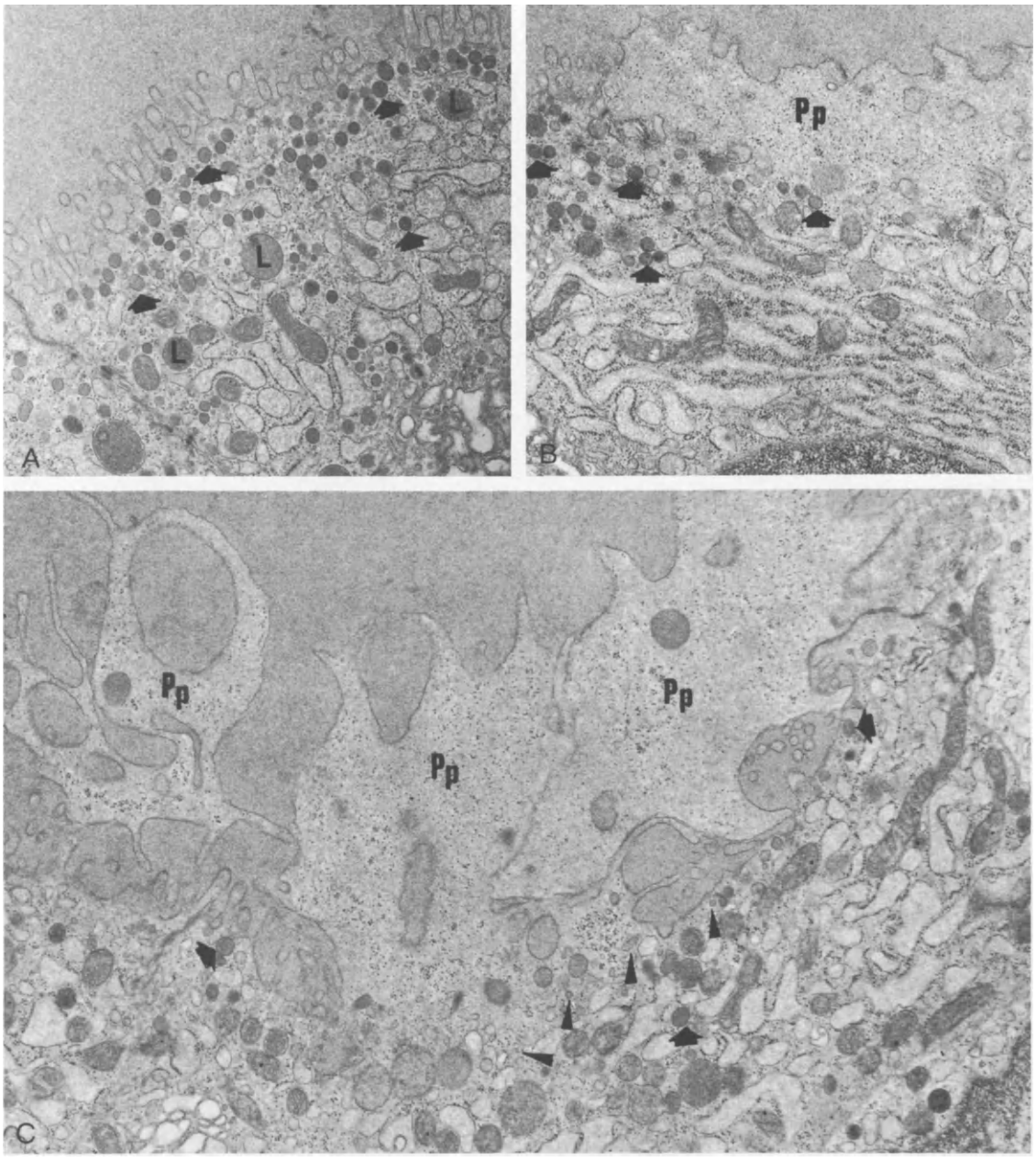

Figure 2–6. Exocytosis and endocytosis shown by electron micrographs of apical portions of rat thyroid follicle cells (×14,500). *A*, Control rat, pretreated with T_4 for 2 days. The apical cytoplasm contains numerous, dense exocytic vesicles, some of which are indicated by arrows. Lysosomes (L) are also present. Pseudopods and colloid droplets are absent. *B*, T_4-treated rat injected with 5 mU thyroid-stimulating hormone (TSH) 20 minutes before fixation. A bulky pseudopod (Pp) protrudes into the follicle lumen. The apical cytoplasm contains exocytotic vesicles, some of which are indicated by arrows. *C*, T_4-treated rat injected with 50 mU TSH 20 minutes before fixation. Three large pseudopods (Pp) belonging to different follicle cells protrude into the follicle lumen. The pseudopods are larger than those induced by the smaller dose of TSH and contain invaginations or colloid droplets, especially the pseudopod to the left. Some exocytotic vesicles remain in the cytoplasm (arrows). Micropinocytotic vesicles are present, some of them indicated by arrowheads. (Reproduced from Engstrom, G. and Ericson, L. E.: Effect of graded doses of thyrotropin on exocytosis and an early phase of endocytosis in the rat thyroid. Endocrinology 108:399–405, 1981, with permission.)

decrease in endocytosis. Thyroglobulin continues to be delivered to the follicle lumen, and this continuing transport is manifested by an increase in thyroglobulin content of the thyroid gland.

The exocytotic vesicles that contain both thyroglobulin and peroxidase accumulate between the Golgi apparatus and the apical plasma membrane (see Fig. 2–6). Microtubulees appear to play a role in this distribution, and disruption of microtubules leads to a scattering of the exocytotic vesicles about the cell.[61] Concomitant with this dislocation is an impaired exocytosis of newly synthesized protein.

The actual mechanism by which exocytotic vesicles are brought into contact with the apical plasma membrane is unknown but charge is thought to be important. Carboxymethylation has been suggested to play a role in cell movement and fusion of membranes (Fig. 2–7), and thyroid cells are rich in carboxymethylase activity.[55] The enzyme protein carboxymethylase donates methyl groups, changing the net charge. Both the secretory granule membrane proteins and the secretory granule proteins themselves have been found to serve as methyl-acceptor proteins. Interest has also focused on actin-containing filaments. Cytochalasin B interacts with these filaments and affects secretion by exocytosis.

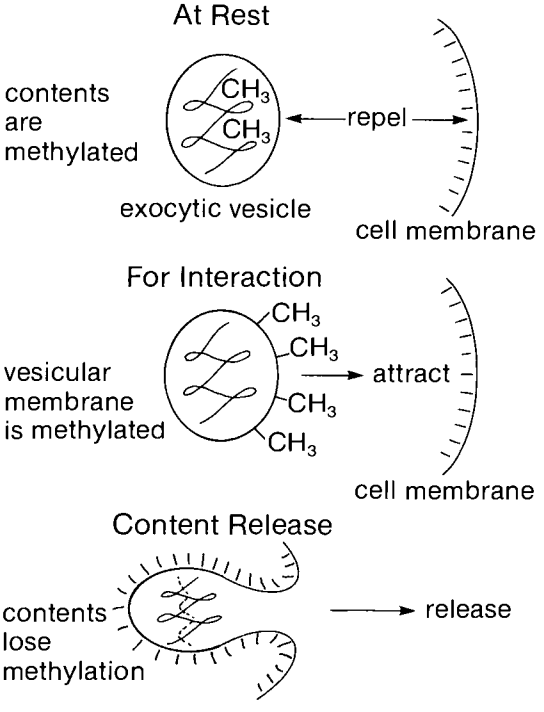

Figure 2–7. Carboxymethylation.

Endocytosis

The ability of the thyroid follicle cell to respond to TSH stimulation with endocytosis appears to be dependent on the pool of membrane available in exocytotic vesicles. TSH stimulation alters membrane distribution in the apical portion of the follicle cell. Total membrane area is not changed. Rather, a redistribution of existing membranes occurs with transfer to the apical plasma membrane during exocytosis and removal during endocytosis.[60]

The rapidity of the endocytotic response to TSH makes *de novo* synthesis an unlikely source for the large membrane requirements of endocytosis in the thyroid cell. The first phase of TSH stimulation is characterized by a transfer of membrane from exocytotic vesicles to the apical plasma membrane.[60] Next, there is a transfer of membrane from the enlarged apical plasma membranes into pseudopods. Finally, there is a shift of membrane material from the pseudopods into colloid droplets. The data suggest that endocytosis is dependent on exocytosis. Acute TSH stimulation induces early phagocytosis of colloid by newly formed apical pseudopods.[135] However, chronic TSH stimulation rarely results in apical pseudopods and intracellular colloid droplets,[83] at least in dog thyroids.

Endocytosis involves the invagination of the apical cell membrane followed by fusion of the neck of invagination and detachment of the fused vesicle. The fused vesicle is transferred to the lysosome.[76, 77] If thyroglobulin is taken up as a function of concentration in the follicle, the process is called fluid-phase pinocytosis. However, if thyroglobulin binds to a receptor in a process, it is called receptor-mediated endocytosis (Fig. 2–8).[71] The concentration of thyroglobulin taken up by this process is much larger than in fluid-phase pinocytosis, since binding to the membrane is a concentration phenomenon. If the binding takes place in coated pits, the vesicle will be coated with clathrin. There is some suggestion that binding of thyroglobulin occurs in coated pits.[65]

Whether endocytosis plays a role in the "first come, first served" phenomenon is unclear. The observation has been made that newly synthesized thyroglobulin is preferentially utilized for thyroid hormone production. Since endocytosis is thought to be the rate-limiting

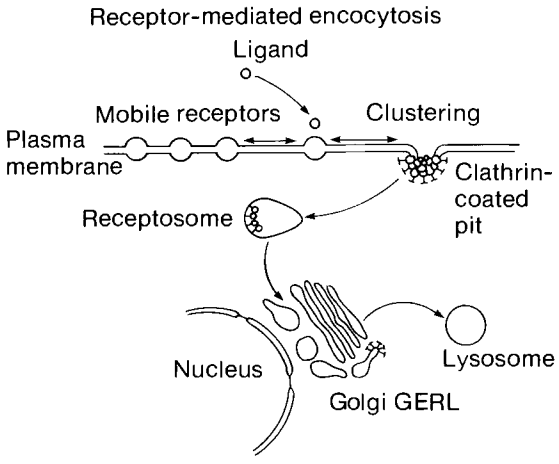

Figure 2–8. Receptor-mediated endocytosis. (Redrawn from Pastan, I. H. and Willingham, M. C.: Journey to the center of the cell: Role of the receptosome. Science 214:504–509, 1981, with permission from the American Association for the Advancement of Science.)

step in thyroglobulin degradation rather than proteolysis, there might be some selectivity for newly synthesized thyroglobulin. Poorly iodinated and therefore presumably newly synthesized thyroglobulin preferentially binds to apical membranes.[36] However, iodine-poor thyroglobulin does not turn over faster than iodine-rich thyroglobulin as might be expected.[38] Whether the newly synthesized, poorly iodinated thyroglobulin binds to the apical membrane where it is more heavily iodinated and temporarily protected from endocytosis remains to be determined. A thyroglobulin receptor has been identified on thyroid cell membranes that requires desialylation prior to receptor recognition.[36] This receptor could function both in the biosynthesis and the biodegradation of the thyroglobulin molecule. Thyroglobulin with a low iodine content appears to have a higher affinity for thyroid membranes than thyroglobulin with a high iodine content.[166] Removal of the penultimate galactose residue to expose *N*-acetylglucosamine residues also increased thyroglobulin binding to thyroid membranes.

Other possibilities exist for membrane recognition. Phosphorylation of carbohydrate residues is an important step in the recognition and pinocytosis of lysosomal enzymes.[109] Rapidity of the uptake of glucuronidase is directly related to its mannose-6-phosphate content, and hydrolysis of the carbohydrate groups results in loss of susceptibility to absorptive endocytosis. Thyroglobulin is also phosphorylated, and this appears to be as mannose phosphate.[55]

Lysosomes

After endocytosis, the endocytotic vesicles fuse with lysosomes to form phagolysosomes where the iodinated thyroglobulin is hydrolyzed and thyroxine and triiodothyronine are ultimately secreted from the cell (Fig. 2–9). Movement of endocytotic vessels toward the center of the thyroid cell and corresponding movement of lysosomes toward the apical region probably depend on a combination of saltatory motion and microtubular guidance.[114] The microtubules radiate out from the perinuclear region toward the cell surface. The saltatory motion of the colloid droplets would be guided in short straight segments toward the cell's center, presumably owing to their association with microtubules. Drugs, such as colchicine which depolymerize cytoplasmic microtubules, inhibit TSH-induced thyroid hormone secretion.[110, 179]

Soon after colloid droplets are formed, signs of fusion with lysosomes may be seen on electron micrographs.[182] There are often several lysosomes clustered around the endocytotic vesicle.[57] Biochemical studies are difficult because of the problem in thyroid cell fractionation without thyroglobulin contamination. However, if thyroid tissue either prelabelled with ^{125}I-iodides or incubated with ^{125}I-thyroglobulin is fractionated on sucrose gradients, the distribution pattern of radioactive iodine is very similar to that of the acid hydrolases indicating that the iodoproteins are sequestered inside particles with the properties of lysosomes.[116, 163]

The turnover of thyroglobulin within the lysosomal fraction is rapid. The proteolytic intermediates obtained from this fraction are similar to those obtained after *in vitro* hydrolysis of thyroglobulin by lysosomal enzymes in the presence of glutathione, suggesting that disulfide bonds play a role in the susceptibility

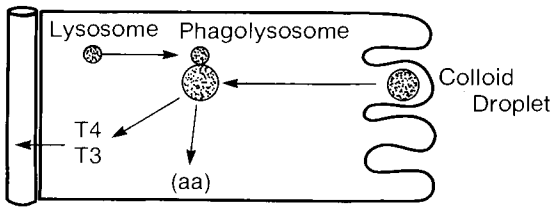

Figure 2–9. Thyroglobulin degradation.

of the thyroglobulin molecule to hydrolysis.[116] Hydrolysis is maximal at pH 4.0.[165]

Glycoside hydrolases, acid proteases, peptidyl amino acid hydrolases, and dipeptide hydrolases are involved in the hydrolysis of the thyroglobulin molecule and all have maximal activity at acid pH.[100] Thyroid tissue contains β-galactosidase, α-amannosidase, β-*N*-acetylglucosaminidase, and β-glucuronidase among the glycoside hydrolases that release carbohydrates from desialated thyroglobulin.[28] Thyroid protease is similar in specificity to pepsin but more restricted. Free thyroxine and free triiodothyronine are preferentially liberated, whereas MIT and DIT seem to remain within oligopeptides. Thyroglobulin hydrolysis is preferential for newly iodinated molecules. Thyroid hormone–rich sequences occur in the thyroglobulin molecule, and it is conceivable that these amino acid sequences are more prone to hydrolysis.[45, 122] With the progression of hydrolysis, the phagolysosome shrinks and migrates toward the basal membrane. Whether the membranes are recycled through the Golgi apparatus is not clear.[77]

Secretion of Thyroid Hormone

Following hydrolysis, thyroxine and triiodothyronine migrate toward the basal surface of the cell where they are secreted into the circulation. Whether this process is simple diffusion due to a concentration gradient or is carrier-mediated is not clear. The most likely explanation is that thyroxine and triiodothyronine, which are polar residues, cross the lysosomal membrane. There may be active transport occurring in lysosomes. Although cytosolic-binding proteins could be implicated in the intracellular transport of these hormones, there is no evidence to support this contention. Most of the iodotyrosine residues are rapidly deiodinated with utilization of the iodide released. Not all the iodide is immediately recycled, and about 50 μgm/day is secreted into the circulation (iodide leak).

The major iodinated compounds found in thyroid venous blood are thyroxine, triiodothyronine, iodotyrosines, thyroglobulin, and iodide. The thyroid produces 80 to 100 μgm of thyroxine daily of which 80% is monodeiodinated to triiodothyronine and reverse triiodothyronine (RT_3) in roughly equal proportions. The thyroidal contribution of these hormones is limited, less than 20% of total hormone production (see Chapter 4). Despite the small amounts, the thyroid secretes triiodothyronine and RT_3 triiodothyronine preferentially to thyroxine.[88] This increased release of T_3 and RT_3 is probably due to both intrathyroidal monodeiodination[88] and selective or facilitated release during lysosomal hydrolysis.[165]

There is a tendency to consider the thyroid gland as a homogeneous collection of follicle cells all synthesizing thyroid hormone at the same rate. However, thyroid cells appear to be polyclonal and neither iodinating capacity nor peroxidase activity is uniformly distributed among follicular cells.[155] Moreover, some follicular cells respond more readily to growth stimuli than others, and the same is presumably true of thyroid hormone secretion. Thyroid hormone secretion may be inhibited by iodides directly.[164] Iodides appear to depress the stimulation of colloid droplet formation by TSH by inhibition at a step after cyclic adenosine monophosphate (cAMP) formation. Lithium, a cation, has a similar inhibitory effect on thyroid hormone release and has been used clinically to inhibit thyroid function.[25, 145]

IODIDE TRANSPORT AND IODOAMINO ACID BIOSYNTHESIS

The complexity of thyroid hormone biosynthesis as well as the complexity of the thyroglubulin molecule itself may result from the use of iodine to stabilize the thyronine molecule, and may derive from an evolutionary adaptation to the variation in iodine supply. Why iodine should play this role when short-chain hydrocarbons are acceptable substitutes and universally available is not clear.[35]

Iodide Transport

Iodide is actively transported into the thyroid gland against both a chemical and an electrical gradient. The accumulation of iodine in the thyroid gland is the resultant of both iodide influx and efflux. Iodide transport is dependent on intact cellular membranes but not follicular organization, since isolated cells can concentrate iodides.[177] The transport is saturated by excess iodide and competitively inhibited by related anions. This iodide concentrating mechanism is localized to the basal membrane.[8] The suggestion has been made that sodium influx may be a determining factor in thyroid iodide transport.[32] TSH would stimu-

late iodide transport by enhancing sodium influx with a resulting decrease in the potential difference across the thyroid follicular basal cell membrane and an increase in the passively distributed component of thyroid iodide.

Clinically, iodide transport can be measured in the thyroid with radioactive iodine tracer. The absolute iodine uptake in the thyroid can be measured by multiplying plasma inorganic iodine concentration by the thyroid iodide clearance that is calculated from the thyroid radioactive iodine (RAI) uptake, i.e., 150 min RAI uptake -60 min RAI uptake/(plasma RAI concentration $\times$ min between the two uptakes). This thyroid clearance represents the quantity of plasma "cleared" of iodine in 90 minutes and when multiplied by the plasma inorganic iodide concentration results in the total amount of iodide taken up by the thyroid.

Transport of iodide in the thyroid is dependent upon cellular ATP,[162] and $Na^+ - K^+$ ATPase is probably involved in the transport. In thyroid slices, ouabain inhibits $Na^+ - K^+$ ATPase activity and accumulation in parallel fashion.[180] However, the specific role of $Na^+ - K^+$–dependent ATPase activity in iodide transport is not clear. TSH does not stimulate enzyme activity, and the thyroid gland from a patient with an iodide trapping defect contained normal amounts of $N^+ - K^+$ ATPase.[152, 178]

The actual transport of iodine across the thyroid plasma membrane has been thought to involve a carrier.[177] Phospholipid fractions can complex iodide and make it soluble, and thyroid phospholipid binding is similar to an enzyme reaction in regard to saturation kinetics, structural specificity, and competition by another anion. The nature of the iodine-lipid linkage is not proved, but its easy reversibility suggests that covalent bonds are not formed. TSH stimulates ^{32}P incorporation into thyroid phospholipids, but the relationship between thyroid phospholipid turnover and iodide transport is unknown.[178] Proteins, particularly albumin, have also been suggested as carriers for iodide in the thyroid.[177]

In addition to iodide, other halides and complex anions are concentrated by the thyroid.[181] Thiocyanate competitively inhibits iodide transport and is not transported. The order of increasing K_i for thyroid iodide transport is as follows: $TcO_4^- < ClO_4^- < SCn^- < I^- < NO_2$. The goitrogenic potency of these competitive amines shows a similar order.[3] Thiocyanate inhibits thyroid iodide accumulation both *in vivo* and *in vitro*, and the perchlorate discharges previously accumulated iodide from the thyroid. The most potent known inhibitor of iodide transport is perchlorate, which appears to inhibit both iodide uptake by the thyroid and reutilization within the gland.[133] Perchlorate also inhibits iodotyrosine formation by a mechanism not connected with iodide transport.[73] Technetium is not a naturally occurring element and exists in a radioactive state available as 99m pertechnetate ($^{99m}TcO^-_4$). A short half-life of 6 hours and no beta energy make it a good agent for clinical scanning of the thyroid. However, technetium does not seem to be bound in the thyroid to any significant degree. Thyroid technetium uptake is increased by TSH and decreased by thyroid hormone administration.[140]

TSH plays the major role in the control of iodide accumulation in the thyroid. The hormone initially causes an increase in the efflux of iodide followed by a late increase in unidirectional clearance with greater accumulation (Fig. 2–10).[177] Presumably, the TSH effects are modulated through cAMP. Dibutyryl-AMP has been shown to reproduce the biphasic effect of TSH on iodide accumulation in isolated thyroid cells.[174] The 7S immunoglobulin

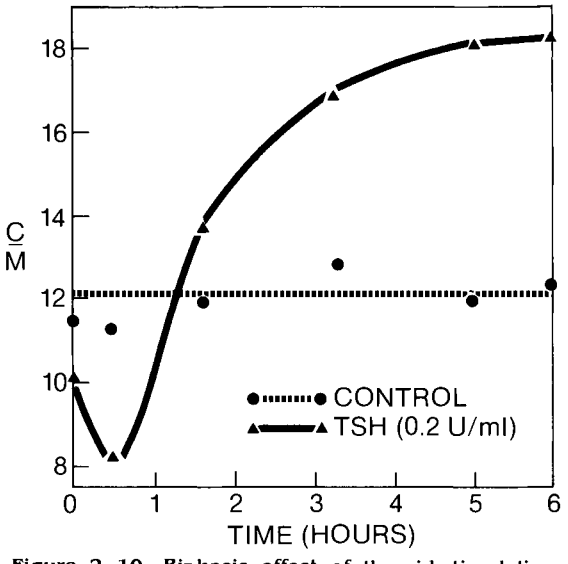

Figure 2–10. Biphasic effect of thyroid-stimulating hormone (TSH) on iodide transport by dispersed bovine thyroid cells. Cells were preincubated for an hour with micromolar ^{131}I-iodide to achieve steady-state ^{131}I-iodide distribution. At time zero TSH was added and the changes in cell-to-medium (C/M) concentration of ^{131}I were observed. (Redrawn from Tong, W.: The use of isolated thyroid cells in studies of thyrotropin action. *In* Tritsch, G., ed.: Axenic Mammalian Cell Reaction. New York, Marcel Dekker, 1969, with permission.)

associated with Graves' disease also stimulates iodide accumulation in thyroid cells.[48] Lithium, a cation, has been reported to inhibit the uptake of iodide but seems to have a greater effect on the release of thyroid hormone.[25, 145]

Wolff-Chaikoff Effect

Pharmacologic doses of iodine transiently inhibit iodotyrosine formation—the Wolff-Chaikoff effect—by affecting iodide transport.[108, 178] This inhibitory effect of iodide is prevented if antithyroid drugs are administered before or during the supplemental iodide administration. The implications of this observation are that the inhibition is not simply due to an increase in extracellular iodide concentration but requires iodotyrosine formation. An inverse relationship exists between the organic iodine content of the thyroid and the activity of the iodide transport mechanism both in the basal state and in response to TSH. As a consequence of this autoregulation, plasma iodide concentrations influence thyroid hormone synthesis by altering iodide transport in the thyroid.[80]

The precise mechanism of the Wolff-Chaikoff effect is unclear. However, in normal individuals, "escape" from this effect occurs despite continued iodide administration. This escape depends on a progressive inhibition of thyroid iodide transport. As a result, the intrathyroidal iodide concentration falls below the level required to sustain the inhibition of iodotyrosine formation.[23] A specific iodinated organic derivative in which concentration and action varies with the total organic iodine content of the thyroid has been postulated. Since iodides have been shown to inhibit cAMP production and the inhibition can be overcome by administration alterations of the antithyroid drugs, inhibition of adenylate cyclase response has been suggested.[121, 170]

Iodination

The unique process of exporting thyroglobulin from the cell for storage in the colloid is directed toward a constant supply of thyroxine and triiodothyronine despite a highly variable iodine intake. Iodination of the thyroglobulin takes place at or near the apical membrane and requires four components—thyroglobulin, iodine, peroxidase, and a source of hydrogen peroxide (Fig. 2–11).[56] Iodide is concentrated by the basal plasma membrane and diffuses freely through thyroid cell cytoplasm. Peroxidase activity has been found predominantly in the rough endoplastic reticulum,[2] with the presumption that the enzyme is transferred to the apical cell surface via Golgi and exocytotic vesicles. Hydrogen peroxide can be generated by several enzyme systems present in the thyroid but which process acts as the substrate for the peroxidase in the iodination of tyrosine moieties in thyroglobulin remains to be determined. These H_2O_2 generating systems are located intracellularly as well as in the apical plasma membrane.[21]

Although some cellular fractionation techniques have suggested that iodination can take place intracellularly, this may be due to contamination with thyroglobulin. Results of electron microscopic studies indicate that the major site of thyroglobulin localization is the apical cell surface (Fig. 2–12).[58, 153] The combination of electron microscopy with radioautography has allowed ^{125}I-labelled thyroglobulin to be located in the cell with a high degree of precision. Administration of TSH, 5 minutes before injection of ^{125}I, and fixation, 30 seconds later, resulted in the distribution of the radioactivity along the membrane of the thyroid follicular cell pseudopods. This distribution correlated well with the distribution of peroxidase and suggested that iodination was catalyzed by peroxidase in the apical plasma membrane.

The acute stimulation of iodination by TSH[1] may be secondary to the stimulation of exocytosis. A temporal relationship exists between exocytosis and iodination.[59] Despite this relationship, iodination of thyroglobulin does not occur exclusively in association with exocytosis. There is good evidence that thyroglobulin that has been stored in the follicular lumen also undergoes iodination.[81, 104] Apparently, thyroglobulin molecules may be iodinated repeatedly at the apical cell surface after export to the colloid.

Iodoaminoacid Biosynthesis

Virtually all the iodide present in the thyroid gland is in the form of the prohormone thyroglobulin. The iodine present in thyroglobulin is in the form of tyrosine derivatives (Fig. 2–13). Only thyroxine and triiodothyronine exhibit biologic activity. About 10 to 15% of the total iodide in the gland is present in insoluble iodoproteins.

The iodine content of 19S thyroglobulin may

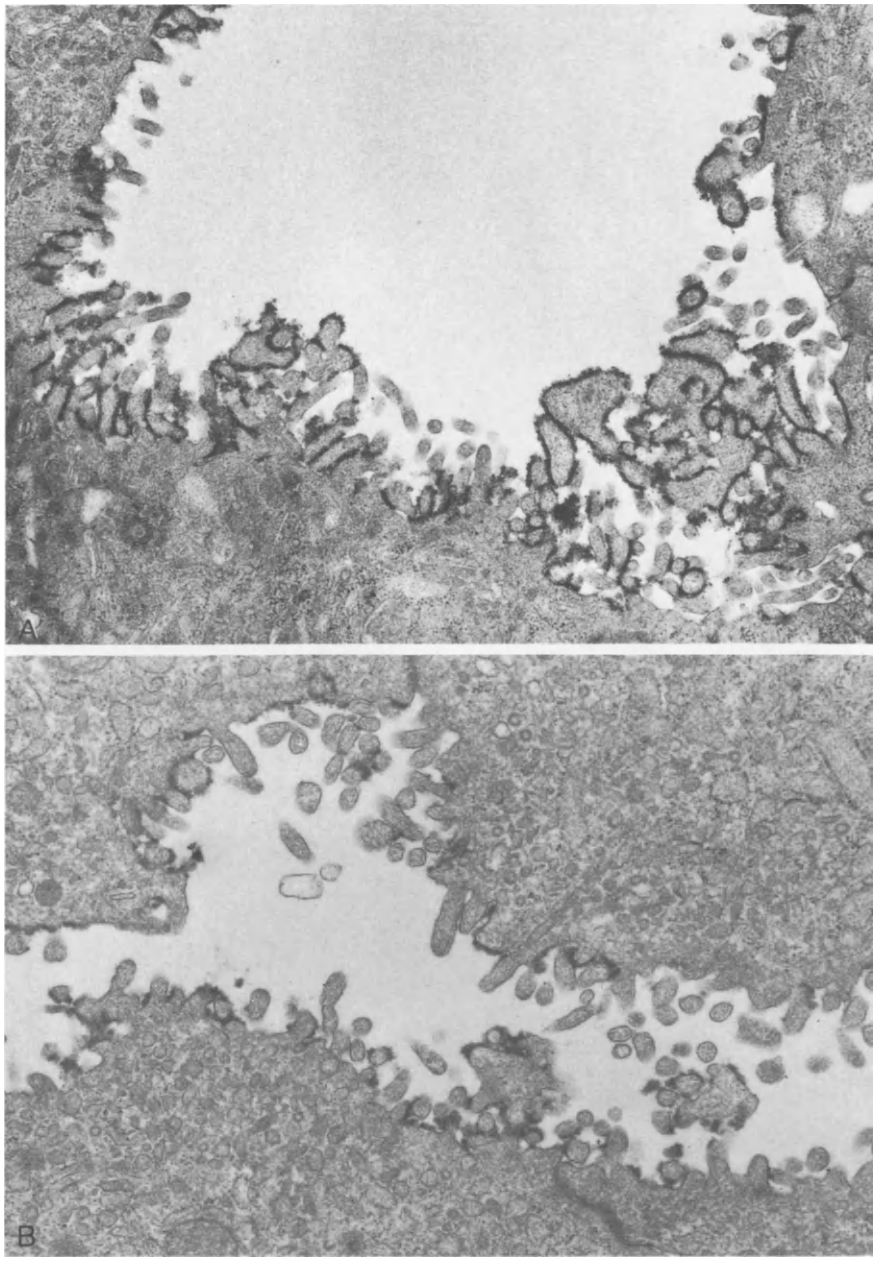

Figure 2–11. Peroxidase activity (in black) is shown along the apical membrane of the thyroid follicular cell. (Reproduced from Björkman, U., Ekholm, R., and Denef, J. F.: Cytochemical localization of hydrogen peroxide in isolated thyroid follicles. J. Ultrastruct. Res. 74:110, 1981, with permission.)

vary from lower than one iodine atom/mole to 15 iodine atoms/mole (1.1%).[41] The thyroglobulin molecule contains 140 tyrosine residues so that theoretically 280 iodine atoms can be incorporated. Only a small percentage of these tyrosine residues are in fact iodinated *in vivo*. However, the iodination is heterogeneous, and thyroglobulin can be fractionated into molecules containing 26 to 70 iodine atoms. This heterogeneity may be related to variations in iodide or thyroglobulin present at the iodination site. If the formation of MIT and DIT is followed, the data are consistent with the hypothesis that the reactivity of the different tyrosine residues and the distribution of iodine are determined by the physical and chemical

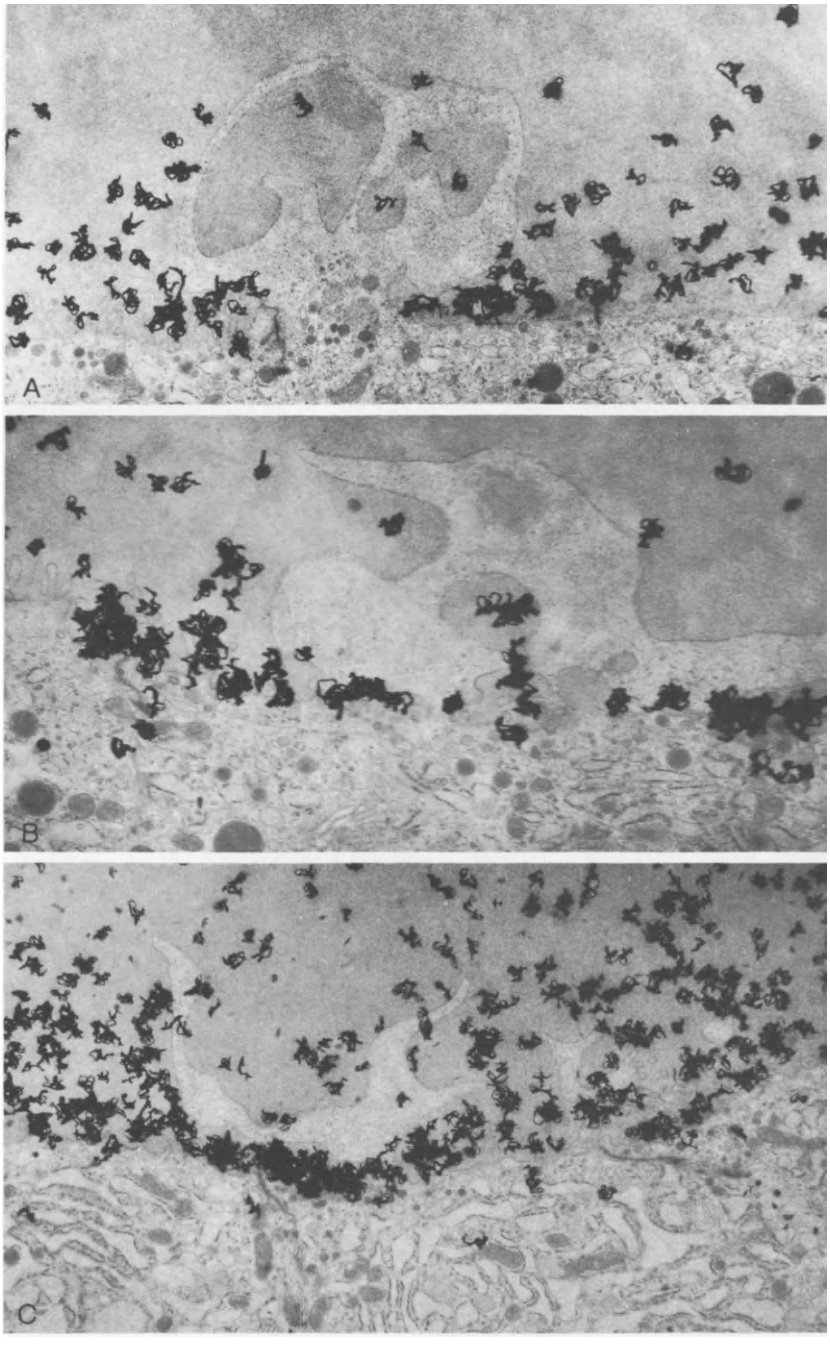

Figure 2–12. Location of iodination within the thyroid cell. *A*, Autoradiographic grains are present along the apical plasma membrane right to the narrow base of the pseudopod and over rounded profiles in the body of the pseudopod. There are few grains along the distal surface of the pseudopod (×12,000). *B*, Silver grains are present along the apical plasma membrane below the broad, irregularly shaped distal part of the pseudopod. Grains are also found over a vesicular structure containing microvilli in the base of the pseudopod and over a rounded profile in the body of the pseudopod. There are practically no grains associated with the distal surface of the pseudopod (×13,000). *C*, Silver grains are concentrated between the apical cell membrane and a pseudopod which in this section has no connection with the distal surface of the pseudopod (×10,000). (Reproduced from Ekholm, R. and Wollman, S.: Site of iodination in the rat thyroid gland deduced from electron microscopic autoradiographs. Endocrinology 97:1432, 1975, with permission.

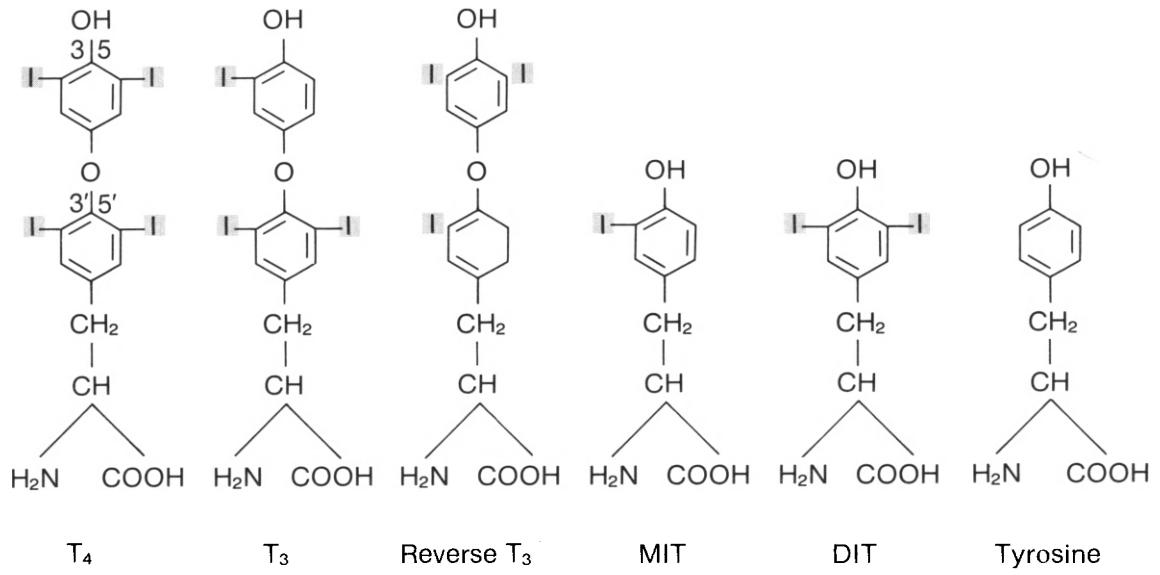

Figure 2–13. Iodotyrosines and iodothyronine. (Redrawn from Nunez, J.: Iodination and thyroid hormone synthesis. *In* de Visscher, M., ed.: The Thyroid Gland. New York, Raven Press, 1980, with permission.)

structure of thyroglobulin.[42] Thyroglobulin contains 3 moles of T_4 or less/mole and 0.3 moles of T_3.[126]

Thyroid Peroxidase

Thyroid peroxidase is a membrane-bound enzyme, which is a hemoprotein,[160] active only in the presence of H_2O_2.[93a] The source of the H_2O_2-generating system within the thyroid is unclear. The iodination of tyrosine catalyzed by peroxidase probably involves free radical mechanisms.[172] The assumption has been made that thyroid peroxidase contains two sites for substrate, iodide and tyrosine (Fig. 2–14).

In addition to catalyzing tyrosine iodination, thyroid peroxidase is also able to catalyze intramolecular coupling of two iodotyrosine residues on thyroglobulin. For this to happen, the tyrosine residues must be aligned correctly in both the primary and tertiary protein structures in order to allow interaction with the enzyme and coupling. The enzymatic coupling mechanism may be the same as the iodination of tyrosine but with DIT bound to each of the substrate sites (Fig. 2–15).

When thyroglobulin is unavailable for iodination because of defective synthesis, other proteins are iodinated instead, notably albumin. Although the suggestion has been made that an albumin-like protein is synthesized and iodinated in the thyroid gland, the balance of evidence indicates that a variety of proteins synthesized elsewhere are iodinated when thyroglobulin is not available.[92, 113]

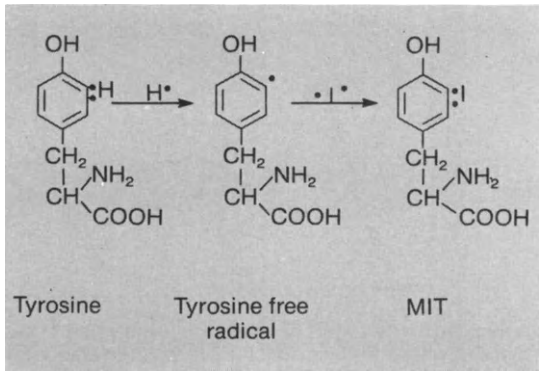

Figure 2–14. Iodotyrosine formation. (Redrawn from Nunez, J.: Iodination and thyroid hormone synthesis. *In* de Visscher, M., ed.: The Thyroid Gland. New York, Raven Press, 1980, with permission.)

Action of Antithyroid Drugs

Iodination catalyzed by thyroid peroxidase can be inhibited by the thioamides, propylthiour-

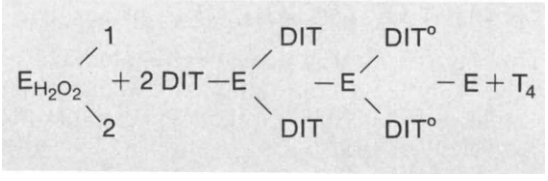

Figure 2–15. The "coupling reaction"—iodothyronine formation. (Redrawn from Nunez, J.: Iodination and thyroid hormone synthesis. *In* de Visscher, M., ed.: The Thyroid Gland. New York, Raven Press, 1980, with permission.)

acil and methimazole. This inhibition could be reversible or could not, depending on the relative concentrations of thioamide and iodide.[138] Iodide was thought to inhibit antithyroid drug action by increasing drug oxidation, reducing peroxidase inactivation, or a combination of both.[47] Further studies indicate that whether the inhibition of iodination is reversible or irreversible depends on the relative rates of peroxidase inactivation and drug oxidation.[39, 138] The rates appear to depend on the iodide to drug concentration ratio. A high ratio favors extensive drug oxidation and reversible inhibition, whereas a low ratio favors thyroid peroxidase inactivation and irreversible inhibition.

Congenital Defects in Iodination

Defects in iodination are expressed clinically as "organification defects" detected by a positive perchlorate discharge test result. Patients with defects in iodination have partial or complete discharge of trapped radioiodine from the thyroid after administration of perchlorate. Total discharge of the trapped iodine is rare and associated with mental retardation. These patients' peroxidase activity is absent.[111, 120] More commonly, patients have goiter and only partial discharge of radioactivity with perchlorate. In some patients, this partial discharge is associated with a defect in the binding of the hematinic prosthetic group to the peroxidase apoenzyme.[111] The most common clinical picture is associated with goiter and nerve deafness. (Pendred's syndrome).[93] There does not appear to be a defect in the apoenzyme or prosthetic group in these patients. The defect could represent a defect in H_2O_2 generation.[27] Although thyroglobulin has been reported to be abnormal in these patients, this finding may be due to decreased iodination, and the thyroglobulin appears to be normal.[54]

STRUCTURE AND MOLECULAR BIOLOGY OF THYROGLOBULIN

The thyroid prohormone thyroglobulin is a large soluble glycoprotein with a molecular weight of 660,000 daltons and a sedimentation coefficient of 19S.[46]

Structure

When care is taken to isolate intact membrane polysomes, large thyroid polysomes can be observed (Fig. 2–16).[41, 134] Nascent peptides from these large polysomes can be precipitated by thyroglobulin antibodies. These large polysomes yield a messenger RNA (mRNA) that is about ten times larger than the average eukaryotic mRNA. The mRNA translated 300,000-dalton peptides, immunologically and chemically related to thyroglobulin. If total mRNA larger than 30S RNA is translated, it is possible to find 660,000-dalton material that cannot be reduced.[50] However, the smaller thyroglobulin mRNA and thyroglobulin subunits reported in the past almost certainly represent nucleolytic and proteolytic cleavage products of the thyroglobulin 33S mRNA and thyroglobulin 300,000 proteins, respectively.

A 12S subunit and a 27S dimer are usually observed on sucrose gradients.[129] The 12S subunits (330,000 daltons) are held together by disulfide bridges as well as by noncovalent links. The number of disulfide bridges increases with the degree of iodination of thyroglobulin with a resulting greater stability to the molecule.[7] Although theoretically, it should be possible to determine the thyroglobulin protomere after reduction, alkylation, and treatment with denaturing agents, wide discrepancies exist in the various reports, ranging from 2 to 8 subunits of thyroglobulin.[20] The most likely explanation for the smaller subunits is proteolysis of the 330,000 subunit. When thyroglobulin was purified in the presence of

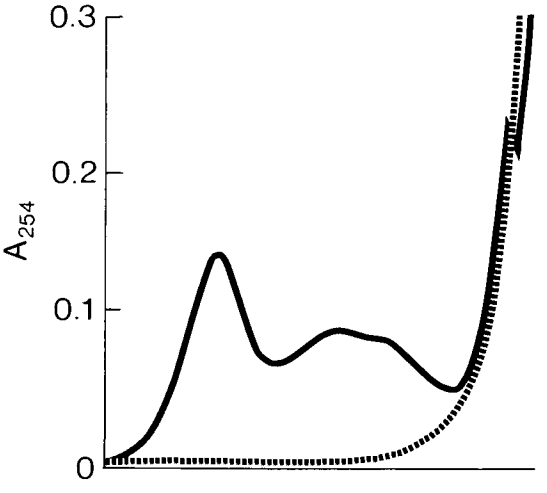

Figure 2–16. Sucrose gradient of membrane-bound thyroid polysomes, demonstrating peak of thyroglobulin-synthesizing polyribosomes. (Redrawn from Davies, E., Dumont, J. E., and Vassart, G.: Improved techniques for isolation of intact thyroglobulin-synthesizing polyribosomes. Biochem. J. 172:228, 1978, with permission of The Biochemical Society, London.)

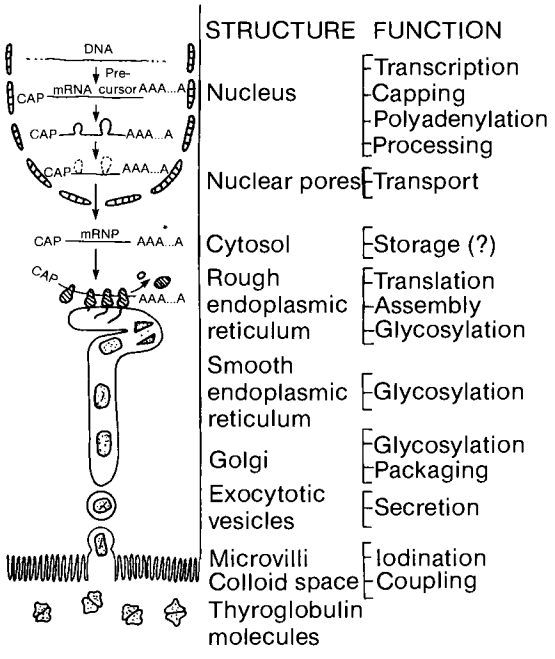

Figure 2–17. Biosynthesis of thyroglobulin. The flow of information is shown from the genomic DNA to the mature iodinated thyroglobulin molecule. The nuclear events include transcription of the thyroglobulin gene that contains putative intervening sequences (thin lines), addition of 7-methylguanosine at the 5′ end of the thyroglobulin messenger RNA precursor (abbreviated as CAP for capping), polyadenylation of the precursor at its 3′ end (abbreviated as AAA. . .A), and splicing of the intervening sequences from the precursor (dotted loops) (mRNP = ribonucleoprotein). (Redrawn from Van Herle, A. J., Vassart, G., and Dumont, J. E.: Control of thyroglobulin synthesis and secretion. New Engl. J. Med. 301:249, 1979, with permission.)

inhibitors of endogenous protease activity, the major band of the reduced and alkylated protein migrated in sodium dodecyl sulfate (SDS) gel electrophoresis was a peptide of about 330,000 daltons.[125] This would indicate that 19S thyroglobulin is made up of two subunits of 330,000. Electron microscopic studies suggest that the thyroglobulin molecule is made up of two symmetric subunits. The suggestion has been made that there are two, nonidentical subunits each with a molecular weight of approximately 330,000 daltons.[168]

Despite the essential role, the number of tyrosine residues in thyroglobulin is not exceptional. Amino acid analysis has been difficult because of blocked *N*-terminal groups.[97] However, short segments, containing thyroxine groups, have been identified.[45, 122] Thyroglobulin contains thyroxine-rich iodopeptides of small molecular size (15,000 to 30,000). Although representing only 3 to 4% of thyroglobulin's weight, these iodopeptides contain about 25% of the thyroxine.[31, 91, 96] In noniodinated preparations of thyroglobulin, there are ten free sulfhydryl groups, which disappear with increasing iodination of the molecule.[123] Conversion of 19S to 27S is thought to occur during the iodination of tyrosyl groups. The 37S is a trimer of 19S and probably results from a similar kind of polymerization.

Biosynthesis

Analytic studies of thyroglobulin structure have been hampered by the large size of the basic subunit and by the susceptibility to proteolytic enzymes.[125] The emerging availability of cloned thyroglobulin DNA may solve many of the remaining mysteries that surround this giant molecule (Fig. 2–17).[51] Studies of nucleic acid sequences, utilizing thyroglobulin mRNA and its complementary DNA (cDNA) have been helpful. Restriction studies of full-length, double-stranded cDNA have demonstrated that the two 330,000 dalton subunits are in fact identical.[171]

In other proteins, correlations have been observed between gene exons and structural as well as functional domains (Fig. 2–18). Therefore, study of gene structure may lead to better knowledge about the structural organization of thyroglobulin. The thyroglobulin gene has been localized on chromosome 8.[169] The entire gene for rat thyroglobulin has been isolated and contains at least 170,000 base pairs. The coding information is contained in

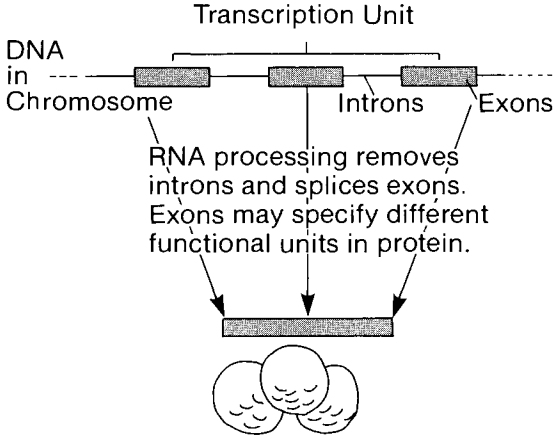

Figure 2–18. Fragmented gene with three coding regions.

9000 base pairs distributed in 42 exons. Both the 3′ and the 5′ ends of the thyroglobulin structural gene appear to be made of repetitive units, which do not show any homology to one another. Two major polypeptide sites for thyroxine synthesis are localized at the two extremities of the molecule and do not show homology.[107] Detailed studies of clones have indicated only one thyroglobulin gene per haploid complement of the human genome. Thyroglobulin mRNA has a half-life of more than 20 hours so that transcription of the gene does not have to be extremely active despite the high rate of thyroglobulin synthesis.[172] This gene appears to be the largest gene yet described and may be as large as 250 kilobases (kb). The intervening sequences or introns are very large, up to 15,000 base pairs and represent as much as 90 to 95% of the gene.[34]

The complete primary structure of bovine thyroglobulin has been derived from the sequence of cDNA, constituting a peptide of relative molecular mass (Mr) 302,253.[103] The amino acid sequence is characterized by a pattern of imperfect repeats derived from three cysteine-rich motifs. The amino terminal portion contains 10 repetitive domains, extending over about 60 amino acids. Over three quarters of the thyroglobulin sequence is involved in some type of repetitive structure, indicating that this enormous protein evolved from the multiplication of a limited number of smaller genetic units.[33, 95, 103]

Four hormonogenic peptides have been localized. Three of them are in the carboxy terminal end and one is in the amino terminal end. There is a remarkable conservation of the amino terminal domain and as much as 50% of the thyroxine in mature thyroglobulin may originate from this domain.[98]

Post Translational Processing

After translation on membrane-bound thyroid ribosomes, nascent thyroglobulin peptides of 300,000 daltons are vectorially discharged into the cisternae of the endoplasmic reticulum.[26] This vectorial discharge occurs through a mechanism that involves an amino terminal signal peptide translated from the 5′ end of the thyroglobulin mRNA that codes for hydrophobic amino acids.[22] According to this signal hypothesis, the nascent thyroglobulin peptide should be synthesized in a "prepro" form. However, discrimination between "prepro" and "pro" thyroid hormone is almost impossible because the basic subunit has a molecular weight of 300,000. Small differences in this size range cannot be determined accurately. Therefore, the alternative approach is to demonstrate nascent thyroglobulin transfer and subsequent glycosylation across microsomal membranes in a cell-free system.[52] In such a cell-free system, thyroglobulin was segregated into microsomal vesicles and core glycosylated as shown by increased protection against proteolytic treatment.[5] The data suggest that the signal peptide is removed and core glycosylation occurs cotranslationally. (Details of further post translational processing have been discussed earlier in the chapter.)

Defects in Thyroglobulin Synthesis

Traditionally, inherited defects in thyroid hormone biosynthesis have been described in relation to iodine metabolism (Fig. 2–19). Congenital defects in thyroid hormone biosynthesis have been viewed in terms of alterations of specific molecular mechanisms involved in thyroid protein biosynthesis (Table 2–1).[128] Similar to the globin genes in thalassemia there will probably be a myriad of defects, insertions, deletions, and so forth, to explain the abnormal thyroglobulin.[124] The major steps in the biosynthesis of thyroglobulin with possible corresponding inherited defects, the phenotypic effect on thyroglobulin production, and some possible examples of such defects are presented in Fig. 2–20. In this scheme, iodination is viewed as only part of the post translational modification of prothyroid hormone, although a very crucial part.

Although the protein structure determines the iodination reaction, iodination in turn strongly affects protein structure. Consequently, any effect in the iodination mechanism is liable to make thyroglobulin appear abnormal with regard to subunit interactions and chain folding. Environmental factors, such as the available iodine supply, will affect the penetrance, the expressivity, and even the apparent mode of transmission of genetic defects in the thyroid. A rich iodine supply, for example, may hide partial deficiencies in the iodination of thyroglobulin.

CONTROL OF THYROID HORMONE PRODUCTION

This complex process to ensure a constant supply of iodothyronine also requires a com-

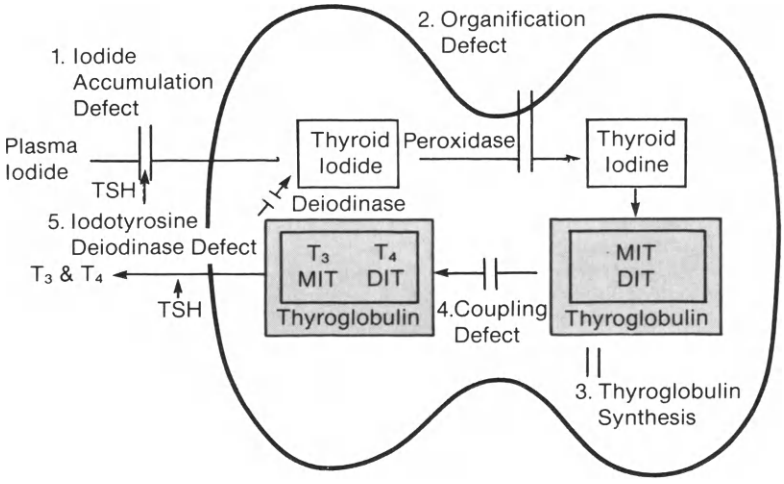

Figure 2–19. Congenital defects in thyroid hormone biosynthesis based on iodine utilization.

plex control system to ensure further that the thyroid gland is responsive to the body's need for thyroid hormone. Although the major signal is thyrotropin, operating in a classic negative feedback system, a number of other agents, including iodine, catecholamines, prostaglandins, epidermal growth factor, and so forth play a modifying role.

The administration of thyrotropin stimulates the secretion of thyroid hormone within minutes. This secretion is accompanied by a variety of cellular responses, including glucose oxidation, phospholipid incorporation, iodide transport, protein synthesis, and glycosylation.[43] TSH stimulation results in increased thyroid protein synthesis, including stimulation of thyroglobulin synthesis.[85] Whether this is specific for thyroglobulin or reflects general thyroid protein stimulation is controversial.[115] The simultaneous stimulation of thyroglobin degradation has made it difficult to document the TSH stimulation of thyroid protein synthesis. These TSH effects may occur at both the transcriptional and translational levels. An increase in thyroglobulin mRNA has been demonstrated in response to TSH stimulation.[30] TSH has also been demonstrated to cause a threefold stimulation in thyroglobulin gene transcription.[167] Forskolin activation of adenylate cyclase could mimic TSH action on thyroglobulin gene transcription. With long-term TSH administration there is an increase in both the size and number of follicular cells, hypertrophy and hyperplasia, and a marked increase in vascularization with large dilated capillaries.

TSH ACTION

Thyrotropin binds to specific receptors on the thyroid plasma membrane and activates adenylate cyclase, which in turn catalyzes the formation of cAMP and in turn, the activation of protein kinase. The activated kinase catalyzes the phosphorylation of serine and threonine groups on proteins.[43] Which particular phosphorylated proteins are responsible for the various actions of TSH remain to be determined. Indeed, how much of TSH action occurs through cAMP and how much through other pathways have not been settled.

TSH Binding to Receptors

Thyrotropin binds to specific receptors on the surface of the thyroid plasma membrane in a reaction that is saturable and reversible with a high binding affinity constant.[173] Receptor binding affinity is generally proportional to the potency of the ligand as an agonist,.[16] "Specific binding" is determined by correction of the total amount of ^{125}I-TSH remaining bound to the receptor by subtracting the ^{125}I-TSH remaining bound to the receptor in the presence of excess unlabelled TSH. "Nonspecific binding" is the residual, nonsaturable binding (Fig. 2–21). High affinity, limited capacity, specific binding interactions are described frequently as a Scatchard plot.[119] This is the plot of an equation that describes the interaction of a hormone (H) with its receptor (R), which, at equilibrium, forms a complex (H-R) with a characteristic affinity constant K.

The ratio of bound to free ligand is ex-

Table 2–1. Congenital Defects in Thyroid Hormone Biosynthesis Based on Thyroglobulin Processing*

Disorders in the biosynthesis of elementary polypeptide chains of thyroglobulin
Pretranscriptional level
Alterations of regulatory genes
Mutations of thyroglobulin structural genes
Transcriptional and post transcriptional levels
Alterations in the formation and processing of mRNA
Translational level
Alterations in initiation, elongation, or termination
Disorders in intracellular post translational modifications of thyroglobulin
Transfer of thyroglobulin from endoplasmic reticulum to follicular lumen
Defects in the segregation mechanisms
Defects in exocytosis
Carbohydrate addition
Defects in core glycosylation
Defects in stepwise sugar addition
Defects in sialyltransferase
Disorders in extracellular post translational modification of thyroglobulin (iodination)
Availability of iodine substrate
The defect in iodine transport
The dehalogenase deficiency
The enzymatic system for iodination
The peroxidase defect
The deficiency of the hydrogen peroxide generating system
Disorders in the reabsorption and degradation of thyroglobulin
Alterations in endocytosis and its regulation
Alterations in the secretory mechanisms
Defects of intrathyroidal mechanisms
Defects in extrathyroidal regulation; defects in TSH production and TSH effects

*Reproduced from Salvatore, G., Stanbury, J. B., and Rall, J. E.: Inherited defects of thyroid hormone biosynthesis. *In:* de Visscher, M. (ed.): The Thyroid Gland. New York, Raven Press, 1980, with permission.

pressed as a function of bound ligand that yields a line in which the negative slope is equal to the binding affinity constant and in which the abscissa intercept is equal to the number of binding sites. There has been controversy as to whether one or two classes of TSH binding sites exist on the thyroid plasma membrane.[118] TSH receptor binding studies have been performed under highly variable conditions, which greatly affect total TSH binding to thyroid membranes and cells. There appear to be two classes of TSH binding sites, but at physiologic pH and temperature in the presence of 50 mM NaCl, the high affinity site is measured and probably reflects the biologically relevant TSH receptors[117] (Fig. 2–22).

The physiologic relevance of two binding sites observed under different binding conditions is not clear. The low affinity site is only sensitive to TSH at pharmacologic concentrations, is relatively nonspecific, and is predominant under nonphysiologic conditions. The high affinity site is likely to represent the biologically relevant TSH receptor in view of its sensitivity to physiologically obtainable TSH concentrations (10^{-12} to 10^{-10}M) and predominance under physiologic incubation conditions.

One of the major questions to be answered is whether all these sites represent true receptors with functional significance or merely hormone binding sites. TSH has also been found to bind to fat and testis membranes.[6, 69] There are data to support the hypothesis that these are true receptors as evidenced by TSH stimulation of cAMP in fat and testis membranes. Binding of TSH to fat membranes has been used to purify radiolabelled thyroid stimulating immunoglobulins for use in binding studies to thyroid membranes. Since the fat membranes do not contain thyroid antigens, they have also been used to study thyroid-stimulating immunoglobulin binding. Thyroglobulin has been shown to inhibit TSH binding, raising the question whether thyroglobulin may have a regulatory effect on TSH binding.[75]

Gangliosides have been suggested to play a role in the interaction of TSH with specific receptors on the thyroid plasma membrane.[90] TSH is thought to bind to the glycoprotein component of the receptor through the beta subunit and then to undergo a conformational change in which the alpha subunit penetrates the membrane and activates adenylate cyclase. This latter step is thought to be mediated by the ganglioside component of the receptor.[4] However, when the role of gangliosides in TSH binding to thyroid cells was studied under physiologic conditions in which the affinity to TSH was high and compared with activation of adenylate cyclase, the findings could not be duplicated.[18]

After binding to the receptor and activating adenylate cyclase, TSH undergoes receptor mediated endocytosis.[11] Fluorescent labelled TSH bound specifically to diffusely distributed membrane receptors that subsequently formed visible patches, which were internalized and degraded. Whether receptor-mediated endocytosis has any significant role other than degradation is not clear. Maximal thyroid cAMP production in response to TSH occurred before internalization. However, down regulation of TSH receptors in response to TSH stimulation

Possible Genetic Defects in Thyroglobulin Synthesis			
Biosynthetic steps	Genetic defect	Phenotype effect	Selected examples
Regulatory gene(s) (?)	?	Low TG synthesis	Human goiters?
↓ TG—DNA	TG gene mutation	Abnormal TG	Merino sheep goiter? Human goiters
↓ Heterogeneous nuclear RNA	Transcriptional defect	No TG?	?
↓ TG—mRNA	RNA processing	No TG	Goat goiter Human goiters?
↓ TG peptide chains	Translational defect	No TG	?
↓ pro-TG	ER segregation defect	Little or no secretion of TG	Afrikander cattle goiter
↓ Glycosylated TG	Glycosylation defect	?	?
↓ Asialo—TG	Exocytosis defects	Little or no secretion of TG	Human goiter
↓ Noniodinated TG (19S)	sialyl transferase defect	Little or no TG	Human goiter Experimental rat tumor
↓ Iodinated TG (19S and 27S)	Iodination defect (e.g. I^- active transport defect, dehalogenase defect, organification defect)	Poorly iodinated or noniodinated TG	Human goiters

Figure 2–20. Possible genetic defects in thyroglobulin synthesis. (Reproduced from Salvatore, G., Stanbury, J. B., and Rall, J. E.: Inherited defects of thyroid hormone biosynthesis. *In* deVisscher, M., ed.: The Thyroid Gland. New York, Raven Press, 1980, with permission.)

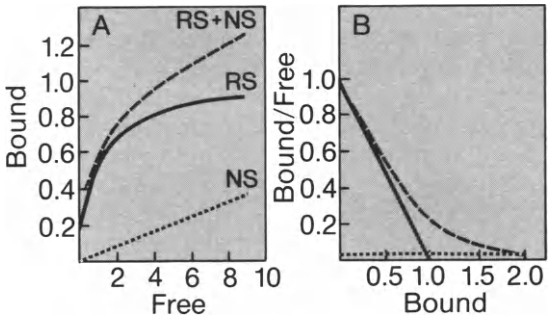

Figure 2–21. Specific and nonspecific binding of hormones to receptors. Data shown in the saturation plot *(A)* have been replotted by Scatchard analysis *(B)*. Total binding in the system (dashed line) is the sum of receptor ligand complexes (solid line) and nonspecific binding (dotted line). (Redrawn from Clark, J. H. and Peck, E. J., Jr.: Female sex steroids: Receptors and function. *In* Gross, F., et al., eds.: Monographs on Endocrinology, Vol. 14. New York, Springer Verlag, 1979, with permission.)

has been considered to relate to the receptor-mediated endocytosis.[71]

A particularly interesting question is whether internalized peptide hormones in some instances circumvent the need for an intracellular messenger.[98] The discovery of intracellular hormone binding sites has led to speculation that peptide hormones mediate their diverse effects on cells through different molecular pathways. Internalization of the hormone, transport to a particular organelle, and binding to a high affinity receptor might elicit a particular cellular response.

Activation of Adenylate Cyclase and cAMP Stimulation

A close relationship exists between TSH binding and activation of adenylate cyclase in thyroid plasma membranes (Fig. 2–23). Adenylate cyclase activity of thyroid membranes is stimulated by the addition of guanosine triphosphate.[43, 44] Therefore, activation of ade-

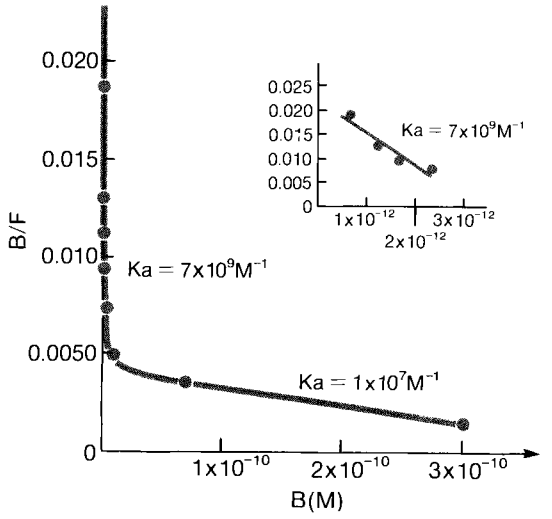

Figure 2–22. Scatchard analysis of thyroid-stimulating hormone (TSH) binding demonstrates two apparent affinity constants, calculated from the limiting tangents. The inset shows the initial part of the plot on an expanded scale. (Redrawn from Pekonnen, F. and Weintraub, B. D.: Thyrotropin receptors on bovine thyroid membranes: Two types with different affinities and specificities. Endocrinology 105:357, 1979, with permission.)

nylate cyclase in the thyroid similar to other tissues appears to involve a guanosine triphosphate (GTP) binding unit, which controls the activity of the catalytic unit (Fig. 2–24).[154]

TSH activation of adenylate cyclase leads to increased cAMP formation and in turn to the activation of protein kinase.[44] The cAMP binds to the regulatory subunit, which dissociates leaving an active catalytic subunit. The cAMP-dependent protein kinases are present in the thyroid and have characteristics similar to those in other tissues. Three peaks of protein kinase activity have been found in the thyroid (Fig. 2–25). Peak I and peak II are stimulated by cAMP, while peak III is cAMP independent.[144]

The two cAMP-dependent protein kinase isoenzymes have identical catalytic subunits while their regulatory subunits are distinct. Only the regulatory subunits of the type II kinase can be phosphorylated by an extramolecular mechanism, and only the type I kinase is present in thyroid membranes.[161] If particular enzymes have an important role in the rapid phosphorylation of some membranous proteins, kinase I is better suited because it dissociates more easily. The concentration of cAMP required for half-maximal activation of protein kinase A is 10^{-7} to 10^{-8}M. These concentrations are not dissimilar to resting cAMP concentrations found in the thyroid and suggest that cAMP may be compartmentalized.[143] Purified thyroid cAMP-dependent protein kinases are half-maximally activated at cAMP concentrations between 4×10^{-8} and 9×10^{-8}M.[11, 183] The resting intracellular cAMP concentration approximates 3×10^{-7}M. If all the cAMP were free to act on the kinase in the thyroid, there would be activation of all protein kinase activity in the thyroid. Compartmentalization implies different roles, and interestingly TSH stimulates organification of iodide in the absence of kinase II.

TSH stimulates an increase in protein kinase A activity.[143, 183] The time course and dose response relationships of TSH activation of protein kinase A are compatible with the concept that the cAMP-mediated effects of TSH are a consequence of changes in the kinase A enzyme activity. Despite impressive evidence that effects of TSH are expressed through activation of protein kinase, specific substrates for this enzyme that affect cell function have not been identified in the thyroid. The cAMP-dependent protein kinase catalyzes the phosphorylation of serine and threonine groups. While it is possible to demonstrate phosphorylation of a variety of thyroid proteins, evidence is lacking that these proteins are responsible for the TSH-induced changes. One of the major TSH effects is pseudopod formation and thyroid hormone secretion.[84] Pseudopod formation is the most rapid TSH effect and the first step in secretion and always follows the rise in cAMP. TSH stimulation results in the phosphorylation of a 26,000 dalton thyroid contractile protein.[87] However, there is no proof that this is the mechanism of TSH action. In fact, with high resolution, two-dimensional gel electrophoresis, TSH stimulation of phosphorylation can be demonstrated with at least eight peptides.[89, 146] Within the thyroid nucleus, TSH also stimulates the phosphorylation of histones HI and HII[37] and certain nonhistone chromosomal proteins.

Until recently, much less attention has been paid to the cAMP independent kinase, protein kinase C.[19, 112] Protein kinase C is activated by diacylglycerol, transiently produced from inositol phospholipids. In general, this turnover of membrane phospholipids is associated with an increase in the intracellular concentration of calcium, which appears to mediate a number of physiologic responses. The activation of protein kinase C and the effects that result

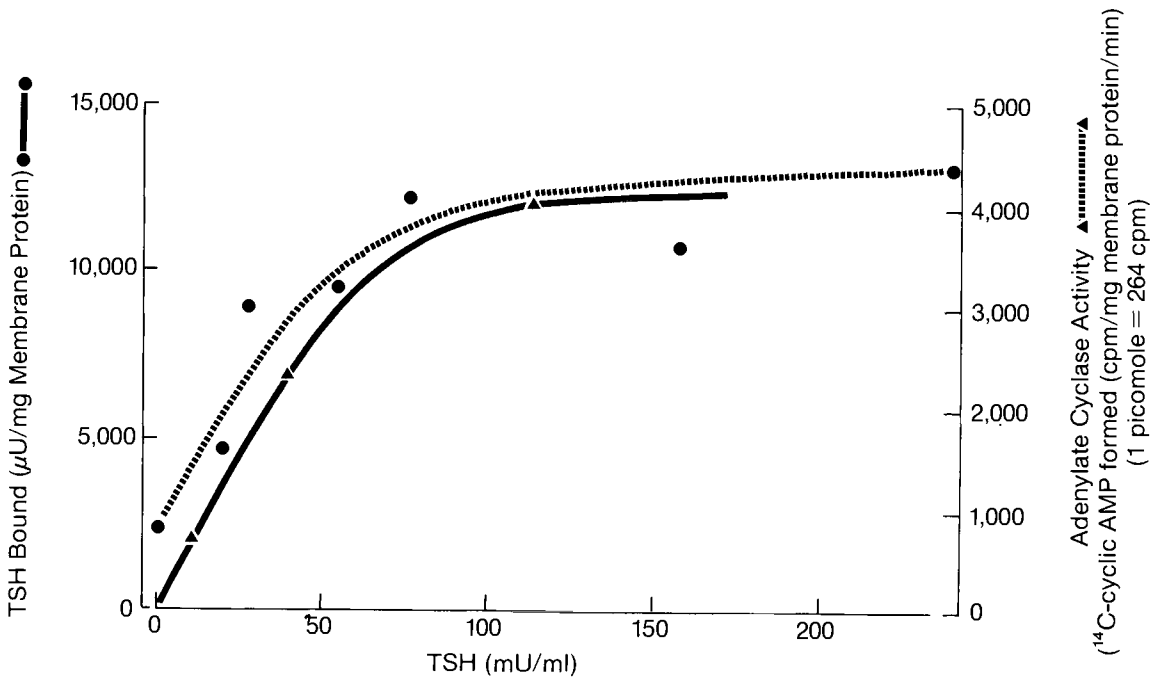

Figure 2–23. Correlation between thyroid-stimulating hormone (TSH) binding and activation of adenylate cyclase in thyroid plasma membranes. (Redrawn from Field, J. B.: Thyroid-stimulating hormone and cyclic adenosine 3′,5′ monophosphate in the regulation of thyroid gland function. Metabolism 24:381, 1975, with permission.)

from protein phosphorylation on serine and thionine groups appear to be distinct from those effects brought about by an increase in intracellular calcium. Protein kinase C is present in the thyroid but the role it plays in the modulation of thyroid function is unclear (Fig. 2–26).[79]

The mechanism of action of calcium is different from other metal ions and closer to the regulatory activity of cyclic nucleotides. The specificity of cAMP is determined by receptors that elevate the cyclic nucleotide and substrates available for the cAMP-dependent protein kinase. A calcium binding protein, calmodulin, has been identified that is capable of binding the estimated intracellular free calcium.[101] Amplification of a calcium signal, similar to the AMP signal, could be accomplished though the activation of specific protein kinases. Calmodulin levels in the cell do not appear to be regulated by trophic hormones. The regulation of calmodulin-mediated enzymes appears to be due to alterations in the net flux of calcium rather than to changes in the cellular content of calmodulin.

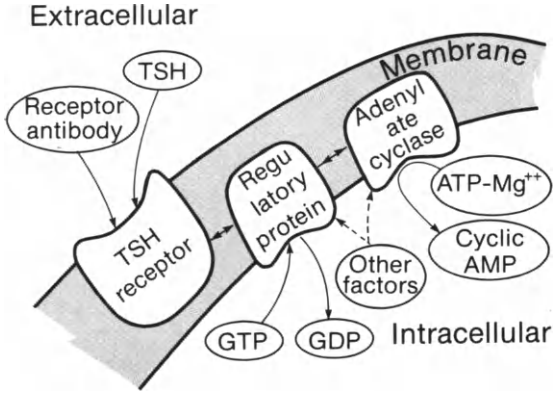

Figure 2– 24. Mechanism of thyroid stimulation by thyrotropin and thyroid-stimulating hormone (TSH) receptor antibodies. (Redrawn from Baxter, J. D. and Funder, J. W.: Hormone Receptors. New Engl. J. Med. 301:1149–1161, 1979, with permission.)

Prostaglandins

Prostaglandins, especially PGE, have been shown to mimic a number of the effects of TSH on the thyroid, including activation of adenylate cyclase, iodide trapping and organification, glucose oxidation, colloid droplet formation, and iodide release.[24, 99] Prostaglandin concentrations in isolated thyroid cells are increased by the addition of TSH, perhaps though stimulation of inositol phospholipids, which results in increased arachidonic acid. However, the exact relationship between pros-

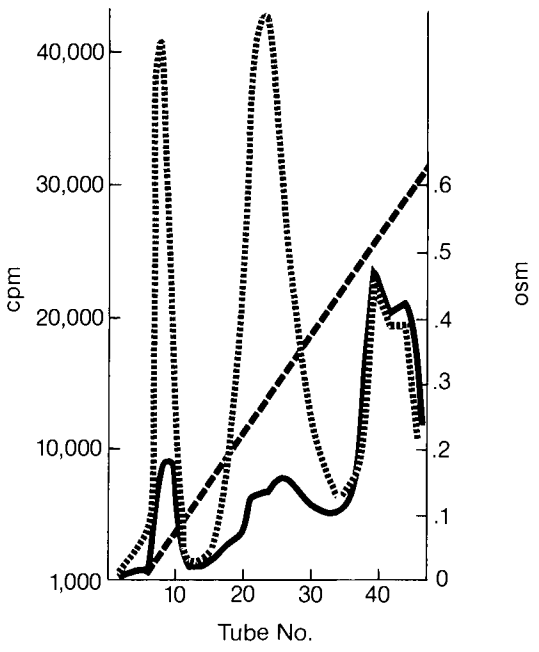

Figure 2–25. Peaks of protein kinase activity in the thyroid. The dotted line indicates assays performed in the presence of cyclic AMP and the solid line indicates assays performed in the absence of cyclic AMP. The change in the phosphate gradient is indicated by the change in osmolarity, shown as a dashed line. Activity is expressed as cpm incorporated into 30 μg of histone/5 minutes' incubation. (Redrawn from Spaulding, S. W. and Burrow, G. N.: Several adenosine 3',5'-monophosphate–dependent protein kinases in the thyroid. Endocrinology 91:1343–1349, 1972, with permission.)

taglandins and cAMP in modulating TSH effects remains controversial.[99, 159] PGE, similar to TSH, increased cAMP-dependent protein kinase activity in the thyroid.[142] However, prostaglandin synthesis is not necessary for TSH action. In addition to the cyclooxygenases, such as PGE and PGF, lipoxygenases, such as leukotrienes, may play a role in modulating thyroid function.[74] Interrelationships between prostaglandins and protein kinases are shown in Figure 2–26.

Down Regulation

Although the diverse metabolic effects of TSH may be mediated through cAMP, less is known about the mechanisms that terminate TSH action. The thyroid gland becomes refractory to subsequent TSH stimulation after exposure to TSH for a period of time.[67, 139] Although the suggestion of down regulation of TSH receptors has been raised, this has not been confirmed.[64, 158] Thyroid desensitization to TSH is associated with decreased adenylate cyclase activity as well as with inhibition distal to cAMP generation. The suggestion has been made that TSH desensitizes by adenosine diphosphate (ADP) ribosylation. Nicotinamide-adenine dinucleotide (NAD^+) serves as a substrate in nonoxidative reactions in which ADP-ribose is bound to cellular substances.[66] The presence of nicotinamide, which blocked ADP ribosylation, inhibited the desensitization phenomenon. Whether desensitization plays a physiologic role remains to be determined.

Other Actions

Iodine

In excess, iodide may block its own accumulation in the thyroid, i.e., the Wolff-Chaikoff effect.[178] In addition, iodide can block TSH-mediated cAMP accumulation.[170] Drugs that block iodide trapping and oxidation, such as perchlorate and methimazole, prevent this inhibition. The effect appears to occur at the level of cAMP synthesis. The suggestion has also been made that short loop, negative feedback exists whereby thyroid hormones directly inhibit their own secretion.[64] However, proof is lacking.

Catecholaminergic and Cholinergic Stimulation

Although catecholamines can be demonstrated to have effects on the thyroid, the physiologic importance remains to be determined. Catecholamines increase colloid droplet formation and thyroid hormone release in non-TSH stimulated glands.[102] The β-adrenergic agonists activate adenylate cyclase and stimulate thyroid protein kinase activity.[143] Catecholamines also effect blood flow to the thyroid.

Acetylcholine increases the calcium level in the thyroid cell, which increases cyclic guanosine monophosphate (cGMP) levels. A physiologic role for cGMP and guanylate cyclase has been postulated.[29] The acetylcholine-calcium system exerts a negative control on cAMP and in turn on thyroid hormone secretion by an effect on the disappearance of cAMP.[43] However, cGMP levels can be stimulated without affecting cAMP levels.[29]

Growth Effects

TSH traditionally has been thought to stimulate growth of the thyroid as well as differen-

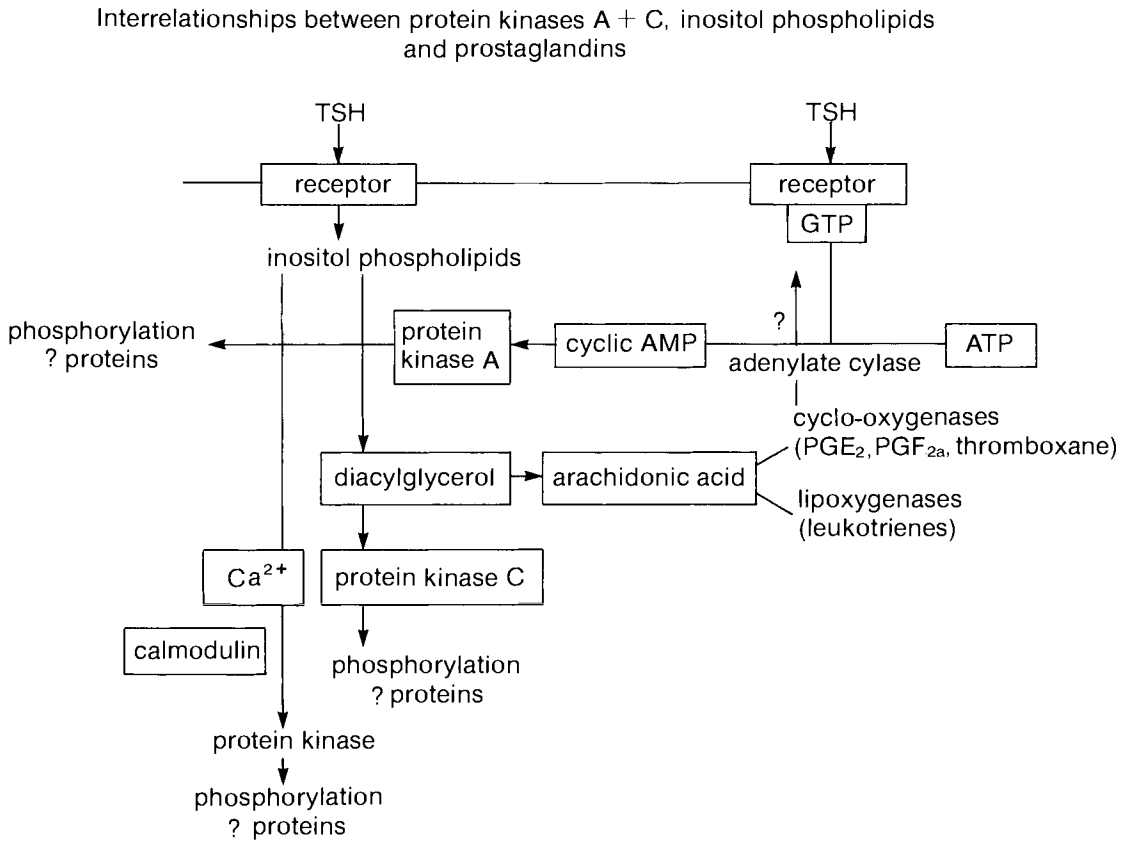

Figure 2–26. Relationships among protein kinases A and C, inositol phospholipids, and prostaglandins.

tiation, e.g., goiter formation. However, further data from thyroid cells grown in culture in the absence of serum raise questions about this concept in sheep cells in primary culture.[176] Epidermal growth factor but not TSH has been demonstrated to stimulate growth of cultured thyroid cells.[49, 175] Interestingly, epidermal growth factor inhibited differentiation, i.e., iodination, while promoting growth.[184] This stimulation of growth may not be modulated by cAMP-dependent protein kinases. Phorbol esters, which activate protein kinase C by substituting for diacylglycerol, also stimulate growth in cultured thyroid cells.[13] Ovine thyroid cells in primary culture have been shown to produce the insulin-like growth factors, IGF-I and IGF-II, and the production of these growth factors was stimulated by TSH.[51, 94] The possibility exists that TSH may not stimulate growth directly but rather a growth-promoting effect may be produced by intermediary growth factors or by involving cells more responsive to circulating growth factors, such as epidermal growth factor.[49, 175]

The polyamines, spermine and spermidine, and their biosynthetic precursor putrescine have been demonstrated to accumulate in rapidly developing cells in concert with increases in nucleic acids. The rate limiting step in polyamine synthesis is the formation of putrescine from ornithine, which is catalyzed by the enzyme ornithine decarboxylase. TSH increases ornithine decarboxylase activity in the thyroid and this is mimicked by the administration of dibutyryl-cAMP.[130, 131, 184]

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3
The Hypothalamic-Pituitary-Thyroid Axis

GERARD N. BURROW

The prolonged half-life of thyroid hormones in the circulation means that minute-to-minute control of thyroid hormonal secretion is not necessary. Unlike insulin secretion, for example, thyroid hormone secretion can be modulated in a much more stately manner. However, there are control systems and, in fact, layers of systems that modulate thyroid hormone secretion.[122] The primary feedback mechanism involves thyroid hormone, thyrotropin, and thyrotropin-releasing hormone (TRH). However, somatostatin, dopamine, and estrogens also play a role, as do catecholamines. These control mechanisms are not simply matters for the neuroendocrinologist to study but are clinically important.

The concept of pituitary control of the thyroid gland dates to 1851 when the observation was made that cretins had enlarged pituitary glands. Approximately 50 years ago, the basic control of the thyroid was postulated to operate through a servomechanism.[4, 77] The neurovascular hypothesis of Green and Harris has been superimposed on this control mechanism, allowing modification of the thyroid-pituitary feedback system by the central nervous system.[66]

THYROID-STIMULATING HORMONE

Thyrotrophs are located predominantly in the anteromedial portion of the pituitary[136] and constitute about 5% of anterior pituitary cells.[48] These cells produce about 165 mU/day of TSH.[128] The plasma clearance rate of TSH has been estimated at 50 ml/min.[154] TSH or a TSH-like substance also has been reported to be produced by lymphocytes.[181]

TSH Secretion

TSH secretion results in a variety of thyroid effects, including thyroid hormone secretion and thyroid gland growth. Since serum thyroid hormone concentrations are fairly steady, serum TSH concentrations are relatively consistent, and, unlike follicle-stimulating hormone (FSH) or luteinizing hormone (LH), useful clinical information can be obtained from a solitary blood sample drawn at any time. Minimal diurnal variation occurs in serum TSH concentrations with a small increase between 11 p.m. and 1 a.m., with the rise beginning before sleep induction.[37, 202] The lowest TSH concentrations occur about 11

a.m. Interestingly, the circadian variation disappears in patients with severe hypothyroidism.[203] How the circadian rhythm for TSH occurs is unclear. The changes are not due to hemoconcentration, and possibilities have included a cortisol effect or release from dopamine inhibition.[169, 203] There is evidence to suggest that TRH controls the TSH rhythm in the rat, which has a well-characterized circadian rhythm.

Exposure to cold increases serum TSH concentrations in neonates but not in adults.[52] Small increases in plasma TSH concentrations in adults have been reported after 2 hours of cold exposure and after a week in the Arctic.[64, 130] Warming decreases serum TSH concentrations in hypothyroid patients perhaps by increasing peripheral metabolism.[130]

The serum TSH concentration remains constant throughout adult life.[146] A decreased TSH response to TRH has been reported for elderly males but not females.[7] Some of the difference might be explained by concomitant nonthyroidal illness, but this would not explain the difference in response between men and women.[185] Hypothyroidism is not uncommon among the elderly, and this finding indicates that serum TSH concentration is a valid measure in the healthy geriatric patient.[36]

Nonthyroidal Acute or Chronic Illness. Significant illness produces changes in peripheral thyroid hormone metabolism (see Chapter 6) that cause major difficulties in the interpretation of thyroid function tests.[200] As a result of impaired conversion of T_4 to T_3 as well as reduced hepatic uptake of T_4, the serum T_3 concentration decreases relative to the serum T_4 concentration.[171] In this clinical setting the ability to diagnose hypothyroidism becomes very difficult but very important. The serum TSH concentration may not be elevated in these situations, since critically ill patients may also receive corticosteroids or dopamine, which suppresses TSH secretion.[91] The TSH response to TRH has been variably reported to be normal or suppressed.[24, 57] However, TSH regulation appears to be abnormal in these ill patients, and a normal serum TSH concentration may not exclude the possibility of hypothyroidism.[114] Fasting induces changes in peripheral thyroid hormone metabolism and blunts the TSH response to TRH. Conceivably, with diminished caloric intake, serum TSH concentrations in critically ill, mildly hypothyroid patients would not be elevated.[19] In fact, with critically ill patients, it may be impossible to accurately determine thyroid function.

The clearance rate of TSH depends on the level of thyroid function not on the presence or absence of thyroid tissue. TSH is metabolized in peripheral tissues, mostly in the kidney or liver. The thyroid gland is not a major site of TSH degradation. TSH secretion has been well studied in patients with chronic renal disease. The serum TSH concentration is normal, and the TSH response to TRH is frequently blunted with a delayed peak response.[90, 107] This delay in response has been attributed to the decreased renal clearance of TSH or TRH.[38]

Iodinated contrast agents, such as iopanoic acid used for cholecystography, also block the iodination of thyroid hormones similar to that seen in acute and chronic illness.[213] Subsequent to the decrease in serum T_3 concentration, there is an increase in the serum TSH concentration and the TSH response to TRH.[93, 192] This increased response differs from that in fasting and nonthyroidal illness in which the reduction in the peripheral conversion of T_4 to T_3 is accompanied by a lower set point of TSH secretion.[53]

Assay of TSH

The development of antibodies to human TSH in laboratory animals provided the means towards development of a specific radioimmunoassay for human TSH.[129] This radioimmunoassy for TSH permitted the separation of abnormal elevated serum TSH concentrations from normal TSH concentrations. However, separation between normal and low concentrations of TSH, e.g., hyperthyroidism, has been difficult. Serum TSH concentrations with standard assays are below the limits of detectability in 10 to 30% of euthyroid individuals.[159] However, the assay can be modified to determine serum TSH concentrations in the low range, and this type of assay will assume increasing importance.[96]

Human TSH is necessary for maximum sensitivity of the assay since TSH has structural species specificity. In addition, anti-TSH sera generally react to some degree with other glycoprotein hormones, such as LH, FSH, and human chorionic gonadotropin (hCG), since all of these hormones have a similar alpha subunit. The sera must be absorbed with a source of alpha subunit, usually hCG. Sufficient specificity can be achieved to demon-

strate that pregnant women with increased hCG and postmenopausal women with increased LH have normal serum TSH concentrations.[17] With such an assay, most but not all normal individuals have detectable amounts of TSH in their sera. The normal values in standard assays range from less than 1 mU/L to 10 mU/L. TSH iodinates easily because of a high tyrosine content and preparations of high specific activity are easily obtained. Reasonable specificity towards TSH is obtained because a large portion of the antibodies raised against purified TSH are directed towards the beta subunit. Antisera against the beta subunit show only slight cross-reactivity against other glycoprotein hormones.[16] Antisera against both alpha and beta subunits of human TSH also have been raised that demonstrate low cross-reactivity towards intact TSH and allow assay of the individual subunits.[100]

Serum TSH concentrations of 2 to 3 mU/L in standard assays may be either normal or low, depending on the conditions of the assay. The TRH test might be considered to increase the sensitivity of the TSH assay in the lower ranges. The TRH test helps to determine whether the value of 2 to 3 mU/L is normal or low, depending on whether there is a TSH response to TRH.

The sensitivity of the assay can be increased to measure low values of serum TSH.[96] Thus, instead of being useful only in the diagnosis of primary hypothyroidism, a sensitive TSH determination may become the single best indicator of abnormal thyroid function and render the TRH stimulation test obsolete. For this change to occur, there must be a low detection threshold, good precision in measuring low concentrations, and a measurable lower limit for the normal range.[73a] The lower normal limit of the sensitive TSH value is about 0.4 mIU/L with an assay detection limit of 0.06 mIU/L. The TSH concentrations in most clinically thyrotoxic patients are suppressed to undetectable levels.[24a, 186a] A sensitive TSH assay would also be helpful in monitoring TSH suppression in patients with thyroid carcinoma who are receiving thyroxine therapy.[104a]

Radioreceptor assays are also possible and of particular interest because the thyroid-stimulating immunoglobulin is presumably an antibody to the TSH receptor.[180] There have been several reports of immunoreactive but biologically inactive TSH.[49, 180] There is a highly sensitive bioassay available based on cytofluorometry, which is more sensitive than any radioimmunoassay for TSH and can detect TSH values to 10^{-15} M.[17, 135] This cytofluorometric bioassay also detects thyroid-stimulating immunoglobulins but the time course is slower than that for TSH.

Primary Thyroid Gland Failure

With thyroid gland failure, for whatever reason, accompanied by decreased serum concentrations of T_4 and T_3, the serum TSH concentration increases in a reciprocal manner. The amount of increase in the serum TSH concentration is widely variable, ranging from 10 to 1000 mU/L (Fig. 3–1). The increases are due to both increased pituitary production and decreased metabolic clearance rate.[96] The absolute level of serum TSH correlates neither with severity nor with duration of the hypothyroidism.[116]

Interestingly, hypothyroidism itself inhibits pituitary function, including TSH secretion. Administration of subphysiologic doses of triiodothyronine to hypothyroid patients resulted in a decrease in basal serum TSH concentration but a significant increase in the serum

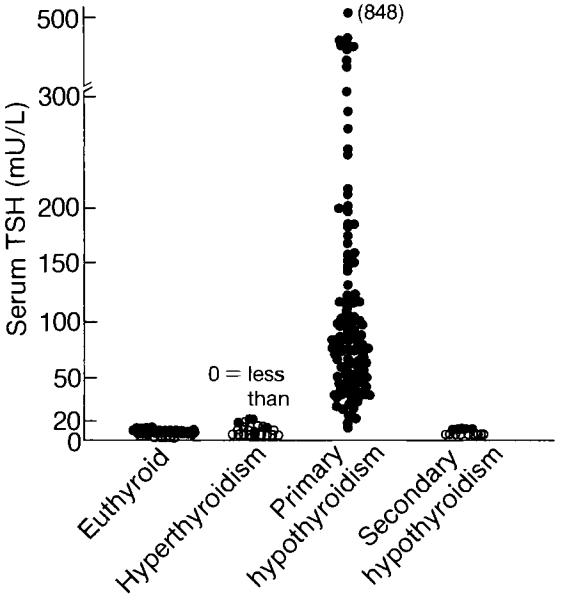

Figure 3–1. Range of serum thyroid-stimulating hormonal (TSH) concentrations in normal subjects and in patients with thyroid disease. (Redrawn from Spaulding, S.W. and Utiger, R.D.: The thyroid: Physiology, hyperthyroidism, hypothyroidism, and the painful thyroid. *In* Felig, P., et al. eds.: Endocrinology and Metabolism. New York, McGraw-Hill Book Co., 1981, with permission.)

TSH response to TRH administration.[152] In patients with pituitary or hypothalamic hypothyroidism, the serum TSH concentrations are usually undetectable. A decreased serum thyroid hormone concentration in conjunction with an elevated serum TSH concentration confirms the diagnosis of primary hypothyroidism. Possible exceptions to this statement would include the production of an immunoreactive but biologically inactive TSH [49, 188] and several instances of hypothalamic hypothyroidism with elevated serum TSH concentrations.[79, 132]

In the absence of prior ablative therapy, e.g., surgery and radioactive iodine, the most common cause of primary hypothyroidism is Hashimoto's thyroiditis. Patients with primary hypothyroidism also have increased circulating alpha and beta subunits of TSH, which come from pituitary secretion.[99] However, these represent only a small percentage of the serum TSH concentration.

Thyroid Hormone Replacement Therapy

Unlike a number of endocrine-deficiency states where the exact hormone replacement cannot be given, e.g. estrogen, thyroid hormone can be replaced, but the ratio of thyroxine and triiodothyronine is controversial. Initially, hypothyroid patients were given replacement therapy with desiccated thyroid hormone, which more or less mimicked the ratio of the two hormones in the thyroid gland. Subsequently, most of the triiodothyronine in the serum was found to result from the peripheral deiodination of thyroxine (see Chapter 4). Studies indicated that some tissues required normal plasma concentrations of both T_4 and T_3 for optimal replacement therapy, and this goal could not be achieved with desiccated thyroid.[106] Furthermore, the rapid absorption of T_3 contained in desiccated thyroid and liotrix resulted in transient elevations in serum T_3 concentrations associated with symptoms in some patients.[83–158, 160, 213]

Patients who received L-thyroxine as hormone replacement did not experience similar fluctuations in serum triiodothyronine concentrations.[160] A replacement dose of 2.25 μgm of T_4/kg of body weight in 44 patients was associated with a mean serum T_4 concentration of 8.1 μgm/dl (104 nmol/L) and a mean serum T_3 concentration of 130 ng/dl (2.0 nmol/L).[189] Comparable values in euthyroid patients were 91 nmol/L and 2.1 nmol/L, respectively. Only two patients had elevated serum thyroxine concentrations with this dose of L-thyroxine. Patients rarely require more than 0.1 to 0.15 mg daily for thyroid hormone replacement.[127] Hormone replacement therapy in patients with primary hypothyroidism should be monitored clinically and with serum TSH determinations. However, a normal serum standard TSH determination does not exclude the possibility of excess L-thyroxine replacement, and the concentrations of both serum T_4 and T_3 are helpful in monitoring therapy.[161, 212] The sensitive TSH assay should be helpful in this regard.

TSH Suppression in Thyroid Malignancy

Although a serum TSH concentration in the normal range may be sufficient for replacement therapy, the goal in thyroid malignancy is to decrease TSH secretion as much as possible. Total suppression of TSH secretion may not be possible despite toxic doses of L-thyroxine. However, TSH may stimulate growth in thyroid malignancies and as complete suppression as possible without inducing thyrotoxicosis seems a reasonable goal. Patients are placed on 0.15 mg of L-thyroxine, and the TSH response to TRH is tested[76] or the sensitive TSH assay is utilized. If there is any TSH response to TRH the dose of L-thyroxine is increased by 0.05 mg and the patient retested 4 weeks later.[47] The sensitive TSH assay would obviously be easier. This process should be continued until no TSH response occurs or thyrotoxic symptoms develop. In patients with uncontrolled metastatic thyroid carcinoma, consideration could be given to hypophysectomy to eliminate any TSH secretion.

Decreased Functioning Thyroid Mass

Elevated serum TSH concentrations have been reported in apparently euthyroid individuals, particularly in those with Hashimoto's disease or treated Graves' disease.[15, 100] Patients were categorized according to increasing severity of thyroid disease, based on serum T_4 concentrations (Table 3–1). Patients in groups I and II may have elevated serum TSH concentrations without detectable clinical evidence of hypothyroidism. Whether these individuals are truly hypothyroid remains unclear. This finding would occur only when thyroid gland functioning capacity is decreased.[208]

Table 3–1. Hypothyroid Patients Classified by Severity on Basis of Serum T_4 Concentration*

Group	T_4 (nmol/L)	T_3 (nmol/L)	TSH (mU/L)	
			Basal	*After TRH (200 μgm)*
Control	91 ± 11.5	1.7 ± 0.4	1.3 ± 0.5	11 ± 4.6
I	77–115	1.8 ± 0.6	5.3 ± 2.3	39 ± 15
II	51–77	1.6 ± 0.3	13 ± 10	92 ± 50
III	25–51	1.5 ± 0.5	63 ± 56	196 ± 120
IV	25	0.6 ± 0.4	149 ± 144	343 ± 326

*From Larsen, P. R.: Thyroid-pituitary interaction: Feedback regulation of thyrotropin secretion of thyroid hormones. N. Engl. J. Med. 306:23, 1982, with permission.

The important question is at which point patients should be treated with thyroid hormone. To wait for them to develop symptomatic hypothyroidism is almost certainly too late. A number of clinicians prescribe replacement doses of thyroxine in patients without complications who have definite elevated serum TSH concentrations greater than 15 to 20 mU/L despite normal serum T_3 concentrations.[106] This approach is based on the unproven consequences of a long-term elevation of the plasma TSH concentration and the fact that replacement therapy is relatively nontoxic. Certainly, if the patient is not treated, she should be followed closely. Hypothyroid patients may not seek medical advice nor complain enough to be brought to medical attention.

Prolactin Hypersecretion in Primary Hypothyroidism

Administration of TRH increases prolactin secretion, which may occur spontaneously in patients with primary hypothyroidism. In premenopausal women with long-standing hypothyroidism, galactorrhea and enlargement of the sella turcica may occur. This clinical picture may be easily mistaken for prolactinoma if the hypothyroidism is not recognized. During a consultation, I saw a patient with an enlarged sella and a serum prolactin concentration of 50 μgm/L who had been referred to a neurosurgeon for transphenoidal hypophysectomy. A decision was made to proceed with the surgery without waiting for the test results despite clinical suspicion of mild hypothyroidism. A TRH test was done preoperatively, and she was started on L-thyroxine 0.1 mg daily. Histologic examination of the pituitary was initially interpreted as chromophobe adenoma. However, after the serum TSH concentration was found to be 350 mU/L preoperatively, immunoperoxidase staining revealed thyrotroph hyperplasia (Fig. 3–2).

Enlargement of the sella turcica has also been reported in long-standing endemic cretinism.[119] Basal serum TSH concentrations have been found to be elevated (4.4±0.5 vs. 2.5±0.2 mU/L) in patients with hyperprolactinemia without evidence of thyroid disease.[45] This hypothesis is compatible with a concept of reduced dopaminergic tone as the cause of both the elevated serum prolactin and serum TSH. However, the hypothesis has been questioned.[15, 155]

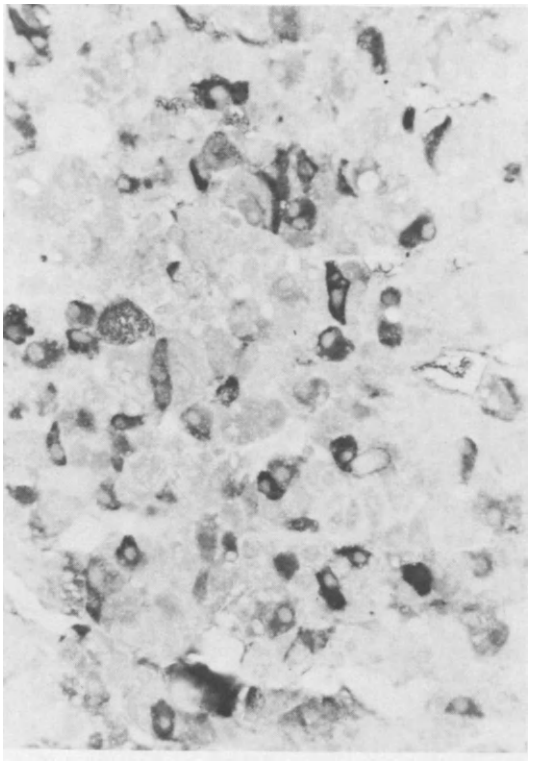

Figure 3–2. Pituitary thyrotroph hyperplasia mimicking prolactin-secreting adenoma. (Reproduced from Khalil, A., et al.: Pituitary thyrotroph hyperplasia mimicking prolactin-secreting adenoma. J. Endocrinol. Invest. 4:402, 1984, with permission.)

Secondary and Tertiary Hypothyroidism

Hypothyroidism may also occur because of a defect at the pituitary or at the hypothalamic level.

Pituitary Hypothyroidism

The most common cause of secondary hypothyroidism is either a pituitary tumor, involving more than 75% of the pituitary, or Sheehan's syndrome. Postpartum hemorrhage with pituitary infarction has occurred in a myxedematous woman.[175] Patients with isolated TSH deficiency have been reported, and it is likely that there are a variety of abnormalities that correspond to the steps in TSH biosynthesis.[193, 209] Abrupt cessation of thyroid hormone therapy in a euthyroid patient on suppressive therapy may result in transient hypothyroidism (Fig. 3–3). Whether this effect is due to pituitary or hypothalamic suppression is not clear but is transient. TSH suppression may also follow the development of antibodies to multiple injections of bovine TSH.[74]

Serum thyroxine and triiodothyronine concentrations are decreased. Basal serum TSH concentrations are not elevated. Standard TSH determinations do not distinguish between normal and low values, and the TRH test is required to make the diagnosis. In a large group of growth hormone deficient children, one third of clinically euthyroid children had abnormal TSH responses to TRH.[120] In children known to have structural damage to the hypothalamic-pituitary axis, more than half had abnormal TSH responses. Treatment of pituitary hypothyroidism may be complicated by the presence of other pituitary deficiencies. Thyroxine replacement therapy in a patient with concomitant adrenal insufficiency may precipitate an addisonian crisis by enhancement of steroid catabolism.[118]

Hypothalamic Hypothyroidism

With the availability of the TRH test, a number of patients with hypothalamic pituitary hypothyroidism have been reported with hypothyroidism, nonelevated serum TSH concentrations, but a relatively normal TSH response to TRH.[50, 132, 139] The majority of these cases have been thought to represent TRH deficiency. The suggestion has been made that prolonged and exaggerated TSH response to TRH with a delayed poor response would differentiate pituitary from hypothalamic disease.[50] However, this has not been a universal finding, and there is no characteristic TSH

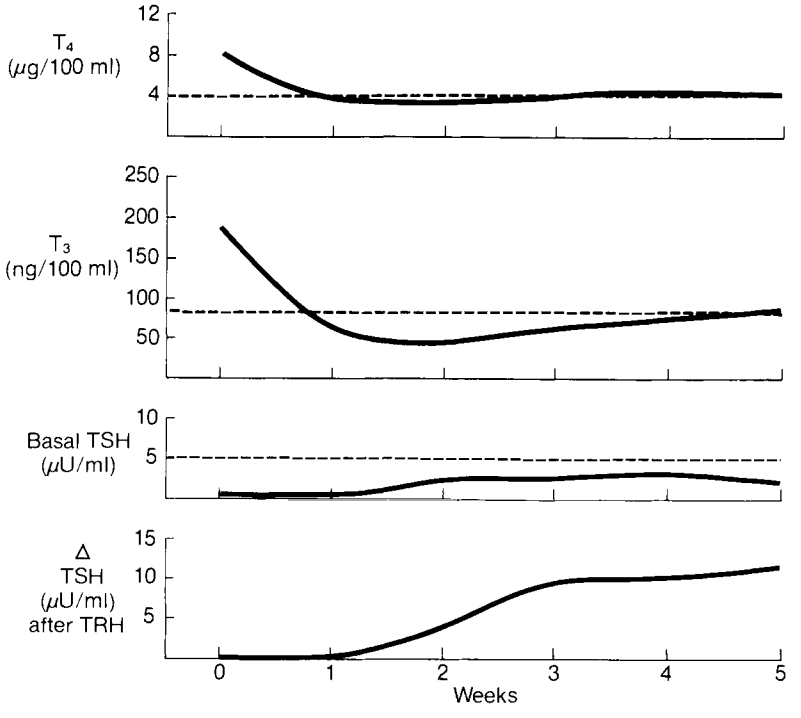

Figure 3–3. Recovery of thyrotropic function after prolonged thyroid suppression. Dashed lines in the two upper panels represent lower limits of normal range for serum thyroxine and triiodothyronine concentrations. The dashed line in the third panel represents the upper limit of the normal range for serum thyrotropin concentration. (Redrawn from Vagenakis, A.G., et al.: Recovery of pituitary thyrotropic function after withdrawal of prolonged thyroid-suppresion therapy. N. Engl. J. Med. 293:681–684, 1975, with permission.)

response that differentiates hypothalamic disease.

Hypothyroidism has been reported to develop in patients during prolonged coma.[105, 158] The etiology is not clear but these individuals have normal TSH responses to TRH, suggesting a hypothalamic etiology. Therapeutic irradiation to the hypothalamic-pituitary area may result in hypothyroidism.[162, 163] Secondary hypothyroidism is a recognized complication of sarcoidosis. Some of these patients with sarcoidosis have been found to have normal TSH responses to TRH, again suggesting a hypothalamic origin.[190]

TSH Secretion in Thyrotoxicosis

In the usual forms of toxic diffuse or toxic nodular goiter, serum TSH concentrations are undetectable.[98] Clinically an euthyroid patient with an autonomous thyroid nodule or Graves' disease may also have an undetectable serum TSH concentration that does not increase after TRH stimulation. If pituitary TSH secretion is the most sensitive indicator of excess thyroid hormone, then these patients should be considered thyrotoxic. However, thyroxine and triiodothyronine have different effects on the pituitary and on other organs, such as the liver and kidneys.[106] Therefore, suppression of the TSH response to TRH may not indicate thyrotoxicosis. TSH secretion is also suppressed in patients with trophoblastic tumors, such as hydatidiform moles or choriocarcinomas. These tumors produce large amounts of hCG, which shares an alpha subunit with TSH, and 1.0 U of hCG is equivalent in thyroid-stimulating activity to approximately 0.3 uU of TSH.[70] Whether thyroid stimulation is due to hCG stimulation or to a substance that is isolated with the hCG peak is not clear.[2, 40] TRH can be produced by the placenta, which raises the possibility that trophoblastic tumors could cause hyperthyroidism in this manner.[174] No documented evidence of ectopic TSH secretion from tumors has been reported, although human lymphocytes have been reported to produce TSH.[97]

Inappropriate Secretion of TSH

There is a heterogeneous group of disorders in which the serum TSH concentration is inappropriately high in the presence of an elevated free thyroid hormone concentration.[98] The patient may have variable degrees of pituitary and peripheral resistance to thyroid hormone action or may harbor a pituitary thyrotropic tumor (Table 3–2). The condition is rare and may be missed, unless the possibility is considered.

Differentiation among patients with inappropriate TSH secretion is important for optimal therapy. Patients with generalized resistance to thyroid hormone need thyroid hormone replacement not thyroid ablation despite elevated serum thyroid hormone concentrations. Distinguishing between those with and those without pituitary neoplasm is important in patients with TSH-induced hyperthyroidism.[97] In general, neoplastic production of TSH appears to be autonomous, i.e., unresponsive to both thyroid hormone suppression and TRH stimulation. TSH secretion is suppressed by glucocorticoids, much less reliably with somatostatin or dopamine.[15] The sella turcica is usually enlarged, and other pituitary hormone concentrations may be increased.[179]

Determination of serum concentrations of individual alpha and beta subunits of TSH has been helpful in differentiating patients with tumors.[97] Patients with TSH-producing pituitary tumors have had markedly elevated serum alpha subunit concentrations relative to elevated serum TSH concentrations but undetectable beta subunits. Nontumorous patients, in contrast, have a predominant increase in TSH secretion with a modest increase in serum

Table 3–2. Inappropriate Secretion of Thyroid-Stimulating Hormone*

- I. Neoplastic production of thyroid-stimulating hormone (TSH)
  - A. Pituitary tumors
    1. Not associated with hypersecretion of other hormones
    2. Associated with hypersecretion of other hormones
  - B. Nonpituitary tumors (ectopic production)
- II. Non-neoplastic pituitary hypersecretion of TSH
  - A. Target organ resistance to thyroid hormone
    1. General-peripheral tissues and pituitary
    2. Pituitary
  - B. Abnormal stimulation of TSH secretion
    1. By thyrotropin-releasing hormone
    2. By other stimulators
  - C. Defective suppression of TSH secretion
    1. By somatostatin
    2. By dopamine
    3. By other suppressors

*From Weintraub, B. D., et al.: Inappropriate secretion of thyroid-stimulating hormone. Ann. Intern. Med. 95:339–351, 1981, with permission.

alpha and beta subunit concentrations.[204] The molar ratio of alpha subunits to TSH may be helpful in distinguishing tumor from other causes of inappropriate TSH secretion. Patients with pituitary tumors have an alpha subunit to TSH molar ratio greater than one, whereas in hyperthyroid nontumorous patients, the ratio is less than one (Table 3–3). However, the ratio was not helpful in differentiating apparently euthyroid nontumorous patients who had normally elevated TSH values and relatively less pituitary resistance.

Several of the patients with pituitary tumor have undergone hypophysectomy and radiotherapy with a subsequent decrease in TSH and remission of hyperthyroidism.[97] In general, the subunit responses have been concordant with the TSH responses, although the alpha subunit may be more helpful in determining the presence of residual tumor. Serum alpha subunit concentrations have also been elevated in patients with gonadotrophin-secreting pituitary tumors and in some patients with chromophobe adenomas. Some caution must be used in interpreting serum alpha subunit values since the alpha subunits of all pituitary glycoproteins are immunologically identical.[138]

Patients without pituitary tumors present more of a therapeutic dilemma. Many of these patients with peripheral resistance appear to benefit from continuous administration of L-thyroxine.[97] Such therapy tends to reduce the serum TSH concentration as well as the goiter. Patients with selective pituitary resistance present the most difficult problem in therapy. They are thyrotoxic, and decreasing serum thyroid hormone concentrations, e.g., antithyroid drug therapy and radioiodine, seems reasonable. However, such reductions invariably result in a significant increase in goiter size and a risk of pituitary hyperplasia.

Table 3–3. TSH and Alpha Subunit Concentrations in Patients with TSH-induced Hyperthyroidism*

	TSH (mU/L)	Alpha Subunit (μg/L)
Normal	<6.0	0.5–2.5†
Graves' disease	0.5	—
Tumor	1.7–88	12.5–105
Nontumor	9.3–160	0.5–5.2

*Modified from Kourides, I. A.: Pituitary thyrotropin secretion in thyroid disorders. Thyroid Today 3:2, 1980, with permission.

†Postmenopausal women have serum alpha subunit concentrations between 1.0 and 7.0 ng/ml.

TSH Biosynthesis

TSH purified by chromatographic procedures contains several closely related biologically active components, ranging from 28,000 to 30,000 daltons. The differences presumably are due mainly to the heterogeneity of the attached oligosaccharide groups.[206]

Chemistry. TSH is composed of two dissimilar, noncovalently bound subunits, alpha and beta.[40] The hormone is related chemically to the pituitary gonadotrophins, LH, and FSH, and to the placental gonadotrophins, chorionic gonadotrophin. Within a species, the alpha subunit from each of these glycoproteins is virtually identical in linear sequence but differs significantly in the carbohydrate moieties.[137, 157] The alpha subunit of TSH has 96 residues with a molecular weight of 14,000. The beta subunit of TSH has 110 amino acids with a molecular weight of 15,000, appears to be unique, and confers immunologic and biologic specificity. Free beta subunits are virtually devoid of receptor binding and biologic activity and require combination with an alpha subunit derived from any of the hormones to become active.[137] Alpha subunits alone are also biology inactive. The TSH molecule contains approximately 16% carbohydrate. Three oligosaccharide moieties are attached to the peptide sequence by asparagine residues. Two are found on the alpha subunit and one on the beta. The role of the carbohydrate is unknown, but the initial glycosylation with mannose enhances the conformation necessary for the alpha and beta subunit combination.[206]

The final complex structure of the secreted TSH carbohydrate seems to be important in determining the intrinsic biologic activity as well as the metabolic clearance rate of circulating TSH.[31a] Some patients with idiopathic central hypothyroidism may be secreting biologically inactive TSH.[8] There is some evidence to suggest that the carbohydrate content may modify the biologic to immunologic ratio of activity.[87]

Synthesis. Biosynthetic studies indicate that TSH is initially synthesized as separate prealpha and prebeta subunit precursors.[28, 101] These precursors are not synthesized in a single chain that contains propeptides such as proinsulin and proparathyroid hormone, but do contain amino-terminal hydrophobic "signal" peptides needed for the vectorial discharge of nascent

alpha or beta subunit peptides into the rough endoplasmic reticulum.[101] These signal peptides are cotranslationally in the intact thyrotroph cell. Subsequent post translational processing involves the asparagine-linked carbohydrate units, which are important in subunit combination.[63, 205] Alpha and beta subunits are synthesized by translation to separate messenger RNA (mRNA) molecules. Recombinant DNA methodology has been used to clone DNA molecules complementary to alpha and beta TSH mRNA.[198] Alpha subunits are synthesized in molar excess whereas the beta subunits appear to be limiting in TSH biosynthesis.[67] The glycosylated alpha and beta subunits are paired into a loose complex, either in secretory granules or at the plasma membrane.[87] The stable active dimer is slowly formed with formation of five disulfide bridges in the alpha subunit and six in the beta subunit.[78] Triiodothyronine administration rapidly decreases the transcription of both the alpha subunit and TSH beta genes. The transcription of the TSH beta subunit gene is affected to a greater extent than the alpha subunit gene.[175a]

HYPOTHALAMIC-PITUITARY-THYROID AXIS

TSH secretion and thyroid hormone production are coordinated so that the supply of thyroid hormone is adjusted to meet the needs of the peripheral tissues. Increasingly, the complexity of this relationship has been appreciated. A number of layers of control have been built upon the system, the classic negative feedback between the thyroid and pituitary.[77] Modulation by TRH as well as by somatostatin, estrogens, and catecholamines also plays an important role.

Pituitary Feedback Control

How thyroid hormone actually alters TSH secretion at the level of the pituitary is of major physiologic and clinical interest. Although the implicit assumption has been made that the major interaction occurred in the pituitary, realization that the nervous system influenced TSH secretion has forced reconsideration of this hypothesis.

Overwhelming evidence indicates that the pituitary is intrinsically capable of an appropriate TSH response to altered thyroid hormone concentrations. While small increases in circulating thyroid hormone concentrations reduce the serum TSH concentrations, small decreases in circulating thyroid hormones elevate the circulating TSH.[129, 160] The plasma TSH concentration is a curvilinear function of the serum T_4 concentration.[34, 151] However, with severe thyroid hormone deficiency, thyroid hormone replacement initially will result in increased TSH synthesis.[134, 201]

Administration of either thyroxine or triiodothyronine to patients with thyroid failure reduces the serum TSH concentration.[151] This effect may be extremely rapid with a decrease in serum TSH occurring 1 to 2 hours after an infusion of T_3 (Fig. 3–4). Thyroxine infusion will also suppress TSH but ten times the dose of T_3 is required, and serum TSH concentration remains suppressed about ten times as long. Acute administration of thyroid hormone suppresses TSH release, whereas long-term administration suppresses TSH synthesis.[39, 71] Even though the serum TSH level falls significantly 1 hour after the administration of triiodothyronine, the pituitary TSH concentration actually increases.[178, 187] The mechanism is not clearly understood but probably involves TRH at some level. Tonic stimulation of TSH release by TRH is necessary to maintain the elevated concentrations of serum TSH characteristic of primary hypothyroidism. Thyroid hormones might regulate TSH acutely by an effect on TRH or by interference with the pituitary response to TRH. This acute suppression of TSH secretion is blocked by compounds

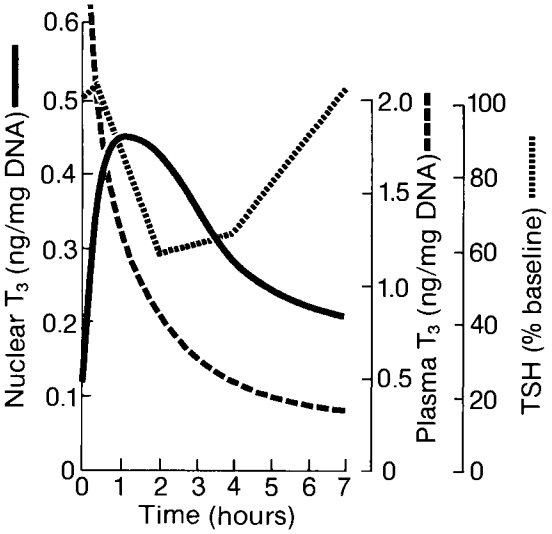

Figure 3–4. Pituitary nuclear T_3 and plasma T_3 and thyroid-stimulating hormone (TSH) concentrations after T_3 administration.

that inhibit protein synthesis.[197] Such an effect would presumably occur at the nucleus and is consistent with the hypothesis that induction of a protein is required for T_3 to suppress TSH secretion. However, the effects are very rapid.[6]

Thyrotrophs appear to respond to physiologic doses of L-thyroxine only after conversion to triiodothyronine, mainly in the pituitary cell.[26, 177] Conversion of thyroxine to triiodothyronine has been demonstrated *in vitro* in the pituitary in both somatotrophs and thyrotrophs.[164] Triiodothyronine inhibits DNA synthesis and cell division in thyrotrophs and stimulates somatotrophs.[6] The converse occurs in hypothyroidism.[5] These data are consistent with the hypothesis that binding of triiodothyronine to nuclear receptors in the thyrotroph is important in the acute suppression of TSH release. The findings also offer an explanation for why serum T_4 concentrations better reflect TSH suppression than serum T_3 concentrations. Decreases in circulating serum T_4 concentrations would result in an increase in serum TSH concentrations, which would increase secretion of T_3 by the thyroid and provide a source of thyroid hormone for peripheral tissues.

Role of the Hypothalamus

When the pituitary gland is separated from the median eminence of the hypothalamus, TSH secretion and thyroid function decrease. The hypothalamus has been postulated to determine the "set point" of feedback control around which the feedback regulating responses are elicited. Whether thyroid hormones regulate TSH secretion by a direct effect on the hypothalamus is not clear. In animal studies with the pituitary transplanted under the renal capsule, implants of L-thyroxine in the hypothalamus have resulted in a decreased circulating thyroid hormone concentration.[89] TRH antibody causes a decrease in serum TSH concentration.[9] Injection of triiodothyronine into the hypothalamus of thyroidectomized monkeys results in a decrease in the serum TSH concentration.[194]

TRH stimulation of pituitary cells results in an increased efflux of calcium, most likely due to redistribution.[60] Pretreatment with triiodothyronine partially inhibits this increase in calcium efflux.[5] Extracellular calcium is required for TRH to induce TSH release, and triiodothyronine could inhibit calcium uptake.[172] The suppression of TRH-induced TSH release by triiodothyronine does not occur immediately and may not be mediated through nuclear receptor binding.

Thyrotropic Area. Ablative studies as well as electrical stimulation and assays for TRH have been used to map the thyrotropic regions of the hypothalamus. The medial basal hypothalamus has been found to be the anatomic region of the hypothalamus involved in TSH regulation.[21, 111] There is a concentration gradient of TRH from the dorsal hypothalamus to the median eminence, which reflects the convergence of TRH-containing nerve fibers towards the pituitary stalk region. The preoptic region is the most anteriorly situated TSH regulatory area and of special interest because temperature sensitive neurons are located in this region. This region of the brain has been thought to integrate all visceral homeostatic mechanisms that maintain body heat and cooling. Some extrahypothalamic regions, including the limbic system, the habenular nucleus, and the globus pallidus of the basal ganglia, have also been implicated in TSH secretion.[150]

Hypothalamic Function in Regulation of TSH Secretion

In 1955, Sir Geoffrey Harris hypothesized that substances produced by nerve cells of the hypothalamus travelled via a capillary plexus to the anterior pituitary.[73] This portal-vessel chemotransmitter hypothesis is now well established. The median eminence of the hypothalamus is a specialized region of the brain that contains fenestrated capillaries that permit passage of large molecules to and from the blood and interstitial space. Hypothalamic neurosecretory cells are activated by neurotransmitters released at synaptic connections from the various afferent neurons that converge on these cells. The neurotransmitters stimulate the cells to secrete one or more of the various releasing or inhibiting factors that regulate pituitary secretion of TSH. These peptidergic neurons within the central nervous system (CNS) may have either neurotransmitter or "neuromodulatory" functions. TRH is released from hypothalamic synaptosomes by depolarizing stimuli.[199] Somatostatin, norepinephrine, and dopaminergic agents have also been reported to increase TRH release from the hypothalamus, but the results have been inconsistent.[75, 110]

THYROTROPIC RELEASING HORMONE

The identification of TRH in 1969 from 50 tons of hypothalamic fragments obtained from 300,000 sheep was a landmark in neuroendocrinology. This tripeptide (Fig. 3–5) was the first of the hypothalamic hormones to be chemically identified, synthesized, and administered to human beings.

Actions of TRH in Normal Individuals

The intravenous administration of TRH is followed by a prompt increase in serum TSH concentrations that reach their peak within 15 to 45 minutes after TRH and then decline to normal in 1 to 4 hours (Fig. 3–6).[184] Although increments in serum TSH levels have been reported to be proportional to the dose of TRH over a range of 15 to 500 μgm, little difference in response has been seen beyond 100 to 200 μgm of TRH. The TSH response to maximal doses of TRH is similar in children and young adults and slightly less in older men.[185] Responses are slightly greater in women than in men, particularly in women during the preovulatory phase.[165] However, evaluations related to age and sex are overshadowed by substantial variation, up to 40%, in peak TSH responses to TRH after repeated tests in the same individual as well as in the whole range of responses found in normal subjects.[166] The TSH response to TRH is one of the few instances in which normal values have been established for response to the hypothalamic releasing hormones. Twenty-two young men were given 500 μmg of TRH on two separate occasions. The mean individual variability of the response was 17% but was as high as 63% in individuals. Peak serum TSH

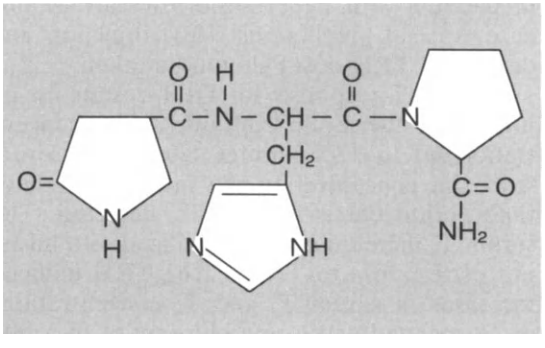

Figure 3–5. Thyrotropin-releasing hormone (TRH) structure.

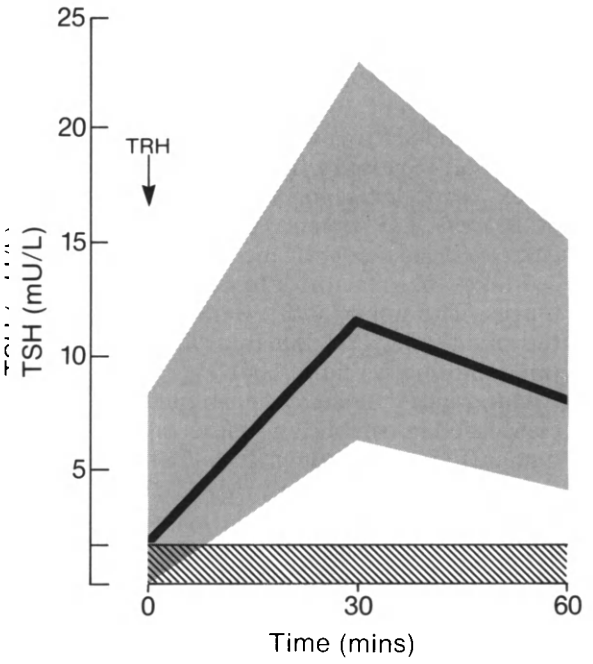

Figure 3–6. Normal thyroid-stimulating hormone (TSH) response to thyrotropin-releasing hormone (TRH). (Redrawn from Jackson, I.M.D.: Thyrotropin-releasing hormone. N. Engl. J. Med. 306:145–155, 1982, with permission.)

response to 500 μmg TRH was 2.7 to 19.5 mU/L. The males' peak TSH responses between 2 and 5 mU/L on one occasion were all greater than 5 mU/L on another. Determination of the peak incremental TSH response to TRH had no clear advantage over that of the absolute peak values. The TSH value obtained 30 minutes after TRH provided as much information as multiple values. Despite the variation, a peak value of greater than 5 mU/L indicated normal TSH response, whereas less than 2 mU/L indicated TSH deficiency and between 2 and 5 mU/L required retesting (Table 3–4). These values are considerably lower

Table 3–4. TSH Response to TRH*

	Peak TSH Value (mU/L)
Normal	>5
TSH deficiency	<2
Retest	2–5

*From Sawin, C. T., and Hershman, J. M.: The TSH response to thyrotropin-releasing hormone (TRH) in young adult men: Intra-individual variation and relation to basal serum TSH and thyroid hormones. J. Clin. Endocrinol. Metab. 42:809–816, 1976, with permission.

than most clinicians use for normal values and emphasize the need to establish normal ranges.

The TSH response to 200 μgm of TRH was determined in 131 normal subjects.[46] The change in TSH was significantly influenced by the basal TSH concentration and the free T_4 index. The change in TSH was the same in both men and women under 40 years but decreased in men with increasing age. Figure 3–7 takes these factors into account for normal ranges. The upper and lower limits vary with the basal TSH and the free T_4 index (FT_4I) (mean normal value of 100).

Differences in assay technique should be considered in establishing these values. Use of human TSH standard (MRC 68/38) rather than the human TSH standard (MRC 63/14) results in about a 30% decrease in measured serum TSH concentrations.[166] A delayed fall in the serum TSH concentration after TRH has been thought to represent a hypothalamic abnormality but occurred in 12 of 22 of these male subjects and should be considered a normal variant. The most significant correlations found were between basal and peak TSH values. TRH effectively increased the sensitivity of TSH in the lower range. TRH is also effective when taken orally, but doses 20 to 40 times higher are needed to produce an equivalent effect on TSH secretion. The increased amount is due to absorption or degradation in the gastrointestinal tract or liver. Repeated oral doses will also increase the serum T_3 concentration and radioactive iodine uptake by the thyroid. The latter effect has been identified in the treatment of thyroid carcinoma. However, the cost of the required large amounts of TRH makes the oral route impractical at present.

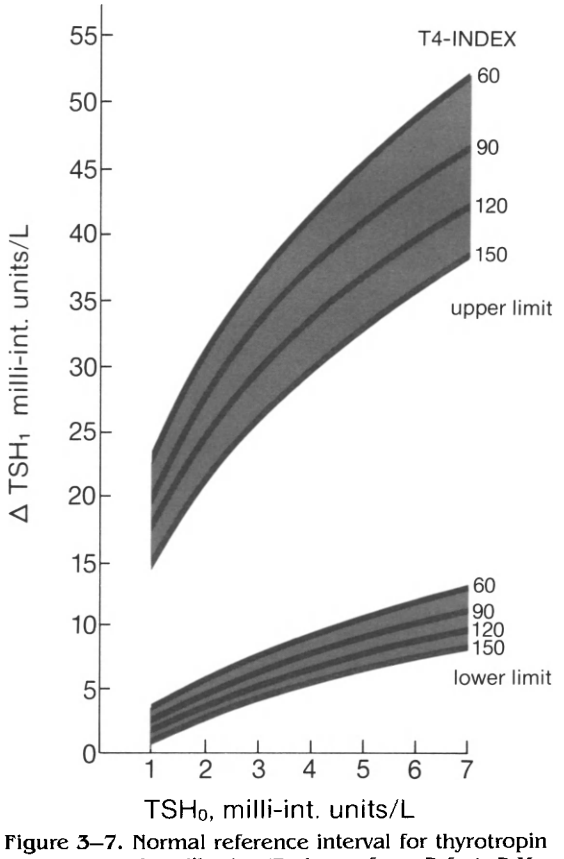

Figure 3–7. Normal reference interval for thyrotropin response to thyroliberin. (Redrawn from Erfurt, E.M., et al.: Normal reference interval for thyrotropin response to thyroliberin. Clin. Chem. 30:196–199, 1984, with permission.)

TRH has been given as part of a "diagnostic cocktail" to test pituitary function. A combination of TRH, 400 μgm, luteinizing hormone–releasing hormone (LH-RH), 100 μgm, and insulin, 0.1 U/kg, allows assesment of all six anterior pituitary hormones simultaneously. The interaction among the hormones does not affect the TSH response, although the prolactin and growth hormone response may be affected.[115] However, the convenience and cost effectiveness make this the procedure of choice for routine clinical evaluation.

Side effects occur in two thirds of subjects administered TRH. These effects are transient and quite mild, lasting 1 to 2 minutes.[3] The suggestion has been made that the side effects are all dose related.[68] The most common include mild nausea, urinary urgency, light headedness, sensation of facial flushing, and a peculiar taste sensation.[11] There may be a slight transient rise in systolic and diastolic blood pressures. All patients undergoing the TRH test should have the blood pressure measured; in patients with hypertension or cardiac disease, special precautions (slow injection and decreased TRH dose) should be taken.[18, 216]

The TSH response to TRH results in an increase in the serum triiodothyronine concentration 60 to 120 minutes later. The serum thyroxine concentration may increase at a later time period but is less readily detected. The serum T_3 increase has been utilized as a measure of response to TRH.[30] The TRH-induced increases in serum T_3 and T_4 concentrations are associated with some blunting of the TSH response to subsequent administration of TRH.[6, 186] Repetitive testing at 3-day intervals

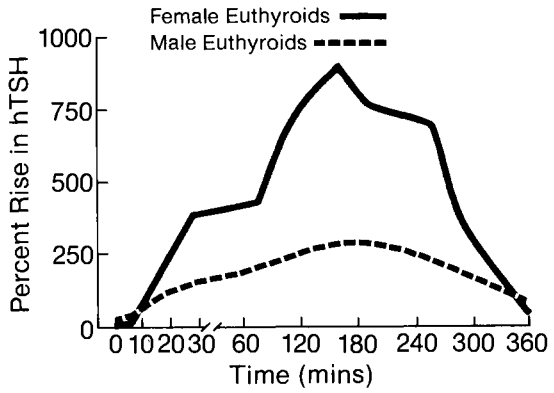

Figure 3–8. Biphasic thyroid-stimulating hormone (TSH) response to thyrotropin-releasing hormone (TRH) infusion. (Redrawn from Chan, V., Wang, C., and Yeung, R.T.T.: Thyrotropin: α and β subunits of thyrotropin and prolactin responses to 4 hour constant infusions of TRH in normal subjects and patients with pituitary-thyroid disorders. J. Clin. Endocrinol. Metab. 49:127–131, 1979, with permission.)

results in a 20% decrease in basal and peak serum TSH concentrations.[186]

The bolus injection of TRH causes a peak TSH response followed by a gradual delay. In contrast, a constant infusion of TRH results in a biphasic TSH response (Fig. 3–8).[20, 25] This biphasic response pattern has been interpreted as evidence for the existence of two TSH pools. The first pool is readily releasable and represents the presynthesized TSH, whereas the second requires longer stimulation and perhaps synthesis before release. Data from hypothyroid patients may also be consistent with a biphasic response with a higher initial pool.[201]

Alpha subunits of TSH also increase after TRH stimulation administered as bolus injection to normal subjects.[43] No increase in beta subunit secretion was noted in these individuals. In hypothyroid patients, increases were found in both alpha and beta subunits after administration of a TRH bolus. However, in response to a constant infusion of 0.4 μgm/min for 4 hours of TRH, normal subjects exhibited biphasic patterns of both alpha and beta subunits.[25] Interestingly, both subunits increased in the serum more rapidly than TSH, indicating direct secretion from the pituitary.

Factors Altering the TSH Response to TRH

Changes in thyroid hormone concentration can significantly alter the TSH response to TRH. Small doses of T_3 or T_4 result in a significant diminution in the TSH response even though serum thyroid hormone concentrations remain within the normal range. The serum TSH response to TRH decreased below normal (maximum change in TSH of 2 mU/L) after L-thyroxine 50 μgm/day for 2 weeks.[167] Even single doses of triiodothyronine resulted in inhibition of the TSH response to TRH 1 to 3 days after the T_3 administration and long past the peak value in the serum.[178] Significant increases in the TSH response are produced by slight reductions in serum thyroid hormone concentrations produced by administration of iodide to normal subjects.[196]

Glucocorticoids

A number of other agents alter the TSH response to TRH but none approximate the potency of thyroid hormones in this regard (Table 3–5). Glucocorticoids suppress TSH secretion from the pituitary and blunt the TSH response to TRH (Fig. 3–9).[147] Pharmacologic doses of dexamethasone (8 to 16 mg) for several days produce a marked decrease in the TSH production rate without a significant change in the metabolic clearance rate. Therefore, the reduction in the serum TSH concentration is due to a decreased pituitary secretion rather than a peripheral effect. Glucocorticoids also decrease the TSH reserve in the pituitary.[131] The dose and timing of the dexamethasone administration are important with effects on the hypothalamus or pituitary.[145] Although dexamethasone suppresses the serum TSH concentration in hypothyroidism, dexamethasone, 2 mg daily for 3 days, failed to inhibit the TSH response to TRH in six hypothyroid

Table 3–5. Factors Influencing the TSH Response to TRH*

Decrease	Increase
Thyroid hormone	Thyroid hormone
Dopamine agonists	Estrogens
Somatostatin	Dopamine antagonists
Glucocorticoids	Pseudohypoparathyroidism
Growth hormone	Cimetidine
Illness	
Starvation	
Renal failure	
Depression (?)	
Calcitonin	
Verapamil	

*Modified from Wood, D. G. and Samols, E.: Thyroid-stimulating hormone. In Rothfeld, B. (ed.), Nuclear Medicine in Vitro, 2nd edition. Philadelphia, J. B. Lippincott Co., 1983, with permission.

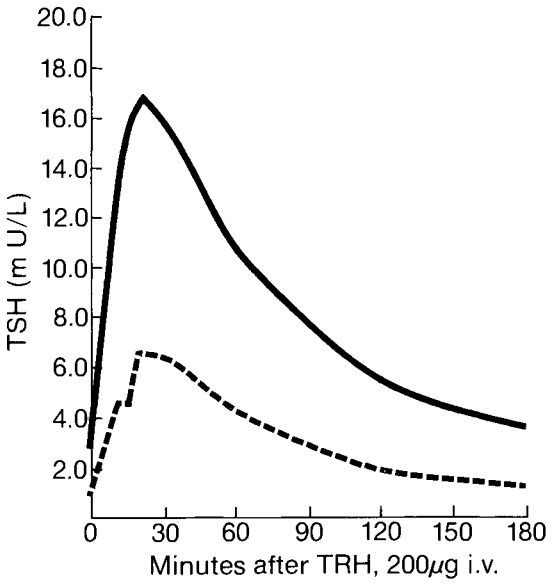

Figure 3–9. Effects of dexamethasone on thyroid-stimulating hormone (TSH) response to thyrotropin-releasing hormone (TRH) in mammals. Response in eight normal subjects before (solid line) and after (broken line) dexamethasone. (Redrawn from Kourides, I.A., et al.: The effect of glucocorticoid administration on human pituitary secretion of thyrotropin and prolactin. J. Clin. Endocrinol. Metab. 43:338–346, 1976, with permission.)

patients.[42] Although pharmacologic doses of dexamethasone appear necessary to inhibit the TSH response to TRH, basal serum TSH concentrations increase in normal subjects after the lowering of plasma cortisol concentrations with metyrapone.[147]

Dopaminergic agonists also play an inhibitory role in the control of TSH secretion with a decreased TSH response to TRH.[13, 33] Basal serum TSH as well as alpha and beta subunits promptly declines in response to intravenous dopamine, 4 μgm/kg.[32] The alpha subunits did not decrease in proportion to the decrease in TSH and beta subunits, which may reflect the presence of an additional gonadotroph pool for the alpha subunit. The dopamine agonist bromocryptine also has been reported to suppress the TSH response.[214] Conversely, administration of dopamine agonists, such as metoclopramide and domperidone, increases the serum TSH concentration.[141, 170]

The failure of domperidone to cross the blood-brain barrier suggests that the dopaminergic inhibition of TSH occurred at the level of the anterior pituitary or median eminence. The blood-brain barrier is an elusive concept and depends on the extent of penetration of trypan blue.[41] Nevertheless, dopamine concentrations in the pituitary portal blood are high (10^{-7} M), and dopamine receptors are present in the anterior pituitary. These data suggest that TSH is under tonic dopaminergic control, probably at the pituitary level. The suggestion has also been made that this dopaminergic inhibition might be modulated by the thyroid hormone concentration.[169] Although animal studies have shown an effect of norepinephrine on TSH release, no consistent effect of alpha or beta adrenergic agonists or antagonists on the TSH response to TRH has been found in human beings.[94, 156]

Somatostatin may also play a physiologic role in the inhibition of TSH release. Infusion of this tetradecapeptide blocks the TSH response to TRH and lowers the elevated serum TSH concentrations in hypothyroid patients.[108, 176] Studies with somatostatin antibodies demonstrated increased basal and peak serum TSH responses to TRH.[51] Thyroid hormones may play a role in modulating somatostatin. Hypothalamic somatostatin content was decreased in hypothyroid rats and restored to normal by triiodothyronine administration.[10] The role of somatostatin in the inhibition of TSH may be a factor in observations that growth hormone deficient children exhibit heightened TSH responses to TRH whereas in acromegalics the response is blunted.[31, 140]

A number of other monoamines and peptides have been implicated in TSH secretion and TRH stimulation but the findings in general have been ambiguous. These include (1) serotonin,[86] (2) neurotensin,[109] (3) opioid peptides,[88] (4) melatonin,[65] and (5) cholecystokinin.[124] Estrogens induce an increased responsiveness of TSH secretion to TRH, which is accompanied by an increase in TRH receptor concentration in the pituitary.[23] The TSH response to TRH is slightly greater in women than in men and particularly in women during the preovulatory phase when estrogens are highest. Women on oral contraceptive steroids, receiving pharmacologic doses of estrogens, have higher TSH responses to TRH than controls.[144]

TRH Stimulation of Other Pituitary Hormones

TRH action on the pituitary is not specific for TSH but consistently causes a release of prolactin (Fig. 3–10).[149] The peak increase in pro-

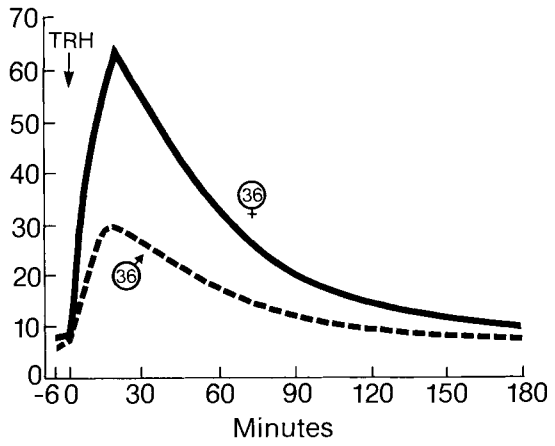

Figure 3–10. Prolactin response to thyrotropin-releasing hormone (TRH) in normal subjects. (Redrawn from Jacobs, L.S., et al.: Prolactin response to thyrotropin-stimulating hormone in normal subjects. J. Clin. Endocrinol. Metab. 36:1069–1973, 1973, with permission.)

lactin with a twofold or greater rise occurs between 15 and 30 minutes after TRH administration. Whether TRH plays a physiologic role in the control of prolactin secretion in men is not clear. Certainly, the role is not major in the regulation of prolactin secretion.[56, 72] Nevertheless, inappropriate lactation with mildly elevated serum prolactin concentration may occur in primary hypothyroidism.[195] The galactorrhea and hyperprolactinemia usually respond to thyroid hormone therapy. The effects of thyroid hormone on the prolactin response to TRH parallel the effects of the TSH response, and both responses are blunted in hyperthyroid patients.[183] However, the prolactin response is not inhibited to the same degree by a given dose of thyroid hormone.

Dexamethasone, which blunts the TSH response to TRH, had no effect on basal prolactin, peak prolactin response after TRH, or prolactin reserve in the pituitary.[43] A blunted prolactin response following TRH also occurs with prolactinomas and has been used to separate prolactinomas from other causes of hyperprolactinemia.[35, 92] However, the test lacks specificity, and TRH has not been as helpful as first thought.

The suggestion has been made that hyperprolactinemia stimulates hypothalamic dopaminergic activity via a short loop, positive prolactin feedback effect. Administration of dopaminergic antagonists to patients with prolactinomas resulted in exaggerated TSH responses consistent with the hypothesis of increased dopaminergic inhibition of TSH release in these patients.[143, 168]

In addition to stimulating prolactin release, TRH causes release of growth hormone in about 50% of acromegalics. TRH does not stimulate growth hormone secretion in normal subjects, however. In fact, TRH prevents the sleep-induced increase in plasma growth hormone concentrations, which suggests that it may have a predominant inhibitory effect on growth hormone release. However, when the central peptidergic or amnergic pathways are disrupted, growth hormone release is stimulated. Whether this TRH effect is due to altered receptors on the somatotroph is not clear.[82] TRH may also stimulate growth hormone release in other conditions, such as anorexia nervosa, renal failure, and liver disease.[121] TRH may also stimulate adrenocorticotropic hormone (ACTH) release in some patients with Cushing's disease and Nelson's syndrome but not in normal individuals.[27]

Metabolism and Assay of TRH

The plasma clearance rate of TRH is extremely rapid with a plasma half-life of 6.2 minutes and a mean plasma clearance of 1500 ml/min.[123] Comparison of the *in vivo* clearance rate of TRH with the *in vitro* destruction rate suggested that intravascular degradation is responsible for about one third of the plasma clearance. The enzymatic breakdown in tissues and body fluids leads to the formation of fragments, which appear to be biologically active.[84, 113] As might be expected, thyroid status modifies the plasma clearance of TRH by effects on the rate of plasma degradation.

The assay of TRH in human serum has been difficult because of rapid degradation of the TRH and low circulating levels compared with the assay's sensitivity, and values in human peripheral blood are controversial, but normal values have been reported between 5 and 200 pg/ml.[85, 103] Human cord blood has been reported to have low TRH-degrading activity.[125] Immunoreactive TRH has been reported in human urine, but this finding is also controversial.[14] The ability to determine TRH in the systemic circulation could be used as a direct measure of hypothalamic function. Unfortunately, the wide distribution of TRH and dilution of the portal vessel blood in the systemic circulation mitigate against the determination of TRH in peripheral blood as a measure of hypothalamic function. Direct assay of pitui-

tary portal vessel blood would be useful but is inaccessible.[207]

Clinical Studies

The availability of TRH has allowed the testing of TSH reserve as well as evaluation of pituitary function. The serum TSH concentration in thyrotoxicosis from whatever causes is low and does not respond to TRH stimulation. Occasionally, there is a small blunted TRH response. The TSH response may return to normal with control of the hyperthyroidism but may remain abnormal for prolonged periods. Patients with Graves' disease who are euthyroid may also have decreased responses to TRH,[29] which implies that circulating thyroid hormone is increased although within the normal range. Similar results may also be found in patients with autonomous thyroid nodules.[22, 58] The majority of these patients do not have thyroid suppression of the radioisotopic thyroid uptake in response to thyroid hormone administration.

Although both the TSH response to TRH and the triiodothyronine suppression of the radioisotope thyroid uptake should measure similar effects, the results of the two tests may be disparate in Graves' disease patients in remission (Table 3–6). Various patterns of TSH responses to TRH are found during and after the treatment of thyrotoxicosis. There tends to be a delay between the return of thyroid hormone concentrations to normal and the return of TSH response to TRH.[153] Subnormal TSH responses to TRH may even be found in some patients soon after treatment. Similar findings occur in euthyroid patients on thyroid hormone suppression following cessation of therapy.[102] Because the patterns of response are so different and may vary with time, little practical information is to be gained by testing such patients. In a patient with thyroid carcinoma in whom complete suppression of TSH is desirable, a TSH response to TRH would be an indication to increase the amount of exogenous thyroid hormone.[171] However, an ultrasensitive TSH assay may provide the same information.[104]

In a patient with hypothyroidism and hypothalamic disease in whom a defect in TRH stimulation might be expected, the TSH response to TRH is usually normal. Patients with hypothyroidism on the basis of intrasellar disease characteristically have low serum TSH concentrations with no response to TRH. Although these are the usual patterns of TSH response to TRH, they are not invariable. On the one hand, patients with significant intrasellar disease may have normal TSH responses to TRH. On the other hand, patients with presumed hypothalamic disease and hypothyroidism may have subnormal TSH responses to TRH. The variable response patterns in patients with hypothalamic or pituitary disease also suggest that no specific disorder, e.g., craniopharyngioma and prolactinoma, is likely to be associated with a characteristic TSH response to TRH. In a series of patients with a variety of significant hypothalamic and pituitary diseases, one third were hypothyroid and all had impaired TSH responses to TRH.[182] Among the euthyroid patients in the series, one quarter had abnormal TSH responses to TRH. In hypothalamic and pituitary disease, TRH testing may be helpful in defining a baseline before pituitary surgery, but otherwise there are no findings that define specific pathologic processes.

Table 3–6. TSH Response to TRH and T^3 Suppression Test in 20 Graves' Disease Patients in Remission*

TSH response†	N	AB	AB	N
T_3 suppression	N	AB	N	AB
No. of patients	7	6	5	2

*From Buerklin, E. M., Schimmel, M., and Utiger, R. D.: Pituitary-thyroid regulation in euthyroid patients with Graves' disease previously treated with antithyroid drugs. J. Clin. Endocrinol. Metab. 43:419–427, 1976, with permission.

†N = Normal response; AB = Abnormal response.

TRH OUTSIDE THE HYPOTHALAMUS

TRH and several other hypothalamic peptides have also been identified in other areas of the CNS and, in some instances, outside.[142] These peptides share the characteristics of wide distribution and a role other than hormonal.

Extrahypothalamic Neural Distribution of TRH

Although TRH concentrations are highest in the hypothalamus, more than 70% of total brain TRH is in other areas of the brain.[85] TRH has been identified in nerve terminals in brain stem motor nuclei as well as in spinal cord motor neurons. Significant concentrations of TRH have been found in the thalamus and cerebral cortex.[95] TRH has been identified in

the fetal cerebellum as early as the 9th week of gestation.[211]

Function of TRH in the CNS

Although TRH crosses the blood-brain barrier with difficulty, TRH does cause CNS effects after peripheral administration.[81] TRH directly affects the electrical activity of single neurons or influences the excitatory or depressant effects induced by norepinephrine and acetylcholine.[210] The location of TRH in motor nuclei suggests a role in the motor side of the nervous system. Although TRH has not been demonstrated to fulfill all the criteria for a true neurotransmitter, TRH may function as a neurotransmitter or neuromodulator.[33]

Extra CNS Distribution of TRH

TRH has also been found outside the CNS in the gastrointestinal tract and pancreas. In neonates, the concentration of TRH is higher in the pancreas than in the hypothalamus but this subsequently reverses.[44] Whether TRH plays some role in opposing the action of somatostatin in the pancreas is speculative.[112] TRH may be part of a diffuse neuroendocrine system, which migrates out of neural tissue to the endoderm during embryonic development.[133] TRH or TRH-like material has been identified in the human placenta, which has a common embryologic origin with neuroectodermal structures.[173, 174, 215]

TRH SYNTHESIS AND ACTION

TRH is released from hypothalamic synaptosomes by depolarizing stimuli.[199] High affinity, high specificity binding sites for TRH have been identified in the anterior pituitary and other areas in the CNS.[61, 104] These receptors are located on the plasma membrane and undergo down regulation.[69, 126] Initially, TRH was thought to act by stimulating cyclic adenosine monophosphate.[55] However, further studies have indicated that the interaction between TRH and the membrane receptor ultimately results in a major burst of free intracellular calcium ions through redistribution.[60] These effects are mediated by phospholipase C catalyzed by dialysis of phosphatidyl inositol to produce diacylglycerol and inositol triphosphate.[59, 79a] The elevation of calcium ions activates the movement of secretory granules to the cell surface and their fusion with the cell surface membrane. There is a parallel activation of protein kinase C by diacylglycerol that also leads to phosphorylation of proteins involved in exocytosis.

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PART III
THYROID HORMONE AND LABORATORY EVALUATION

4
Distribution and Metabolism of the Thyroid Hormones

JACK H. OPPENHEIMER

Although it is customary to separate the processes of distribution and metabolism, it should be emphasized that both occur simultaneously and both determine the instantaneous concentration of iodothyronines in plasma. Since the laboratory-based diagnosis of thyroid disease rests in large part on the concentrations of plasma hormones, an understanding of these factors assumes major clinical relevance. On the one hand, the term "metabolism" will be used in the sense of irreversible exit of iodothyronine from the body, whether this is accomplished via conventional biochemical transformation, such as deiodination or deamination, or simply excretion via the urinary or fecal route. The term "distribution," on the other hand, will refer to those reversible processes that operate to determine the partition of hormones between the various constituents of plasma, between plasma and various cellular pools, and most importantly among the individual subcellular compartments. Viewed in this fashion, the concentration of hormone in plasma or in any given body compartment will be uniquely determined by the combined effects of distribution and metabolism.

DISTRIBUTION OF THYROID HORMONE

Iodothyronine Plasma Binding Proteins and Iodothyronines in Plasma

For the purpose of our discussion, we shall confine ourselves to the following two generally recognized thyroid hormones: L-thyroxine (T_4) and 3,5,3′-L-triiodothyronine (T_3). Recent studies from many laboratories have clearly shown the presence of other iodothyronines in plasma, including 3,3′,5′-L-triiodothyronine, the acetic acid analogues of T_4 and T_3, as well as trace quantities of various forms of diiodinated thyronines and deaminated and ether-cleaved products.[42] Nevertheless, currently available data do not suggest a major biologic function for these substances. In human serum, the estimated concentrations for T_4, from most laboratories, range from 5.0 to 11.0 μgm/dl (64.3 to 141 mM) and for T_3, 80 to 180 ngm/dl (1.27 to 2.76 mM). Further reports have quoted mean concentrations of rT_3 from 14 to 32 ngm/dl.[31, 55, 80, 91, 100, 109, 121, 149, 211] The concentrations of triac have been estimated to vary from 2.6 to 9.0, [56, 122] and that of tetrac, 8 ngm/dl.[33] The concentrations of the three diodothyronines (3,5-T_2,3,3′-T_2,

and 3′,5′-T_2) are probably less than 8 ngm/dl,[42] whereas the concentration of 3′-monoiodothyronine has been reported to be about 1.4 ngm/dl.[28]

Hormone Plasma Binding Proteins in Plasma

In 1957, the concept was first formulated quantitatively that the vast bulk of circulating T_4 and T_3 was reversibly bound to specific plasma proteins and was in a state of equilibrium with a small "free" or nonprotein-bound fraction.[160] At that time, the importance of T_3 in determining net thyroidal status had not yet been fully appreciated. Accordingly, the focus of scientific and clinical attention remained on T_4. The specific T_4-binding or "transport" protein had earlier been identified as thyroxine-binding globulin (TBG) by means of paper electrophoresis of serum to which tracer concentrations of ^{131}I-T_4 had been added.[63, 104, 161] A major radioactive peak appeared in the inter-alpha globulin area. Approximately 85% of the total tracer was bound to this protein, with the remaining 15% bound to serum albumin. By eliminating barbital from the electrophoretic buffer, an additional protein component became apparent, thyroxine-binding prealbumin (TBPA most recently redesignated transthyretin), which exhibited an electrophoretic mobility anodal to that of albumin.[85] Although electrophoretic studies had originally suggested that as much as 30% of total circulating T_4 was bound to TBPA,[132] subsequent studies involving immunoprecipitation revealed that only approximately 15% of total T_4 is bound to this protein fraction.[160] Approximately 75% of circulation is associated with TBG and 10% with serum albumin. Electrophoretic studies suggested that about 70% of T_3 is also bound to TBG with the remaining 30% associated with serum albumin. Although electrophoresis had suggested that TBPA does not bind T_3,[217] more recent investigations have suggested that T_3 does bind to TBPA minimally.[105] Theoretic calculations based on the dissociation constants of T_3 to individual binding proteins have suggested a rather surprising distribution of T_3 among the established binding proteins. Thus, only approximately 38% of plasma T_3 was estimated to be bound to TBG, 27% to TBPA, and 35% to albumin.[146, 157]

TBG has been purified by use of affinity columns [140] followed by a multiple step procedure.[120] The molecule is a glycoprotein rich in sialic acid with a molecular weight of 55,000. Vigorous treatment of TBG with guanidinium hydrochloride has not revealed any subunits,[58] a conclusion that has been confirmed by *N*-terminal amino acid analysis.[23] A variety of physicochemical techniques, including circular dichroism, show that TBG is a compact molecule without evidence for attached subunits.[59]

The binding capacity of TBG is approximately 20 μgm/dl as determined by electrophoretic analysis with one binding site for T_3 and T_4 and an estimated affinity constant of 10^{10} M^{-1} for T_4 and an approximate concentration in plasma of 1.0 to 1.5 mgm/dl.[159] Under physiologic conditions, approximately one third of the sites are occupied. From studies of acutely isolated monkey hepatocytes, it appears highly likely that TBG is synthesized in the liver.[61] The liver also plays an important role in the removal of TBG from the circulation. Thus, the desialylated protein displays a characteristically slower anodal electrophoretic mobility and accumulates in the plasma of patients with hepatic disease.[120] Recent studies in normal volunteers injected with ^{125}I-labelled TBG have shown a plasma disappearance curve with a half-time of about 5 days, a distribution volume of 7 L, a metabolic clearance rate of 0.8 L/day, and a daily production rate of 17.8 mg (Table 4–1).[21]

The circulating concentration of TBG is strongly influenced by the levels of estrogens and androgens.[37, 41] The high level of TBG in pregnancy, approximately twice normal, is the consequence of a marked increase in estrogen production rate.[38] Conversely, patients treated with testosterone or related anabolic steroids exhibit a marked decrease in the plasma TBG content.[41, 43] However, no major differences exist between TBG levels in males and nonpregnant females, and the concentration is not age related.[159] The increase in TBG produced by estrogen is due to an increase in protein mass, not to an increase in binding affinity.

Table 4–1. Common Kinetic Parameters of Thyroid Hormone

	T_4	T_3
Total distribution volume (L/kg)	0.18	0.44
Metabolic clearance rate (L/kg/d)	0.013	0.32
Fractional plasma removal rate (d^{-1})	0.069	0.72
Half-time of plasma disappearance (d)	10.0	0.96
Residence time (d)	14.4	1.3

Studies with acutely isolated hepatocytes from estrogen-treated monkeys[60] and *in vivo* studies in these animals with ^{125}I TBG[62] support the concept that estrogen stimulates TBG production by the liver.

Pathologic stimuli may influence the level of TBG. Acute hepatitis may cause an increase[208] and hepatic cirrhosis a decrease in TBG.[83] TBG may also be elevated in acute intermittent porphyria.[77] Of particular importance from a clinical point of view is recognition of familial congenital decreases[154] and increases[87] in TBG binding capacity. These changes appear to be transmitted primarily as X-linked recessive traits. The impact of changes in TBG levels on the levels of total and free iodothyronines will be examined subsequently.

Historically, there has been a great deal of interest in TBPA. Amino acid sequence elucidation[88] and crystallographic studies[14–16] have allowed a virtually complete structural definition of this protein. TBPA has a molecular weight of 56,000 and consists of four identical subunits so arranged as to create a central channel at either end of which is situated a T_4-binding site (Fig. 4–1). There is a strong negative cooperative interaction between the two sites. When T_4 occupies one site, the binding site of the other becomes exceedingly weak. For this reason, early estimates had suggested only one binding site for T_4.[150] The protein has an effective binding affinity of 1×10^8 M, a binding capacity of approximately 300 μgm/dl, corresponding to a circulating protein concentration of 30 mgm/dl. TBPA also binds in a noncovalent manner the retinol-binding protein, a moiety of M_r 21,000.[150] Failure to dissociate this protein probably resulted in the initial overestimation of the molecular weight of TBPA.[150] Unlike TBG, TBPA contains no carbohydrate and is exceedingly stable, probably because of the high content of beta structure. Some of the properties of the thyroid hormone binding proteins are summarized in Table 4–2.

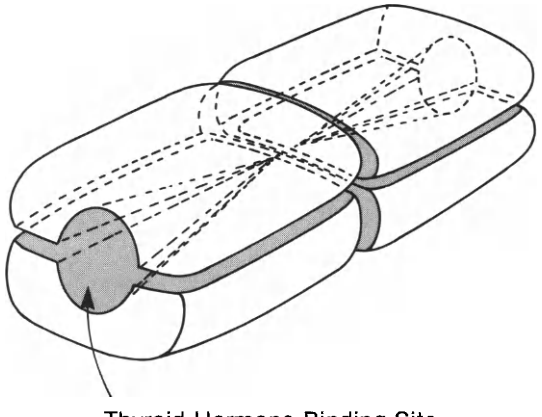

Figure 4–1. Schematic representation of the tetrameric structure of thyroxine-binding prealbumin (TBPA) protein. There are four identical subunits that create a central channel, narrowing towards the center. This narrowing creates two binding sites for T_4. A cooperative interaction diminishes the affinity for T_4 of the second binding site after the first is occupied. (Based on a modification of Apriletti, J.W., et al.[3a] of a computerized model presented by Blake, C.C.F. and Oatley, S.J.[15])

Initially, interest in TBPA was stimulated by the lability of the plasma protein concentration. A prompt decrease follows a variety of catabolic stimuli including nonspecific nonthyroidal illnesses and general surgery.[10, 132, 201] In severe illness, this binding protein rapidly disappeared from the circulation primarily as a consequence of a marked reduction of snythesis and a half-time of only 1 day.[135, 192] These findings suggested the possibility that a reduction in circulating TBPA resulted in an increase in the dialyzable fraction with a temporary increase in free T_4.[10, 132, 201] Subsequent studies that indicated only 15% of circulating T_4 is bound to TBPA, however, raised questions about the biologic impact of these changes on peripheral tissue metabolism.[217] The relatively small fraction of circulating T_4 normally bound to TBPA also makes it unlikely that a reduction in overall TBPA binding of T_4, noted in many patients with severe nonthyroidal illness, is due to a primary decrease in TBPA, as first supposed. This problem will be considered in greater detail in Chapter 6.

The detailed structural information available for TBPA prompted the suggestion that the surface of the TBPA molecule contains two depressions that are complementary to double-stranded helical DNA.[15] This similarity raised the possibility that TBPA could serve as a model for the interaction of T_3 with the nuclear receptor. Despite this intriguing structural analogy, there is little to suggest that TBPA or for that matter any of the plasma-binding proteins enter cells in significant quantities to interact directly with DNA. Similar to TBG, the volume of distribution of TBPA corresponds to that of serum albumin, a protein that is known to have an almost exclusively extracellular distribution.[135] Moreover, there are marked differences between the binding of

Table 4–2. Properties of the Thyroid Hormone Binding Proteins

	TBG	TBPA	Albumin
Concentration (mg/dl)	1.5	25	4200
T_4 binding capacity (μgm/dl)	20	300	"Unlimited"
Effective affinity constant (M^{-1})	1.7×10^{10}	2.3×10^{8}	6.2×10^{5}
Binding sites			
Strong	1	1	1
Weak	—	1	5
Polypeptide chains	1	4	1
CHO content (%)	23	6	6
M_r	54,000	54,000	66,000
Other functions	—	Associated with retinol-binding protein.	Oncotic pressure binding of other hormones, metabolites, and drugs.

analogues to TBPA and the nuclear receptor.[155]

Despite the remarkable evolutionary conservation of many components of the thyroid hormone system, there is considerable diversity among animal species with regard to the nature of the thyroid hormone binding proteins.[153] Thus, TBG and TBPA are restricted to the human and simian species. The binding proteins in the rat display distinctive physicochemical properties, though the net intensity of binding of iodothyronines by these species is quite similar. Serum albumin in all species, however, appears to play a role, even if minor, in the binding of T_3 and T_4. This protein is unusually suited to binding a broad spectrum of ligands, including hormones, metabolites, and drugs. The binding affinity of albumin for T_4 is relatively low compared with those of TBG and TBPA with an association constant of approximately 1×10^5 M.[193] However, since binding capacity is virtually unlimited, about 10% of circulating T_4 is bound to this protein.

The Multiple Equilibrium State

The original formulation of the free T_4 hypothesis held that unbound or free T_4 was in reversible equilibrium, with T_4 bound to the various iodothyronine-binding proteins (Fig. 4–2). Calculations suggested that the actual concentration of free hormone was exceedingly small compared with the total content of hormone in blood, perhaps on the order of 1/3,000 of the total.[158] Despite the small magnitude of free T_4 concentration in extracted fluids, the theory predicted that the free T_4 concentration would be more important as a determinant of the hormonal status of a tissue than the total plasma hormone concentration. Only the free hormone could penetrate the cells. Perturbation of plasma protein binding was postulated to result only in an evanescent change in the level of free circulating hormone but no changes in steady-state levels. Accordingly, for diagnostic purposes it appeared more relevant to assess the steady-state free rather than the total hormone concentration. Although important developments over the past 30 years have wrought basic changes in concepts of thyroid hormone distribution, metabolism, and action, the conceptual framework of the free thyroxine hypothesis has remained intact. This hypothesis also stimulated the acceptance of analogous interpretations of the plasma concentrations of other protein-bound hormones, metabolites, and drugs.

The fundamental relationship between free and bound hormone is provided by the law of mass action. Thus, if T_f = the concentration of free hormone in plasma; TP = the concentration of hormone bound to binding protein P; M = the total number of binding sites ("maximal binding capacity"); and K_a = the affinity

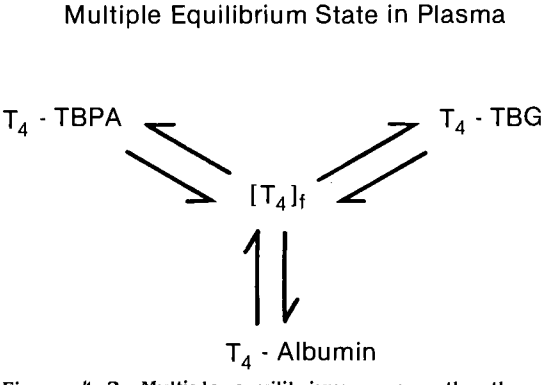

Figure 4–2. Multiple equilibrium among the three major binding proteins in serum (TBPA = thyroxine-binding prealbumin; TBG = thyroxine-binding globulin; $[T_4]_f$ = free thyroxine).

constant, the following relationships are apparent:

$$T_f + P \rightleftharpoons TP \quad (1)$$

$$T_f = \frac{TP}{(M - TP)K_a} \quad (2)$$

$$\frac{T_f}{TP} = \frac{1}{(M - TP)\,K_a} \quad (3)$$

For the sake of simplicity, the existence of only a single class of binding sites is assumed. The value in parentheses in equations 2 and 3 clearly represents the concentration of free binding sites (=P). The ratio of the free to bound hormone on the left side of equation 3 provides a measure of the overall intensity of binding of T_4 by plasma proteins. As indicated by the equation, this parameter will be inversely proportional to the product of the unoccupied binding sites and the association constant. From equation 2, it is apparent that as the concentration of total T_4 in the system increases and the number of unoccupied binding sites falls, the free/total T_4 ($=T_f/TP$) rises. This is one of the reasons that in hyperthyroidism free T_4 rises to a greater extent than does total T_4. Conversely, removal of T_4 from the system, as in severe hypothyroidism, will result in an increase in the denominator of equations 2 and 3 and a fall in the ratio of free T_4 to total T_4 in equation 3. As discussed further in Chapter 6, hyperthyroidism and hypothyroidism are also accompanied by alterations in the concentration of individual binding proteins. These will also influence the fraction of T_4 in the free form.

Since there are at least three serum proteins that participate in the binding process (TBG, TBPA, and albumin), the relationship is more adequately represented by a multiple equilibrium state.

$$T_f = \frac{TP_{TBG} + TP_{PA} + TB_{Alb}}{K_{TBG}(M_{TBG} - TP_{TBG}) + K_{PA}M_{PA} + K_{Alb}M_{Alb}} \quad (4)$$

where the subscripts TBG, PA, and Alb refer to the three major binding proteins. Since the binding capacity of both TBPA and albumin greatly exceeds the concentration of T_3 bound to each of these moieties (i.e., $M_{TBPA} >> TP_{TBPA}$ and $M_{Alb} >> TP_{Alb}$), the terms for albumin and TBPA in the denominator of equation 4 can be simplified as indicated. The numerator represents, as a first approximation, the total T_4 in the serum. Analagous to equation 3, the following expression designates the strength of the overall binding in a three-component system:

$$\frac{T_f}{TT_4} = \frac{1}{K_{TBG}(M_{TBG} - TP_{TBG}) + K_{PA}M_{PA} + K_{Alb}M_{Alb}} \quad (5)$$

Comparison of equation 5 with equation 3 reveals that the contribution of the two large capacity binding systems to the equilibrium relationship limits the excursion of T_f/TT_4 produced by a given change in T_4. In general, any analytic binding system in which the effects of TBPA and albumin are minimized and the effects of the limited capacity TBG are maximized will be more sensitive to a given change in T_4. Since barbital inhibits binding of T_4 to TBPA, the addition of barbital thus sensitizes the system to T_4.[85]

Measurements of Free T_4

Although the free T_4 hypothesis was developed without the benefit of direct measurements of free T_4, the application of equilibrium dialysis has made it possible to provide reasonable approximations of unbound T_4 in serum and other body fluids. Despite the fact that there are both practical and theoretic limitations in the interpretations of the results of equilibrium dialysis, the technique continues to be widely regarded as the "gold standard" for estimating the free T_4 concentration. In principle, the method is simple. Radioactive T_4 is added to the test serum in tracer quantities (Fig. 4–3). The mixture is added to a compartment separated from adjacent aqueous buffer by a semipermeable membrane and allowed to equilibrate, generally for 18 to 24 hours. Small molecules such as T_4 readily cross the membrane; larger molecules such as the binding proteins do not. At the end of the equilibration period the fraction of total counts in the system in the free form is determined, simply by multiplying the counting rate in the dialysate (free radioactive T_4 counts) by the total volume of the system and dividing the product by the total counting rate (free + bound) in the whole system. This value is designated as the dialysis fraction (DF). The product of the DF and the total gravimetric T_4 concentration as determined by radioimmunoassay or any other chemical methods will yield the absolute concentration of free T_4.

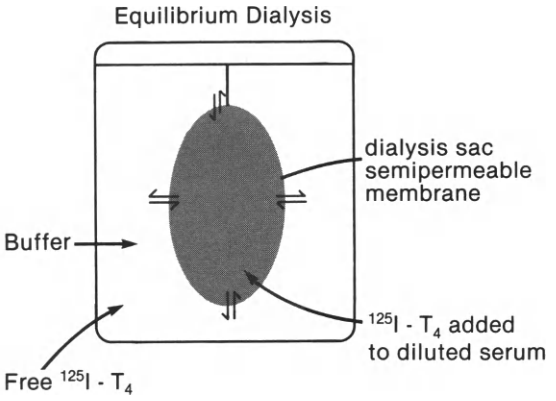

Figure 4–3. Equilibrium dialysis. ^{125}I-T_4 and ^{125}I-T_3 are added to serum in tracer quantities. Diluted serum is added to a bag of semipermeable membrane, which allows the free transport of ligand but not of protein. Equilibration is complete within 24 hours. The free T_4 concentration is determined by multiplying the fraction of total ^{125}I-T_4 added that is in the free form by the concentration of the T_4 in the undiluted serum, applying a correction factor for serum dilution.

An important technical problem in the determination of free T_4 by equilibrium dialysis deserves special emphasis, the contamination of preparations of radioactively labelled thyroxine with radioactively labelled iodide. Because of the strong binding of T_4 by serum and the relatively weak binding of iodide, the predominant form of radioactivity in the dialysates of undiluted sera will be in the form of iodide. This makes it virtually impossible on a routine basis to determine the concentration of radioactive T_4 in the dialysates. Even if a preparation of ^{125}I-T_4 is prepurified, a slow but continuous process of deiodination will continue and result in the formation of radioiodide that is not protein bound. In general, from 1 to 5% of total radioactivity in the radioactive preparation is in the form of iodide.

In the original method for performing equilibrium dialysis, an exceedingly complex series of separate steps, including evaporation of the dialysate and extraction of butanol and water followed by paper chromatography, was required.[196] A simple stratagem for overcoming this problem and for increasing the proportion of total radioactivity in the dialysate in the form of T_4 was subsequently introduced.[132] This involved the dilution of the serum that served to diminish the strength of protein binding and allowed the escape of a larger fraction of radioactive T_4 into the free form. The ratio of T_4 to iodide in the dialysate was thus increased, and quantitative separation between radioactive iodide and radioactive T_4 made technically feasible. The dialysate was subjected to trichloroacetic acid (TCA) precipitation, following the addition, to the dialysate, of out-dated serum obtained from blood banks. Labelled T_4 was precipitated and labelled iodide was not. The effect of serum dilution was taken into account in the final calculation of the concentration of free hormone in serum. The relevant principle applied in this approach is that the DF is approximately, inversely proportional to the dilution factor.[132] An alternate method for simplifying the separation of T_4 from iodide in the dialysate was the use of Mg^{++} to precipitate T_4.[194] Many of the current methods for measuring free T_4 employ both diluted serum and Mg^{++} precipitation.

The concentration calculated by equilibrium dialysis, however, must be considered to be simply an approximation. The diluting buffer used in the analysis does not faithfully represent the ionic composition of serum and thus may distort the binding of the individual proteins. The DF itself is also very temperature sensitive.[10] Since the dialysis is routinely performed at 37°C, determination of the DF may not reflect the effect of a fever, which in the patient may have raised the *in vivo* value. Lastly, the dilution may have a variable effect on the inhibitors of protein binding. Ultrafiltration overcomes some of these problems.[132, 181] Despite these limitations, equilibrium dialysis is generally considered to be superior to the so-called direct methods, which have been introduced commercially for clinical analyses.[40]

The DF as determined by equilibrium dialysis can now be related to the effect of individual binding proteins by application of equation 5. Thus,

$$\frac{DF}{1 - DF} = \frac{T_f}{TT_4} = \frac{1}{K_{TBG}(M_{TBG} - TP_{TBG}) + K_{PA}M_{PA} + K_{Alb}M_{Alb}} \quad (6)$$

The analysis used for T_4 can similarly be applied to the binding of T_3 as well. Radioactive T_3 is added to serum to determine the DF. The dilution of serum that is required to optimize the T_3/I^- ratio is not as large as that required to determine the DF for T_4, since T_3 is bound to plasma proteins approximately 1/10 as tightly as T_3. The approximate free T_3 concentration is 8×10^{-11} M^{-1}, with a DF corrected for a dilution of 1/15; the corresponding

value for T_4 is 2.5×10^{-11} M^{-1} for a dilution of 1/150.

Kinetic and Functional Relationship Between Thyroid Hormones in Blood and Tissues

Although thyroid hormones in plasma have always been considered "en route" from the gland to the target and metabolizing tissues, the overall relationship between tissue and plasma hormone pools has only been clarified within the last 20 years or so. Tracer analysis of radioactively labelled T_4 and T_3 has clearly shown that there is "two-way traffic" between plasma and tissue pools of hormones.[22, 40, 124, 126, 144, 181] In essence, kinetic analysis of the plasma disappearance curve of intravenously injected tracer T_4 and T_3 over a sufficiently long period allows calculation of the fractional rate of irreversible exit from a central distribution compartment. This can be identified anatomically with the plasma volume and those components of the interstitial compartment that are in rapid equilibrium with it. If during the early period following injection the tracer disappears from the central compartment more rapidly than the calculated rate of irreversible exit, one must conclude that the tracer is entering some portion of the external compartment only to return to the central compartment at a later time.

The volume of internal and external compartments can be calculated by standard tracer analysis. Although for many years single compartment kinetics had been applied to the analysis of plasma disappearance curves of T_3 and T_4, such an analysis, under certain circumstances, may lead to considerable error, especially in the case of T_3.[127] Preferable approaches include noncompartmental[127] and multicompartmental analyses.[34] In noncompartmental analysis, only a central distributive compartment and an aggregate external compartment are defined. No explicit assumptions are made regarding the specific size or interrelationships of the various kinetic subcompartments, which may constitute the external compartment. The only basic question addressed is how long an average molecule of tracer requires to complete its circuit from the central compartment through the external compartment and back. The information obtained allows calculation of common kinetic parameters, such as the distribution volume, the average half-time, and the metabolic clearance rate.[127] In contrast, compartmental kinetics makes precise inferences about the number and the specific relationships of various compartments. In theory, compartmental kinetics can provide more information than noncompartmental kinetics. The relative advantages and disadvantages of these computational approaches have been reviewed.[34]

It is important to emphasize that the analysis, whether noncompartmental or multicompartmental, ordinarily estimates the distribution and metabolism of only *plasma-derived* hormone. This type of analysis does not lead to any significant error in the case of T_4, since it can be safely assumed that the only source of T_4 is the thyroid and the secretions of the thyroid gland are immediately poured into the central plasma compartment. As discussed subsequently in this chapter, however, the principal source of T_3 in humans, under baseline physiologic conditions, is the monodeiodination of T_4 by peripheral tissues. For most tissues and presumably for the bulk of exchangeable T_3, the equilibration between the tissue source of T_3 and plasma is rapid. However, further studies have shown that with certain tissues, such as brain and pituitary, a relatively slow rate of equilibration leads to significantly higher specific activities of T_3 than in plasma.[107] Any T_3 that is formed in the peripheral tissues but is metabolized before going to the plasma will not be registered in these analyses. The overall error introduced by making the assumption that all T_3 is plasma derived has not been fully evaluated. In the rat, however, calculation of the total distribution volume and metabolic clearance rates by special methods designed to obviate the problems posed by the generation of T_3 from T_4 in slowly equilibrating pools [35] has yielded values similar to those estimated by noncompartmental methods.[97] It is reasonable, therefore, to assume that the analytic error in humans is also relatively small. Additional studies, however, are required to settle this issue.

Given these limitations in analysis, current estimates in humans suggest that the total distribution volume (central compartment + external compartments) for T_4 is approximately 6.5 L/m^2 and that for T_3, 13.7 L/m^2.[97] Moreover, both for T_4 and for T_3, both slowly and rapidly exchanging external compartments have been defined. The rapidly exchanging compartment has been identified anatomically with liver and kidney,[126] and the slowly equilibrating compartment, with muscle, skin, and

brain.[136] In humans, equilibration between the plasma T_4 and the rapidly exchanging compartment is essentially complete within 4 hours after the intravenous injection of the tracer.[126] The slowly equilibrating pools require about 24 hours.[124] Approximately 58% of the total distribution volume of T_4 represents T_4 bound to plasma proteins, 29% to the rapidly exchanging compartment, and the remaining 13% to the slowly exchanging pool.[133] With T_3, approximately 30% of T_3 is associated with plasma protein and the remainder with the cellular pool. The fractional turnovers of T_4 and T_3 are approximately 11% and 117%/day, yielding average half-lives of 6 and 0.5 days, respectively (see Table 4–1).[133]

The role of the interstitial (extravascular extracellular) space in the equilibration process deserves some comment. Wherever measurements have been made, such as in the cerebrospinal fluid,[68] all three thyroid hormone–binding proteins have been demonstrated. In comparison with serum, there appears to be a somewhat higher relative content of TBPA in the cerebrospinal fluid. The overall content in thyroid hormone–binding proteins reflects the marked reduction in the protein content in cerebrospinal fluid compared with serum. Moreover, within the experimental error of the assay of free hormone there are no marked differences from serum in the concentrations of free iodothyronines in these body fluids.[68, 205] This is not surprising in view of the principle that the concentration of free hormone is generally independent of dilution.[132]

The rate of equilibration of thyroid hormones between the vascular, the interstitial, and the cellular compartments appears to be greatly influenced by the fractional rate of exit of protein through capillary pores.[134] The effective size of these pores may be of critical importance in determining the rate of equilibration. In the liver, electron microscopy reveals no effective anatomic barrier between the lumen of hepatic sinusoids and the parenchymal cell, and the rate of equilibration between these anatomic compartments is rapid. In contrast, the rate of equilibration is slow in muscle in which the capillary appears to be lined by a continuous basement membrane.[9] The rate-limiting factor in the transport of iodothyronines from plasma to cells may be the exit of the hormone–binding unit from the capillary.[125]

Overall, the distribution of T_4 or T_3 in the body can be viewed as an exchange between cellular and plasma components.[133] In analyzing the kinetics of the iodothyronines, it may be useful to divide total body T_3 or T_4 into two composite compartments, one representing the iodothyronine bound to plasma-binding proteins, whether in the plasma or extravascular space, and the other representing the aggregate cellular compartment. The partition of body T_4 or T_3 will represent under steady-state conditions the net balance of the net effect of plasma protein binding and what can be termed "tissue binding." The net plasma protein binding can be evaluated mathematically simply from the product of the strength of plasma protein binding determined by equilibrium dialysis ($b_p = 1/DF$) and the net distribution volume of the plasma binding proteins (V_p), which can be approximated by the distribution of ^{125}I-albumin.[133] Thus, $B_p = V_p b_p$, where B_p is the total body plasma protein binding. From the operational definition of cellular binding offered and the fact that the mass of free hormone in the body is negligible in comparison with bound hormone, it follows that the ratio of total cellular to total plasma protein binding will be proportional to the pool size of cellular and plasma protein-bound hormone. In other words

$$\frac{B_c}{B_p} = \frac{(V_T - V_P)\, c_4}{V_P c_4} = \frac{V_T - V_P}{V_P} \qquad (7)$$

where V_T = total body distribution volume of ^{125}I-albumin, which can be readily determined by standard isotopic techniques, and c_4 = concentration of T_4 in plasma. Equation 7 simply states that net cellular binding stands in the same ratio to the net plasma protein binding, as the volume of extra plasma protein to the plasma protein volume. By simple rearrangement of equation 7 and substitution of $b_p V_p$ for B_p, we derive the relationship

$$B_c = (V_T - V_P)\, b_P \qquad (8)$$

Equation 8 can be evaluated from experimentally determined albumin spaces and the DF of the serum. As previously indicated, approximately 58% of total body T_4 and 32% of total body T_3 are situated in the plasma protein compartments. One can therefore calculate that the ratio B_c/B_p for T_4 is 0.72 and for T_3, 2.1. Since overall plasma protein binding of T_4 is approximately 10-fold greater than that of T_3 (i.e., $(b_p)_4/(b_p)_3 = 10$), the overall

cellular binding of T_4 in humans exceeds that of T_3 by a factor of about 3.4.

The concept of cellular binding deserves additional clarification.[127, 134] It represents the sum of factors that are responsible for the retention of hormone in the cellular compartment in competition with the effects of plasma protein binding. The ability of both T_3 and T_4 to penetrate the cells and to bind in a nonspecific fashion to all of the subcellular fractions and organelles has been demonstrated both by ultracentrifugal fractionation and by radioautography.[128, 134, 182] Thus, cellular protein binding per se contributes in a major fashion to the retention process.[129] In addition, however, any energy-dependent process that contributes to the cellular accumulation of hormone would also serve to increase the estimated cellular binding. In theory, the metabolism of iodothyronine in the cellular compartment would tend to decrease the apparent cellular binding by lowering the cellular content of hormone. In practice, however, the fractional exit rate representing metabolism is very small compared with the fractional rate of interchange with plasma and therefore, can be disregarded as a first approximation.

Determinants of Common Kinetic Parameters

The utility of the concept of cellular binding becomes apparent in considering the factors that determine the commonly used kinetic parameters, including the total distribution volume (V_T), the fractional turnover (k), and the metabolic clearance rate (MCR) of the hormone. The MCR represents the plasma volume equivalent of a hormone that is irreversibly degraded or excreted per unit time; V_T, the plasma volume equivalent containing the entire body pool of hormone; and k_T, the fraction of total body hormone irreversibly removed per unit time. In practice, these measurements are made with the assumption that all portions of the cellular hormone pool are exchangeable with the plasma pool. Each parameter is influenced by the total plasma protein binding ($B_p = V_p b_p$), the cellular binding as previously defined (B_c), and the fraction of cellular hormone irreversibly metabolized per unit time (k_c). It can be shown that the following relationships apply:[127]

$$V_T = V_P + B_c/B_P \tag{9}$$

$$k_T = k_c B_c/(B_c + V_P b_P) \tag{10}$$

$$MCR = B_c k_c/b_P \tag{11}$$

These equations resolve the individual parameter into tissue and plasma protein determinants. This distinction assumes practical importance from the generalization that alterations in plasma protein binding of thyroid hormones do not in a steady state influence the biologic behavior of the organism. Thus, if kinetic measurements are made in a given pathophysiologic setting, such as hyperthyroidism, it is possible to determine whether the changes in V_T, k_T, and MCR are due simply to the effects of hyperthyroidism on plasma binding proteins, are due to direct effects on the tissues, or are due to both. Rearrangement of equation 11 shows that the cellular component of the metabolic clearance rate, $k_c B_c$, equals $(MCR)b_p$. This value represents the metabolic clearance of free hormone. The principle of tissue binding is equally applicable to individual tissues.[72] For studies with rat, the equilibrium ratio of cellular hormone per gram to plasma hormone per milliliter is defined as cellular binding. This value thus provides an index of the overall intensity with which a given tissue can compete with plasma proteins for T_4 or T_3.

Mechanisms Underlying Plasma-Tissue Hormone Exchange

Some of the most controversial and poorly understood aspects of iodothyronine kinetics relate to the specific molecular mechanisms responsible for the interchange of hormones between plasma and tissues. The magnitude of this interchange is illustrated by the following examples.

In humans, one can calculate from a variety of kinetic parameters that the quantity of T_4 that enters the liver per minute (unidirectional flux) is 3.1 μgm.[126] This value represents approximately 2.2% of the delivery rate of total hormone as calculated from the product of the hepatic blood flow and the total T_4 concentration. This quantity, however, is about 45-fold greater than the entire body T_4 turnover per minute. It is, therefore, obvious that the vast bulk of T_4 that penetrates the liver must return to the plasma without being metabolized or excreted. The magnitude of the exchange is even more startling when expressed not in terms of total T_4 but of free T_4. Thus, the

unidirectional clearance rate of plasma-free T_4 calculated from the quotient of the unidirectional flux (3.1 μgm/min) and the average free T_4 concentration in plasma (15 ngm/L) yields a value of 106 L/min or approximately 206-fold the hepatic blood flow. Since there is no evidence to support the supposition that thyroxine-binding proteins can actually penetrate the hepatic cells, it appears very likely that a rapid exchange of T_4 between plasma proteins and elements of the hepatocellular plasma membrane allows the multiple recirculations of the effective free hormone during a single pass of blood through the hepatic sinusoids.

Similar considerations are applicable to experimental studies in rat tissues. Thus, calculations based on the unidirectional transfer constant from plasma to liver suggest that essentially all of the T_4 delivered to the liver (hepatic blood flow × plasma T_4) is taken up by the hepatic parenchyma.[72] However, making the limiting assumption that all of T_4 is metabolized in the liver, it can be shown that over 99% of the T_4 taken up by the liver must be returned to the plasma without prior metabolic transformation. The free T_4 clearance thus is about 3000-fold the hepatic blood flow.

These considerations explain experiments in which prealbumin and albumin have had no significant effect in retarding the transfer of T_4 from plasma to liver in single-pass perfusion experiments.[139] The rate of dissociation of T_4 simply exceeded the ability of the specific protein to retain T_4 in a single-pass perfusion experiment. However, the results of these single-pass perfusion experiments have prompted the expression of fundamental reservations about the usefulness of the free thyroxine hypothesis.[139] These investigators, instead, propose that the rate of transfer of T_4 and T_3 from plasma to certain tissues is mediated by albumin or prealbumin and that, in general, transport is governed by the length of the capillary path and by specific physicochemical conditions at the endothelial level, which are postulated to facilitate the rate of dissociation of T_4 bound to proteins.[139] The concept that certain hormone-binding protein complexes serve as a source of "available" hormone for given tissues has also been advanced in connection with these studies.

Other observers, however, have pointed out that single-pass perfusion experiments in which only the unidirectional egress of T_4 from the capillary is measured cannot predict the steady-state partition of hormone between plasma and cells.[40, 156] There is an almost equally rapid backward movement from liver to plasma, and both the forward and backward rates will determine the final distribution of hormone. Since there is a rapid exchange of hormone among all binding protein species, it may be inappropriate to define hormone bound to one species of protein as "available" and hormone bound to another species as "unavailable."

In comparison, the single-pass perfusion studies as well as the kinetic studies cited do focus attention on what are important and still largely unresolved questions with respect to the mechanism of transfer of protein-bound ligands from one macromolecule to another. Although some fragmentary data regarding the rate of association and dissociation of T_4 have been published[74-76] and appear to support the possibility of the rapid interchange,[156] additional physicochemical studies are needed, as well as a detailed mathematic treatment that takes into account an accurate estimation of the values of the binding constants and the physiologic data already reviewed. Although the free T_4 hypothesis probably will undergo further refinement with respect to the details that describe the approach to the steady state, the overall validity of the hypothesis in predicting the steady-state partition of hormone appears assured.

METABOLISM OF THE IODOTHYRONINES

General Patterns

Following the original identification of T_3 in plasma in 1952,[183] several investigators proposed that T_4 could be monodeiodinated by peripheral tissues to the more potently calorigenic T_3.[65, 67, 113, 114] This concept, however, did not gain credence initially, in large part because of a report[108] that suggested that the small quantities of ^{131}I-T_3 demonstrated in the sera of patients injected with ^{131}I-T_4 represented the residual ^{131}I-T_3 contaminating the injected ^{131}I-T_4 dose. This publication constituted a retraction of a previous publication in which they had suggested that T_4 to T_3 occurs in human beings and is physiologically important.[143] In retrospect, this retraction appeared unfounded, since the rapid metabolism of contaminating T_3 in the patient would have resulted in substantially lower quantities of T_3 than were actually found. Under any circum-

stance, the possibility of peripheral monodeiodination as a source of T_3 remained dormant until 1970 when the concept was revived by the finding of unlabelled T_3 in the plasma of thyroidectomized patients maintained on replacement doses of T_4.[17] Peripheral monodeiodination of T_4 with the formation of T_3 was rapidly confirmed both in humans[36, 211] and in rats.[183] Moreover, evidence came to light that suggested that T_4 acted in large part as a prohormone for T_4.[86, 131] The phenomenon of T_4 to T_3 conversion by peripheral tissues is now recognized as a central physiologic control mechanism in the regulation of thyroid hormone action. These developments have stimulated a great deal of interest in the broader issues that relate to T_4 and T_3 metabolism and the mechanisms responsible for their deiodination.

The overall pattern of iodothyronine metabolism has been extensively reviewed[42] and is summarized as follows (Fig. 4–4). The peripheral deiodination of T_4 results in the formation of T_3 and rT_3, substances that subsequently are sequentially deiodinated with the terminal formation of T_0. Such deiodination results from the action of several enzymes that display both substrate and tissue specificity. There are two 5′-deiodinases, designated as type I and type II, both of which remove iodine from either the 3′ or 5′ position of the tyrosyl ring and are thus responsible for the peripheral generation of T_3. Because of the free rotation about the ether linkage of T_4, these enzymes do not distinguish between 3′ and 5′ iodines. The two species of 5′ deiodinases are characterized by a selective tissue distribution, an inhibitor specificity, and a distinctive set of kinetic reaction mechanisms, which will be reviewed subsequently. The action of the T_4 5′deiodinase results in the formation of rT_3. Other enzymatic processes involved in the biotransformation of iodothyronines include deamination; sulfation; glucuronidation; and,

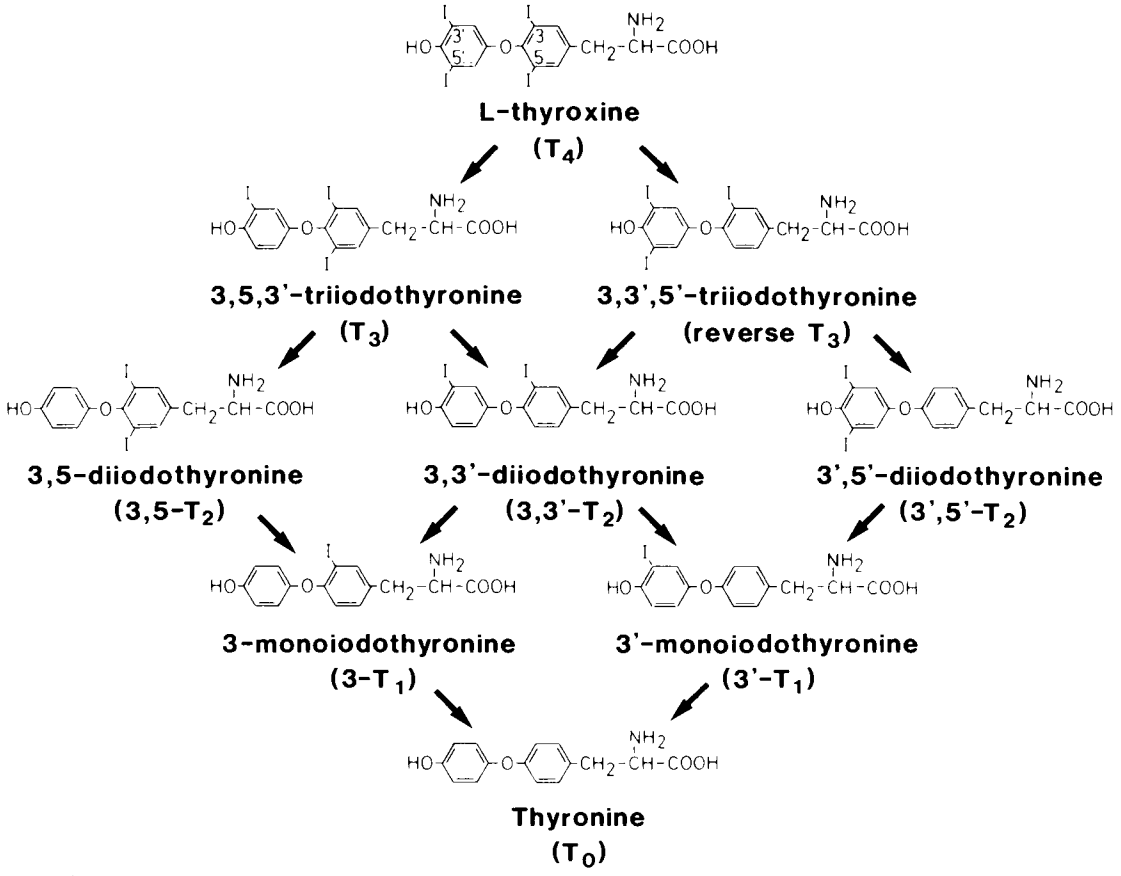

Figure 4–4. Pathways of thyroxine metabolism via the deiodinating pathways. (Modified from Engler, P. and Burger, A.: The deiodination of iodothyronines and their derivatives in man. Endocr. Rev. 5:151–184, 1984, with permission.)

to a limited extent, ether cleavage under oxidative conditions.

The importance of iodine in thyroid hormone economy and the ready availability of 5′-radioiodine labelled iodothyronine have directed considerable attention to the fate of labelled iodine. In humans, approximately 15% of the iodine in the 5′ position is eventually excreted via the fecal route either as an altered T_4 or as T_4 glucuronide; the remaining 85% is initially distributed to the thyroid gland where it is reutilized for T_4 formation and to the kidney where it is excreted in the urine as iodine.[162] In contrast, in the rat approximately 50% of labelled T_4 is excreted by the fecal route; the remainder is distributed to the urine and thyroid.[1] In both species, only trace quantities of labelled T_4 are excreted either as iodothyronines or as iodotyrosines in urine. The partition between thyroidal and urinary accumulation of iodine reflects a level of thyroidal activity. In a population with a high iodine intake, approximately 15% of any absorbed iodide dose is accumulated in the thyroid; the remaining radioactivity is excreted in the urine. With iodine deficiency or with thyroidal hyperactivity for other reasons, a substantial increase in the accumulation of iodide by the gland occurs.

Quantitation of Overall Metabolism

Although there are multiple approaches to the measurement of thyroid hormone metabolism, perhaps the most widely used technique depends on only the serial measurement of plasma radioactive hormone levels following the intravenous injection of 125- or 131-labelled iodothyronine. As previously indicated, the plasma disappearance curve can be analyzed by either compartmental or noncompartmental kinetics. Of these, the more popular and simpler has been the noncompartmental approach, although as discussed, more information is obtained from compartmental analysis.[34] Briefly, the following equations can be applied[127] to determine the MCR, the mean residence time of hormone ($\bar{t}$), the total distribution space (V_T), and the average fractional disappearance rate (k_T):

$$MCR = D/\int_0^\infty c^*dt \tag{12}$$

$$\bar{t} = \int_0^\infty c^*tdt/\int_0^\infty c^*dt \tag{13}$$

$$V_T = MRC(\bar{t}) \tag{14}$$

$$k_T = 1/\bar{t} \tag{15}$$

where c^* is the instantaneous concentration of hormone in plasma, and t is time. The integrals in these equations can be evaluated by graphic techniques[127]; alternatively, the values for the parameters are readily solved by computer techniques.[34] The results of such analysis have been discussed and are summarized in Table 4–1.

The interpretation of plasma tracer disappearance curves is clearly dependent not only on the mathematical model assumed but on the fidelity with which the generating curve represents the disappearance of the iodothyronine traced. Thus, both the presence of radioactive contaminants in the dose injected and the generation of radioactive products, including iodothyronines, iodoproteins, and iodide, can introduce serious error unless appropriate plasma separatory techniques are applied.[199, 215]

Quantitation of T_4 to T_3 Conversion

As previously discussed, the recognition of the pivotal role of T_3 in determining the thyroidal status of tissues has stimulated enormous interest in the peripheral conversion of T_4 to T_3. The quantitation of this process has presented a special challenge, especially in view of the obvious clinical and physiologic implications of this process. The problem also raises several theoretic and practical issues, deserving special consideration.

The fundamental parameter that must be determined to solve this problem is the conversion ratio of T_4 to T_3 (CR_{4-3}), which is defined by the following equation:

$$(MCR)_3c_3 = S_3 + (CR)_{4-3}(MCR)_4c_4 \tag{16}$$

where S_3 is the thyroidal secretion rate of T_3; c_3 and c_4 are the concentrations of T_3 and T_4 in plasma, respectively; and MCR is the metabolic clearance rate with the subscripts referring to T_3 and T_4. Rearranging equation 16 we obtain

$$(CR)_{4-3} = \frac{(MCR)_3c_3 - S_3}{(MCR)_4c_4} \tag{17}$$

Although it is relatively simple to measure the MCRs as described in the previous section, direct measurement of thyroidal secretory rate

is difficult. It is therefore necessary to quantitate directly the conversion ratio by application of isotopic techniques to the unmanipulated animal or to estimate it indirectly by first eliminating thyroidal secretion and subsequently supplying exogenous T_4 at a fixed rate. In theory, the direct measurement of ^{125}I-T_3 following the administration of ^{125}I-T_4 would appear to be straightforward and readily quantitated. However, the equilibrium ratio of ^{125}I-T_3/^{125}I-T_4 achieved ranges from 1/100 to 1/200. Such a preponderance of ^{125}I-T_4 makes it exceedingly difficult to separate the two labelled hormones by the most readily available chromatographic techniques. In humans, efforts to circumvent this problem have been cumbersome. In one approach, iodothyronines labelled with separate nuclides were used.[142] This technique clearly is not suitable for the study of multiple patients. Moreover, the calculated contribution of conversion to the total production rate fell sharply below subsequent estimates by other methods.

In order to bypass this problem the conversion ratio of thyroidectomized patients maintained on a constant replacement dose of T_4 has been evaluated by determining the turnover of T_4 and T_3 by standard isotopic techniques.[202] Under these circumstances, S_3 in equation 16 is reduced to zero. It is therefore possible to quantitate the conversion ratio simply from the turnover of T_3 $[=(MCR)_3c_3]$, determined from the plasma disappearance curve of ^{131}I-T_3 and the radioimmunoassay of serum T_3, and the turnover of T_4 $[=(MCR)_4c_4]$, calculated from the disappearance curve of ^{125}I-T_4 and the radioimmunoassay of serum T_4. This approach was feasible, since it was technically simpler to measure the concentration of both nonradioactive iodothyronines by radioimmunoassay procedures than to separate with equal precision a higher ratio of radioactive T_4 to T_3 in serum. It should be noted that since the T_4 5′-deiodinase attacks both the 3′ and 5′ positions of T_4, only one of which is labelled, deiodination will result in the generation of only one labelled molecule of T_3 for every two molecules generated. The results of this study suggested the average conversion ratio in seven patients studied was 42.6% with a range from 30.7 to 50.8%. Moreover, the plasma level of T_3 generated by peripheral conversion, 136 ngm/dl, was 93% of the average concentration of T_3 in these patients. These findings therefore suggested that the large bulk of circulating T_3 arose from peripheral conversion of T_4 rather than from direct thyroidal secretion. An essentially similar conclusion was reached in other studies using this approach,[11, 82] although the estimates of the percentage of T_4 production rate converted to T_3 was less, 25%[11] and 28%.[82] In another study approximately 33% of exogenously administered T_4 was converted to T_3.[18]

In a study[44a] of thyroidal conversion in 19 hypothyroid patients evaluated with the replacement method introduced by some members of the same group some 13 years earlier,[197] the average conversion ratio was found to be 26 ± 4 (SD)%, significantly less than the original estimates reported. Thus, there appears to be general agreement in later studies about the lower values for the conversion ratio.

The most important limitation of this approach is that it can be performed only in a thyroidectomized or hypothyroid patient on a constant maintenance dose of levothyroxine. The procedure is based on the assumption that the conversion ratio in a treated euthyroid patient is equivalent to that in a normal subject. From an analytic point of view, the results are contingent on the precision of the radioimmunoassay of both serum T_3 and serum T_4.

The direct method for assessing the conversion ratio was originally applied to a study of monodeiodination of T_4 in the rat.[183] The equilibrium radioactive T_3/T_4 ratio in total body carcass together with supplementary data on the turnover kinetics of T_4 and T_3 permitted calculation that showed that in the rat 17% of the T_4 turnover resulted in T_3 formation. Since T_3 is preferentially concentrated in the tissues, the use of the total body homogenates resulted in a lower equilibrium radioactive T_3/T_4 ratio than in serum and thus facilitated accurate quantitation of the conversion ratio. The direct approach has also been successfully used in determining T_4 to T_3 conversion in normal humans.[11] In this study, two methods of calculation were used, the convolutional method (a noncompartmental approach) and the T_3/T_4 equilibrium value method. The adoption of a sensitive gel chromatographic technique for separating radioactively labelled T_3 and T_4 in serum made it possible to analyze the plasma disappearance curves. These workers also compared the values for the conversion ratio obtained in T_4-treated hypothyroid patients with the two direct methods and with the indirect method previously described. All three approaches yielded essentially identical results and suggested an average conversion

ratio ranging from 25 to 30%, depending on the method and patient group studied. Approximately 72% of exchangeable T_3 originated from conversion.

Efforts have been made to simplify quantitation of T_4 to T_3 conversion by measuring radiolabelled T_3 in urine after the intravenous injection of ^{125}I-T_4. The assumption is made that there is a direct precursor-product relationship between injected T_4 and urinary T_3.[18] However, the claim has been made that urinary measurements lead to a systematic overestimation of the conversion ratio.[12] This problem has been attributed to an underestimation of total body deiodination by the restricting of measurements to urinary iodide. In essence, the most current data suggest that the average conversion ratio of T_4 to T_3 in human subjects falls between 25 and 30%, and approximately 82% of T_3 production is due to peripheral conversion with the remaining 18% supplied by direct thyroidal secretion.

Deiodination at the Tissue Level

Deiodination reactions constitute central mechanisms both in the generation of T_3 from T_4 and in the degradation of iodothyronines. The balance of the processes plays a critical role in establishing the tissue effects of any given rate of thyroidal secretion. Substantial research in the past decade has been designed to define individual deiodinases and to describe their molecular action. The overall strategy in achieving these objectives has included an analysis of the *in vitro* enzymatic deiodinating activity of individual tissues and an effort to correlate such activity with the results of experiments in which the distribution and metabolism of injected radiolabelled iodothyronine in whole animals are quantitated. The approach is further refined when the study of inhibitors, such as propylthiouracil, and stimuli, such as starvation, are compared at both levels of organization.

Although there has been substantial progress in this area, the field is hampered by two major problems. First, none of the deiodinases has so far been isolated and characterized. This is undoubtedly related to the fact that they are membrane bound and therefore not readily solubilized. A second problem is the difficulty of determining in whole animal experiments the metabolic contribution of specific tissues to the net deiodination as measured by the tracer methodology described previously. This is related to the slow rate of irreversible metabolism by the tissues in comparison with the rapid rate of hormone delivery by the circulation. As a consequence, the net arteriovenous extraction is too small to measure.

Recent studies, however, have been helpful in drawing attention to the action of deiodinases that are responsible for the creation of significant concentration gradients in certain tissues.[32, 106, 107, 187–189] In essence, the content of T_3 in these tissues, including pituitary and brain, is greater than can be attributed to an external T_3 source, feeding into the plasma compartment. This finding can be demonstrated by the simultaneous intravenous injection of ^{125}I-T_3 and ^{131}I-T_4. The tissue concentration of T_3 derived from T_4 can be assayed by the content of ^{131}I-T_3 and the concentration of T_3 derived from plasma by the content of ^{125}I-T_3. In liver, kidney, and heart tissue, T_3 content could be calculated from the tissue/plasma ratio of ^{125}I-T_3 and the plasma T_3. The production of T_3 in these tissues by deiodinases did not result in local accumulation. These results confirmed those of earlier studies.[198] In pituitary and brain, however, the content of T_3 was calculated to be substantially higher than predicted from the equilibrium tissue/plasma ratio. In the pituitary, approximately 50%[187] and in the brain, approximately 70% of T_3 were of local origin.[32]

These observations thus emphasize the potential impact of local T_4 monodeiodination in pituitary and brain. Some investigators have suggested that local deiodination in the pituitary may provide an explanation for the unusual sensitivity of pituitary TSH release to administered T_4.[188] The ability of T_4 to shut off TSH secretion almost as rapidly as T_3 had been puzzling in light of the greater biologic activity of T_3. The explanation provided suggests that local deiodination of the administered T_4 results in the rapid accumulation of intracellular T_3. As a consequence of an interaction of this T_3 with the specific nuclear receptors, TSH release is shut off. However, later clinical data suggest that in humans, plasma T_3 rather than T_4 modulates pituitary TSH release.[44a]

The ability to monitor the cellular T_3 content derived from local intracellular deiodination in pituitary and brain has provided an opportunity to correlate the *in situ* behavior of the deiodinase in these tissues with enzymatic activity as measured *in vitro*. From a teleologic perspective, the ability of a given tissue to

contribute selectively to the T_3 content of that tissue could provide a distinctive biologic advantage to the organism. As discussed subsequently, the ability to maintain the necessary T_3 content in brain under conditions of iodine deficiency and borderline systemic hypothyroidism mitigates impaired central nervous system developments, which characterize neonatal hypothyroidism.

The basis of preferential accumulation of T_3 in certain tissues is apparent in the following kinetic considerations. In the model of tissue-plasma exchange illustrated in Figure 4–5, the concentration of T_3 in the tissues under steady-state conditions is provided by the following formula:

$$C_3 = \frac{k_1 P_3 + S_{3-4}}{k_3 + k_2}$$

where S_{3-4} is the rate of local T_3 production in the specific tissue. P_3 is the tissue concentration of T_3, k_1, k_2, and k_3; the fractional transfer constants as illustrated in Fig. 4–5. For any given rate of local T_4 deiodination S_{3-4}, the determining factor is the relationship of k_1P to S_{3-4}. If k_1, the fractional forward rate constant from plasma to the cell, is sufficiently large to make $k_1P >> S_{3-4}$, the effect of local deiodination is negligible. The opposite is true if the value of k_1 is low. For a given rate of local monodeiodination, the contribution of local deiodination hinges substantially on the magnitude of the forward rate constant. The more rapidly T_3 enters from the blood, the less will be the effect of local deiodination on the tissue T_3 content.

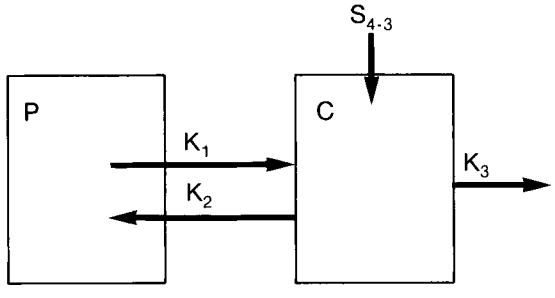

Figure 4–5. Kinetic representation of transfer of iodothyronines from plasma to tissue. The relationship between the rate of delivery of hormone to the tissue (k_1P) and the rate of conversion of T_4 to T_3 (S_{4-3}) in the tissue will determine whether the tissue will develop a local T_3 tissue/plasma gradient (P = plasma protein compartment; C = cellular compartment); K_1, K_2, and K_3 = fractional transfer constants..

Most of the information about iodothyronine deiodinases has been obtained from the study of the deiodination reaction carried out *in vitro* with the use of homogenate preparations. In general, the analyses of such reactions have measured the reaction kinetics with several substrates at varying concentrations and temperatures. The effects of cofactors and inhibitors are exploited to characterize the enzyme. The deiodinases are designated generically by what is considered to be the principal biochemical action and its biologic substrate. Current classifications are based entirely on functional criteria. With the development of more refined techniques for characterizing the deiodinases, additional subdivision in classification can be anticipated. An example of this is the recent recognition of two types of T_4 5′-deiodinases.

Deiodinases have been detected in human tissues, including kidney slices,[2] placenta,[178] and fibroblasts, and liver and kidney cells in culture.[152, 195] Current concepts hold that there are two major classes of enzymes acting upon the 5′ and 5 ioidine substituents of the T_4 molecule, T_4 5′-deiodinase and T_4 5-deiodinase, respectively. Although the speculation was originally advanced that deiodination of T_4 was a random process,[200] it is now firmly established that deiodination is enzymatic in nature and is regulated by pathophysiologic determinants. The action of T_4 5′-deiodinase results in the formation of T_3, whereas the action of T_4 5-deiodinase generates rT_3. The results of several *in vivo* metabolic studies in humans suggest that approximately 41% of T_4 is degraded via T_3, 38% via rT_3, and the remaining 21% via other pathways, including sulfation, glucuronidation, deamination, and ether cleavage (Fig. 4–6).[42]

The bulk of information regarding the deiodinating enzymes comes from the study of rat homogenate preparations. T_4 5′-deiodinase activity has been demonstrated in liver, kidney, heart, pituitary,[26, 29, 73, 93, 96, 147, 213] and in brown fat adipose tissue.[112] Although the tissues showing the most active deiodination activity *in vitro* are liver and kidney, it cannot be automatically assumed that these tissues provide a commensurate share of T_3 in the intact organism. Both of these tissues accept rT_3 preferentially as a substrate with the formation of 3′5′-diiodothyronine. The deiodination reaction requires the presence of an SH-containing group of compounds, such as dithiothreitol, and suggests that such substances act as nec-

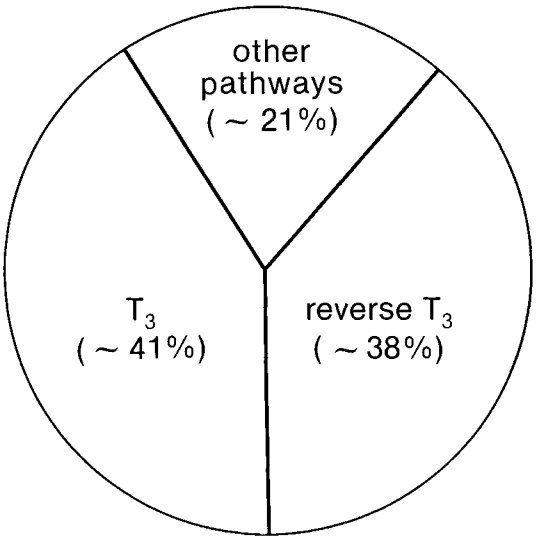

Figure 4–6. Partition of degradation pathways of T_4 via the formation of T_3, reverse T_3, and other metabolic products. (Modified from Engler, P. and Burger, A.: The deiodination of iodothyronines and their derivatives in man. Endocr. Rev. 5:151–184, 1984, with permission.)

essary cofactors *in situ*. The SH-containing groups are probably involved in a reduction of the iodothyronine substrate.[30, 214] The 5′-deiodinase enzyme has been reported to be localized in the microsomes of the liver[44, 214] and the membrane fractions of the kidney.[26, 115, 118]

Starvation of rats characteristically reduces the activity of hepatic T_4 5′-deiodinase but fails to produce changes in either kidney or cerebral cortex.[4, 5, 54, 70, 93] In the hypothalamus, starvation is reported to be associated with an increase in the rate of deiodination.[90, 94] It is not clear whether the reduced enzyme activity in starvation is due to a reduction in cofactor or enzyme concentration. Recent studies favor the view that a decreased enzyme concentration is primarily responsible.[27, 56a, 57, 180] The effect of starvation on rat hepatic 5′-deiodinase has frequently been used as a model for the study of the diminished T_4 to T_3 conversion in humans subjected to caloric deprivation.[206] However, recent studies have shown that in the rat, the reduction in serum T_3 produced by starvation is due to a diminished thyroidal secretion of T_4 and not to an overall reduction in the peripheral conversion of T_4 to T_3.[98] This finding implies that in the rat, the reduced hepatic 5′-deiodinase activity is offset by increased deiodination in other tissues or that the *in vitro* measurement of hepatic deiodination does not reflect hepatic activity *in situ*.

Thyroidal state also influences the activity of hepatic T_4 5′-deiodinase, with a decrease in hypothyroidism and an increase in hyperthyroidism.[7, 71, 89, 92] In general, the alterations in kidney enzyme follow those in liver. The responses in brain and pituitary, however, diverge from those in liver and kidney. Thus, hypothyroidism is associated with an increase in T_3 production in brain[96] and pituitary.[207]

Propylthiouracil (6-propyl-2-thiouracil, PTU) also exerts major effects on the deiodination of T_4, effects that have been recognized for three decades.[25] PTU has further been shown to inhibit T_4 to T_3 conversion in whole rat experiments.[131] The inhibition, however, was only approximately 50%, in contrast to subsequent *in vitro* experiments in which maximal concentrations of PTU could achieve a nearly 100% inhibition of T_4 5′-deiodinase.[25, 26, 81, 214] This discrepancy is apparently due to the heterogeneity of T_4 5′-deiodinase in the rat. Mercaptoimidazole, another thionamide commonly used as an antithyroid agent, does not share with PTU the capacity to inhibit deiodination.

The mechanism of PTU that inhibits deiodination has been investigated.[113, 114] Two half reactions have been postulated, the first involving the transfer of an iodinium ion from the substrate to an essential SH-containing group on the enzyme and the second involving a reduction of the iodinium-enzyme complex by the cofactor. PTU is postulated to compete with the substrate for the essential SH-containing group on the enzyme. In turn, dithiothreitol will compete with PTU for the same group and thus restore enzymatic action.

Reference has been made previously to the heterogeneous nature of the monodeiodinases and to the characteristic differences in tissue distribution and response of these enzymes. Further additional evidence has come to light that fully justifies the reclassification of T_4 5′-deiodinases into two subgroups, type I and type II (Table 4–3).[187, 189, 210] Type I enzymes are inhibited by PTU, starvation, and the hypothyroid state; in kinetic studies with homogenate preparations, they exhibit a relatively high K_m and a sequential reaction pattern. Type II enzymes, by comparison, are not inhibited by PTU, are generally unaffected by starvation, are markedly stimulated by hypothyroidism, and exhibit a low K_m and an alternating enzyme reaction pattern. Type I enzymes are located in liver and kidney, whereas type II enzymes are found in pituitary, brain,

Table 4–3. Characterization of 5′-Iodothyronine Deiodinase*

	Type I	Type II
Effect of PTU	Inhibition	None
K_m for T_4	"High"	"Low"
Tissue localization	Liver, kidney	Pituitary, CNS
Response to hypothyroidism	Decrease	Increase
Response to hyperthyroidism	Increase	Decrease
Possible physiologic role	Provides T_3 to serum	Provides T_3 to serum

*Adapted from Silva, J. E. and Larsen, P. R. (1983). Thyroid Today Vol. 6, No. 4.

and brown fat adipose tissue. It appears possible that tissues may have a mixture of enzymes, recent studies having suggested that both are present in pituitary.[209] Of interest is that ipodate and iopanoic acid, gallbladder dyes which have been noted to reduce the concentration of serum T_3,[20] are effective in inhibiting both type I and type II enzymes.

As previously mentioned, the striking increase in type II T_4 5′-deiodinase activity in response to partial hypothyroidism may be of major adaptive value in maintaining a high degree of saturation of brain nuclei with T_3. The profound effect of thyroid hormone deprivation at critical stages of ontogenesis involving the central nervous system is widely recognized, even though the precise biochemical and molecular details of these effects remain obscure. Deiodinase type II appears responsible for maintaining a high degree of saturation of the nuclear receptors, estimated to be over 90% in contrast to the more typical 50% saturation in other tissues.[32] Moreover, when varying degrees of hypothyroidism were created in neonatal rat pups with the administration of increasing doses of methimazole to the mothers, the level of the concentration of T_3 in the cortex was selectively maintained by a 10- to 15-fold increase in type II deiodinase.[186] At the same time, the level of activity of three brain enzymes (aspartic transaminase, succinic dehydrogenase, and Na/K ATPase), which serve as markers of thyroid hormone status, was maintained as a consequence of the activation of type II T_4 5′-deiodinase.

Of considerable interest is the mechanism responsible for increase in type II T_4 5′-deiodinase activity.[116] The T_4-induced fall in cerebral deiodinase activity over an interval of 4 hours was not inhibited either by cycloheximide or by actinomycin despite an appropriate reduction in protein and RNA synthesis. This finding would appear to represent a clear extranuclear biologic effect of thyroid hormone. Another report, however, has suggested that the decrease in pituitary type II enzyme produced by T_3 is dependent on protein synthesis.[119]

The possibility that type II deiodinase activity in addition to regulating the intracellular T_3 content in certain tissues may also affect the plasma hormone concentration has been raised.[117] Cessation of T_4 administration to euthyroid and athyreotic subjects resulted in a pronounced increase in the T_3/T_4 ratio. Since preferential T_3 secretion by the thyroid could be ruled out as an explanation, the autoregulatory phenomenon observed must have been due to differential peripheral generation of T_3 or to differential metabolism or distribution. Further studies to decide among these possibilities will be of interest. Studies in the hypothyroid rat do in fact support the concept that the type II deiodinase contributes a significant fraction of T_3 to the circulating pool.[190]

The identification of type II T_4 5′-deiodinase in brown fat adipose tissue has raised some interesting physiologic possibilities.[112] Brown fat adipose tissue in the rat is known to contribute to heat production as a result of sympathetic nervous stimulation following cold exposure or food ingestion.[50, 123, 177] A later report indicates that noradrenaline and cold exposure increase deiodinase activity in brown adipose tissue through alpha$_1$-adrenergic receptors, whereas catecholamine depletion with alpha-methyl-*p*-tyrosine prevents the effect of cold but not of noradrenaline.[185]

Unfortunately, comparatively little is known regarding the deiodinases that are responsible for the degradation of the other iodothyronines. Thus, it is believed that rT_3 also serves as a substrate of the 5′-deiodinase.[93] This may account for the slow fractional removal rate of rT_3 and its accumulation in plasma when the level of activity of 5′-deiodinase is reduced with starvation or drug treatment. Degradation of T_3 in brain appears to proceed largely via tyrosyl-ring deiodinase.[95] The possibility that fractional removal of T_3 in the neonatal brain is reduced in the hypothyroid rat pup in an effort to maintain intracerebral T_3 content has also recently been suggested.[191] If this can be established, T_3 deiodinase may also serve a regulatory function.

Other Metabolic Transformations

Glucuronidation of the phenolic hydroxyl group of T_4 and other iodinated thyronines

also occurs and contributes to their inactivation and biliary excretion.[45, 169, 174–176, 204] In general, T_4 and iodothyronines with two substituents in the phenolic group are most susceptible to conjugation. Following the intravenous injection of ring-labelled ^{131}I-T_4, 40% of the injected tracer appeared in the bile as glucuronide conjugates.[49] Ordinarily, the glucuronide conjugates are hydrolyzed in the gastrointestinal tract, a fact that accounts for the low levels in plasma and the failure to find large amounts in the feces.[163] Glucuronidation occurs not only in the liver but in extrahepatic tissues as well.[48] Although the concept of extensive enterohepatic circulation in the rat has been proposed,[1] more recent data have challenged this finding.[52] This question has not yet been investigated in humans.

Sulfoconjugation is carried out by phenol sulfotransferases, primarily in the liver and, secondarily, in extrahepatic tissues such as the kidney.[49, 164, 165, 167, 170] Unlike the glucuronides, substantial levels of the sulfate esters of iodothyronines can be identified in plasma, as a consequence of either absorption or extrahepatic secretion into the blood stream. Iodothyronines with one iodine substituent in the phenolic ring appear to be better substrates than compounds with two iodine substituents.[184] A novel function for sulfotransferase action has been suggested, namely, that antecedent sulfoconjugation facilitates the deiodination of certain iodothyronines. Thus, there is a correlation between phenolic ring deiodination of 3,3′-T_2 by rat hepatocytes and sulfotransferase activity.[137] Moreover, the rate of tyrosyl deiodination of T_3-S to 3,3′-T_2S is substantially faster than the rate of deiodination of the unconjugated T_3.[212] Of interest is that this facilitation occurs with a relatively low K_m and therefore does not require saturation of the deiodinase enzyme.[212] It should be interesting to determine whether a similar reaction pattern can be demonstrated *in vivo* and whether glucuronidation plays an analogous role with T_4 and other iodothyronines that contain two iodines in the phenolic ring.

Deamination and decarboxylation of T_4 and T_3 result in the formation of their acetic acid analogues, tetrac and triac. These pathways have been demonstrated both in animal studies [47, 53, 171–173] and in humans.[211] Unlike many of the metabolites of T_3 and T_4, triac is biologically active and binds to nuclear receptors.[64] However, because of its rapid metabolism, the concentration of triac in plasma [56a, 122] is probably too low to make a significant contribution to the thyroid status of the tissues.

There has been considerable debate about the quantitative importance and physiologic significance of the oxidative deiodinative processes that are responsible for the cleavage of diphenyl ether and the production of peripheral iodoproteins identified as nonextractable iodine (NEI). Some reports indicate that ether cleavage does not occur under physiologic circumstances in the rat.[141] Further studies, however, have suggested that ether cleavage does occur, albeit to a limited extent, perhaps involving 6% of the total turnover of injected T_4.[8] Ether cleavage has been demonstrated in rat liver of homogenates,[6] as well as in phagocytic leukocytes.[19] Phagocytosis stimulates both T_4 uptake and oxidative deiodination by leukocytes, and the latter process is in turn coupled with ether cleavage and protein iodination.[173] The hypothesis has been advanced that iodination of bacteria, which occurs as a result of augmented oxidative deiodination by leukocytes, could serve an antimicrobial function[173] and contribute to the overall increase in T_4 turnover that has been noted in some patients with bacterial disease.[216] It appears possible that the operation of oxidative deiodination at low levels is also responsible for the formation of nonextractable iodine in plasma and tissues under basal conditions.[199, 203]

CONCLUSIONS

Thyroid hormones are associated with specific binding proteins in plasma and are in equilibrium with tissue pools. The rate of such equilibration is characteristic for each tissue and may depend on the rate of transit of protein across the capillary bed supplying the tissue. The common kinetic parameters of T_4 and T_3, including the fractional turnover rate, metabolic clearance rate, and total volume of distribution, depend on plasma protein and cellular binding as well as intrinsic cellular metabolic processes. Perturbation in plasma protein binding does not appear to result in any changes in hormone flux or hormonal status under steady-state conditions. Measurement of the plasma-free hormone concentration by equilibrium dialysis provides for an effective correction for alterations in plasma protein bindings and, as such, serves as a more reliable guide to the influence of the hormone at the tissue level than does the concentration

of total homone. Binding proteins, both vascular and cellular, stabilize the concentration of hormone at the critical intracellular receptor sites. The conversion of T_4, the principal product of thyroidal secretion, to the metabolically more active T_3 by deiodinases in the peripheral tissues is central to the operation of the thyroid system. In certain tissues, such as brain and pituitary, nuclear T_3 is derived in large part from local deiodination of T_4 within the cell, whereas in other tissues such as liver, kidney, and heart, the principal source of T_3 is plasma. This system provides certain tissues a degree of autonomy in the regulation of their nuclear receptor T_3 content. Although the deiodinases have not been isolated, their functional and kinetic behavior indicates that there are at least two T_4 5′-deiodinases, type I and type II. The latter is found in brain, pituitary, and brown fat, and shows increased activity in the hypothyroid state, presumably as an adaptive phenomenon. A major challenge for future research is to define the contribution of individual tissues to total T_3 formation and to achieve a greater degree of understanding of the coordination between tissue production and utilization of T_3 and the optimal tissue concentration of T_3 for any given physiologic setting.

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5
Tissue and Cellular Effects of Thyroid Hormones and Their Mechanism of Action

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Despite the long-standing general interest in the subject, current concepts of thyroid hormone action at the tissue level remain largely fragmentary and, for the most part, descriptive. The activity of thyroid hormones is generally understood in terms of the catalogue of clinical and experimental observations, which have been accumulated over the past century describing the clinical, physiological, and biochemical changes wrought by thyroid hormone excess and deficiency. With the advent of the newer approaches of cellular and molecular biology, however, data are beginning to emerge that promise to provide more precise understanding of both the initiating events in hormone action and the sequence of subsequent reactions, which result in the ultimate expression of hormone function at the tissue level. The molecular data also promise to provide more general insight into the evolutionary significance of thyroid hormone action and perhaps begin to provide some unification in the diversity of phenomenology that now characterizes the field.

In the first part of this chapter, I attempt to provide a broad overview of the biologic impact of thyroid hormones. In the second part, attention is directed to further studies at the cellular and molecular levels. Where possible, an effort is made to relate these processes to the observed effects of thyroid hormone at the biologic and clinical levels.

TISSUE EFFECTS OF THYROID HORMONES

Historical Perspectives

Unlike many hormones with highly targeted sites of action, such as thyroid-stimulating hormone (TSH), thyroid hormones exhibit multiple and often apparently unrelated biologic effects in diverse tissues. Broadly speaking, these biologic effects can be classified as "metabolic" and "developmental." These functions were first recognized as being integrally related to thyroidal activity in the last half of the 19th century. The syndromes of sporadic and endemic (iodine-deficient) cretinism were characterized by severe developmental defects, including dwarfism and mental retardation, and were associated with distinctive clinical symptoms, reflecting the metabolic functions of thyroid hormone. The recognition that the two forms of cretinism represented disturbed thy-

roid function and bore a relationship to the metabolic signs and symptoms of the surgically created athyreotic state in the adult led to the recognition of the role of the thyroid gland.[1] Experimental studies in animals, which demonstrated the histologic effects of thyroidectomy in monkeys supported the clinical observations. These relationships were codified in the landmark report of the Committee of the Clinical Society of London in 1888.[2] The relationship of oxygen consumption to the thyroid gland was also first recognized clinically by Magnus-Levy in 1895.[3] Further, with the introduction of the Benedict-Roth respirometer, measurement of the basal rate of oxygen consumption (basal metabolic rate) became the only standard clinical laboratory assay of thyroid function until the development of techniques for measuring hormonal iodine in the 1940s.[4] Although Bauman first recognized the association of iodine with thyroidal secretion in 1896,[5] the actual identification of the two major thyroidal secretory products did not occur until later. Kendall isolated thyroxine (T_4) in 1914,[6] Harrington characterized it in 1926,[7] and Gross and Pitt-Rivers identified triiodothyronine (T_3) in 1952.[8] Although the claim that T_4 served as the precursor of T_3 was made soon after the discovery of T_3,[9–12] these reports were generally disregarded until the demonstration of T_4 to T_3 conversion in humans by Braverman and coworkers in 1970.[13] Quantitation of T_4 to T_3 conversion in the rat,[14] and evidence that the biologic effects of thyroxine could be effectively reduced when the conversion of T_4 to T_3 was blocked by propylthiouracil[15] led to the proposal that the principal function of T_4 was as a precursor of T_3 and that most of the hormonal effects of thyroidal secretion were mediated by T_3. These proposals were strengthened by the finding that the nuclear receptor showed a considerably higher affinity for T_3 than for T_4.[16]

Evolutionary Considerations

Thyroid hormones are characteristically vertebrate hormones (Table 5–1). Although monoiodotyrosine and diiodotyrosine can be demonstrated in invertebrates as can trace quantities of T_4 and T_3, these substances are generally believed to be formed as a consequence of nonenzymatic processes.[17] Neither thyroid hormone–producing cells nor thyroid globulin has been identified in such species, and hormones do not circulate in the blood stream. Thus, most observers do not ascribe any function to these compounds in invertebrates.

The appearance of circulating thyroid hormones with discrete cellular sites of origin is coincident with the appearance of the earliest vertebrate forms some 500 million years ago. Surprisingly high levels of T_3 have been reported in the ammocoete stage of the lamprey,[18, 19] a cyclostome and descendant of one of the earliest vertebrate species to diverge from the main evolutionary course. Plasma concentrations of T_3 of 3000 ngm/dl are far in excess of values found in other species and appear not to be due to an unusually strong plasma protein binding.[19] T_4 is also present in the lamprey ammocoete, albeit in lower concentrations, and nuclear T_3 receptors in the livers of such animals exhibit the same spectrum of T_3 analogue binding as do mammalian receptors. An abrupt fall in T_3 concentration occurs in the transition of the ammocoete form to the more mature transformant stage. Both T_3 and T_4, as well as nuclear receptors, have been demonstrated in teleosts, and their physicochemical characteristics and analogue binding properties appear to be indistinguishable from those found in mammals, amphibians, and birds, thus suggesting a high degree of conservation.

Despite the presence of what appears to be an initiating apparatus, it has been exceedingly difficult to understand the function of thyroid hormones in fish. Although the normal smolting patterns of the salmon appear to be dependent on thyroid hormones,[20] thyroid hormones do not influence oxygen consumption or the level of activity of the hepatic enzymes, which are characteristically thyroid hormone responsive in mammals.[19] There has been little convincing direct evidence that thyroid hormones are involved in growth and development in fish. This factor may have been due to the difficulty in producing the hypothyroid state in such animals at the appropriate stage of development and the failure in administering the thyroid hormone at the critical time. As pointed out subsequently, thyroid hormone characteristically functions in coordination with other hormones and metabolic factors, and if the coordinate signal is lacking, thyroid hormone may not produce any effects.

The bias that thyroid hormone action in early vertebrate species is related to growth and development of the organism is based on the classic experiments of Gudernatsch,[21]

Table 5–1. Evolution of Thyroid Hormone Effector System*

Differentiated (million years)	Orders	Lipogenic enzymes	T_3	T_4	Nuclear receptor	T_3-stimulated		Homeotherm
						Lipogenesis	*O_2 consumption*	
< −500	Prevertebrates	+	−	−	−	−	−	−
−600	Agnatha	+	−	−	−	−	−	−
−250	Teleosts	+	+	+	+	−	−	−
−150	Birds	+	+	+	+	+	+	+
−100	Mammals	+	+	+	+	+	+	+

*Approximate dates of differentiation of species; the appearance of lipogenic enzyme, thyroid hormones, and nuclear receptors; and responses of lipogenic enzymes and oxygen consumption to thyroid hormones. These correlations suggest that hormonal stimulation of lipogenesis and oxygen consumption may have been necessary prerequisites to the development of the homeothermic species.

which demonstrated the role of thyroid hormones in the metamorphosis of the free-swimming aquatic tadpole to the terrestrial-based, lung-breathing frog. As demonstrated by many observers,[22–24] this involves an extraordinarily extensive rearrangement in structure and function and is contingent upon an increase in the thyroidal secretion by the tadpole. In the frog, however, the thyroid hormones have not been assigned a clear-cut function.

There is every reason, therefore, to suppose that the general metabolic effects of thyroid hormone represent a recent evolutionary acquisition. Increased oxygen consumption of the tissues from thyroid hormone–treated animals is not apparent in evolutionary development until the appearance of homeotherms approximately 200 million years ago. Similarly, the tissues of early vertebrates fail to respond to thyroid hormone administration with an increase in the activity of lipogenic enzymes.[19] As discussed further, these enzymes may play an important role in fueling thyroid hormone–stimulated thermogenesis. The maintenance of a constant temperature under all environmental temperatures by the homeotherm may well be contingent on the development of a more specific set of metabolic functions of the thyroid hormones.

Developmental Effects of Thyroid Hormones

Amphibian Metamorphosis

As indicated, the phenomenon of amphibian metamorphosis has received considerable attention in the past as an implicit model of the developmental actions of the thyroid hormones.[22–24] Thyroid hormones are essential for metamorphosis, and administration of thyroactive preparations markedly accelerates this process. Changes that occur in the transition of the larval tadpole to the adult frog include extensive structural alterations, such as resorption of gills and tail with the development of lungs and limb buds. On a biochemical level, the transition from the ammonia-excretory mechanisms of the tadpole to the urea-excretory mechanisms of the frog requires the appearance of urea cycle enzymes. The transition is also accompanied by a marked increase in serum protein concentration, including serum albumin. Tail resorption is associated with an augmented level of hydrolases and with locally diminished protein synthesis. Larval tadpole hemoglobin is replaced by adult frog hemoglobin and the transformation of the retinal pigment from porphyropsin to rhodopsin.

The time frame in which these changes occur has been divided into three stages: premetamorphosis, characterized by rapid body growth without differentiation; prometamorphosis, in which rate of growth diminishes and differentiation begins; and metamorphic climax, associated with complete cessation of growth and maximal rates of differentiation. The duration of each phase varies from species to species. In *Rana catesbeiana* tadpoles, premetamorphosis requires more than 1 year, prometamorphosis several weeks, and metamorphic climax only 2 weeks. Of considerable interest with regard to the role of thyroid hormones is the finding that levels of T_4 and T_3 are virtually undetectable at the premetamorphic stage but are present in small concentrations during prometamorphosis. During metamorphic climax, there is a surge in the concentrations of both T_4 and T_3, which reach apical values during midclimax. Thereafter, the levels of T_4 and T_3 fall again to virtually undetectable levels in the adult frog. As previously pointed out, although the tadpole is sensitive to thyroid hormone for almost its entire larval development, thyroid function in the adult frog is more difficult to demonstrate.

Developmental Effects of Thyroid Hormone in Homeotherms

The developmental effects of thyroid hormone so dramatically apparent in amphibian metamorphosis continue to play an essential role in the ontogenesis of higher vertebrates. As in metamorphosis, thyroid hormone participates in the development of the animal at a well-defined stage.[25] For example, in mammals, thyroid hormones have conventionally not been regarded as playing an important role in intrauterine development until very late in gestation. The precise time at which thyroid hormone begins to influence development in a given species is related to the duration of gestation and the degree of maturity of the offspring at birth. Thus, in the rat and in humans, prenatal thyroidectomy or the spontaneous absence of the thyroid does not result in altered birth weight. Clinical recognition of hypothyroidism at the time of birth is notoriously difficult even though epiphyseal changes may be demonstrable by radiologic examination. Hypothyroidism may not be clinically apparent until approximately 1 month after birth. This fact underscores the importance of early laboratory-based diagnosis of neonatal hypothyroidism with measurements of TSH and T_4 levels in order to institute early replacement therapy designed to forestall the associated catastrophic mental retardation and developmental defects. Similarly, in the rat, the diminished growth rate characteristic of hypothyroidism is not apparent until the neonate is 8 days old.

In both species, the relatively normal presentation at the time of birth contrasts with the development of fetal thyroid function at a much earlier intrauterine stage. Moreover, in the rat, low levels of thyroid hormone are detectable in fetal serum at midpregnancy. Conventionally, such low levels of thyroid hormone have not been regarded as being functionally important, although some studies[26] have raised the possibility that even small quantities of thyroid hormone transferred during midpregnancy may play a role in the development of the brain. This issue remains controversial. The major effects of thyroid hormone in the human and the rat appear to become manifest following a surge of hormone coincident with an increase in T_4 deiodinase, which occurs at approximately 8 days in the rat[27] and on the first day of life in the infant.[28] This situation is thus strikingly analogous to the surge in T_3 concentrations that precedes amphibian metamorphic climax.

In mammals, the manifestations of neonatal hypothyroidism are protean and are most prominently expressed in generalized retardation of body growth and marked skeletal retardation with profound disturbances in central nervous system (CNS) function. The abnormalities associated with thyroid hormone deficiency involve retardation of both general body growth and maturation of individual cells and tissues. The role of thyroid hormones is clearly intertwined with the developmental program encoded in the DNA of the organism. Whereas in the adult animal most of the deficiencies of thyroid hormones can be easily reversed, this is not the case in the neonatal animal. Thus, abnormalities in the development of the CNS can be effectively prevented only if the thyroid hormone is given during a specific "time window." Thyroid hormones can also accelerate such programs, since the early administration of excess thyroid hormone may actually advance the appearance of specific developmental traits, such as the opening of the eyes in the rat and the appearance of early ossification centers. Thyroid hormone appears to function both by an increase in the general growth of the animals and by an acceleration of the differentiation of specific cell types. Thyroid hormone is essential for the production of pituitary growth hormone[29] and perhaps of the as of yet unidentified growth factors.

Despite the paucity of external manifestations, approximately half of the infants born with congenital hypothyroidism have significantly late bone maturation. The absence of femoral epiphyses in newborns with a birth weight above 2500 gm is suggestive of thyroid hormone deficiency. After several weeks, a delay in the appearance of ossification centers soon becomes evident and probably accounts for the typical cretinoid facies, including a depressed nasal bridge, relatively narrow forehead, and mandibular hypoplasia. There are a characteristic retardation in the closure of the fontanelles and, as emphasized, delayed appearance of ossification centers, depression of the radial long bone growth, and retardation in dental age. In general, alterations in skeletal development can be rectified prior to epiphyseal closure. Moreover, in hyperthyroid states, accelerated maturation is characteristic.

Studies in the rat have indicated that the appearance of ossification centers and linear growth of bone are affected.[25] The appearance

of the ossification centers is a direct effect of thyroid hormone, whereas the linear growth of bone most probably results from the increased multiplication of cartilaginous cells under the influence of pituitary growth hormone secretion, which is dependent upon the thyroidal status of the organism. Thyroid hormones are known to stimulate endochondrial ossification and are essential for maturation of bone cells, leading to increased deposition of matrix and calcification of cartilage. Both in the rat and in humans, treatment with large doses of thyroid hormones leads to acceleration in skeletal development.

Effect of Thyroid Hormones on CNS Development (Figure 5–1)

The extraordinary impact of thyroid hormone deficiency on the normal maturation of the CNS has been the stimulus for considerable research in this area over the past 50 years. Nevertheless, our knowledge of the effect remains largely descriptive. As emphasized, the diagnosis of hypothyroidism at the time of birth is difficult from clinical considerations, but within several weeks the diagnosis becomes obvious. The tongue is thick; the fontanelles are open; and an umbilical hernia and typical cretinoid features are present. Further, there is failure of normal progression of motor skills. Untreated, congenital hypothyroidism leads to major mental retardation, which frequently requires institutionalization. Unless treatment is started within the first 3 months, the effects of hypothyroidism on mental retardation cannot be completely reversed.

Considerable attention has been directed to an analysis of the effect of thyroid hormone deprivation on the morphology of the rat brain.[30] In the rat, major deviations from normality in brain weight and structure are not apparent until 10 to 15 days after birth. As indicated, thyroid hormone is effective in nor-

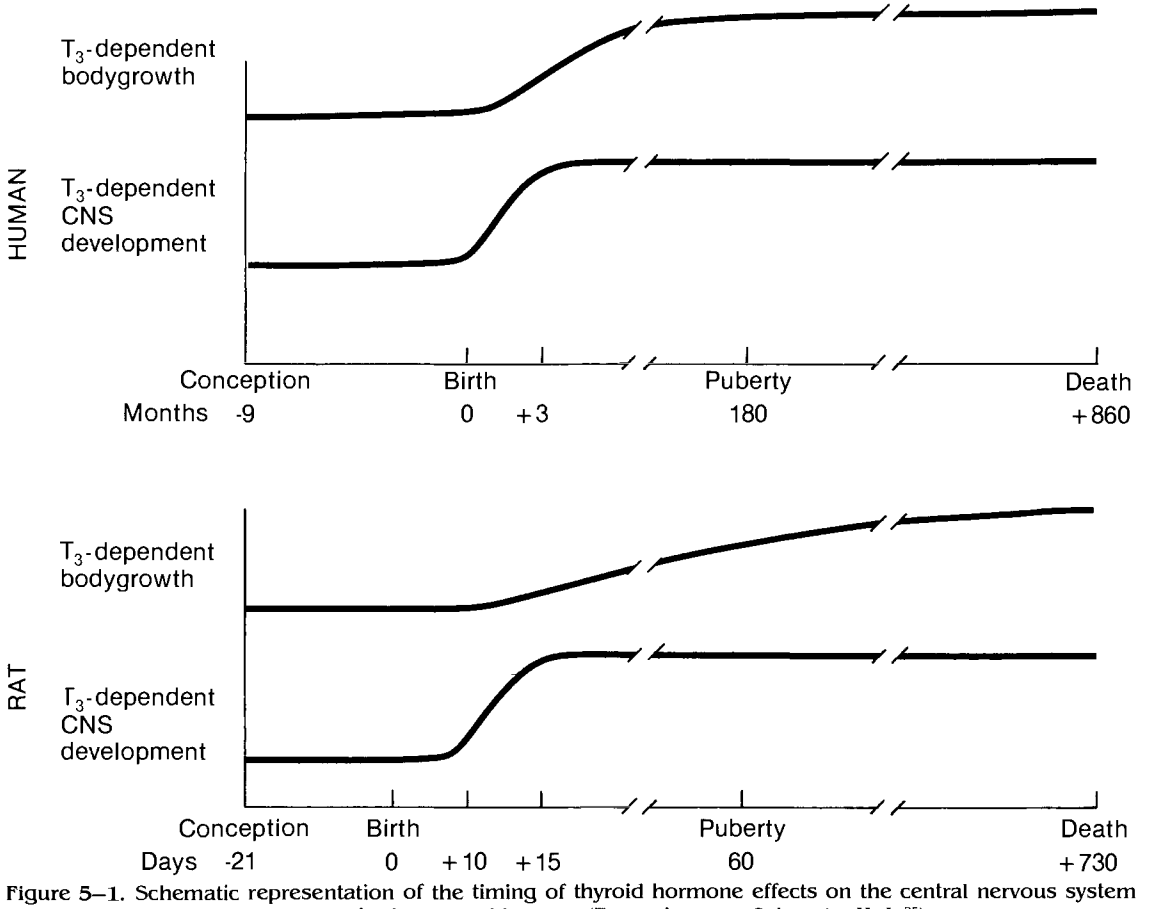


Figure 5–1. Schematic representation of the timing of thyroid hormone effects on the central nervous system and general somatic development in the rat and human. (For review, see Schwartz, H. L.[25])

malizing brain structure only if it is made available prior to this period.[31-34] Histologically, the neonatal hypothyroid state is characterized by a reduction in the branching of dendrites and axons and a deficiency in axonal myelin formation. Ruiz-Marcos and associates[35] have also reported that the number of spines on the apical shafts of pyramidal neurons is severely reduced in the hypothyroid state.

The thyroid-deficient state is also characterized by alterations in the morphology of specific regions in the brain, such as the cerebellum. In addition to a marked reduction in the arborization of Purkinje's cells, there is a delayed migration of the external granule cells towards the center of the cerebellar cortex. The finding of the general slowing in the developmental processes prompted Hamburgh and colleagues to suggest that the primary function of thyroid hormone in brain is to switch cells from a proliferative mode to one of differentiation.[36] These morphologic changes are accompanied by retardation in overall motor development and the orderly development of automatic behavior.[37]

Unfortunately, the biochemical basis of these changes remains obscure. As discussed subsequently, specific nuclear T_3 receptors are present in fetal, neonatal, and adult brain.[38, 39] In regard to conventional physicochemical and analogue binding characteristics,[38] these receptors are similar to receptors in other tissues. An increase in nuclear receptors occurs during the first 4 postnatal days. This surge occurs just prior to the increase in T_3, which takes place between days 4 and 8. Moreover, the increase in T_3 immediately precedes the appearance of the thyroid-induced structural changes that occur between days 10 and 15. This pattern of maturation is suggestive of the orderly appearance of components of the developmental machinery. The precise gene or genes that are regulated by the thyroid hormone in this organ, however, have not been identified.

The adult brain, along with testis and spleen, fails to respond with an increased oxygen consumption to the administration of thyroid hormone.[40] Although claims were advanced that the neonatal brain, in contrast to the adult brain, responded to T_3,[41] these have not been confirmed in later experiments.[39] Brain also fails to respond to thyroid hormones with an increase in the levels of malic enzyme and mitochondrial α-glycerophosphate dehydrogenase, enzymes that show a brisk rise in liver and that are expressed under basal conditions in brain. Several enzymes, however, do show changes in the hypothyroid neonatal brain and are normalized when thyroid hormones are administered prior to days 10 to 15 of neonatal life. These enzymes include succinic dehydrogenase, glutamate dehydrogenase, aspartate aminotransferase, γ-aminobutyric-acid transaminase, and cholinesterase. Furthermore, the enzymes involved in myelin synthesis are diminished in proportion to the reduction in total brain myelin content. (For review, see Schwartz.[25])

Nunez and colleagues have suggested that thyroid hormones may have direct effects on the formation of proteins that are responsible for microtubular organizations.[42] Both glial and neuronal cells have been reported to contain nuclear T_3 receptors, and thyroid hormones produce structural effects on both cell types in culture.[43, 44] Since glial cells are known to exert a profound influence on the maturation and organization of neuronal cells, it is impossible from currently available data in the whole animal to infer whether the reported structural or biochemical effects of the neuronal cell in hypothyroidism are mediated by an antecedent effect of thyroid hormone on glial cells. Moreover, the possibility that growth factors mediate these changes is under active investigation.[45] These considerations emphasize the general dilemma encountered in attempting to dissect the molecular circuitry responsible for the observed effects of thyroid hormone. Resolution of these issues probably will be contingent on the development of appropriate cell culture systems to allow a detailed biochemical and structural analysis of these processes under highly standardized conditions.

Energy Metabolism, Oxygen Consumption, and Thermogenesis

As previously discussed, there is an historical association among the action of thyroid hormones, energy metabolism, and thermogenesis. The thyroid hormone–induced augmentation in oxygen consumption observed by respirometry in the whole animal is reflected by an increase in oxygen consumption of tissue slices and homogenates.[40] As previously noted, the only tissues that appear not to respond to thyroid hormones with an increase in oxygen consumption are brain, testis, and spleen. The

increase in respiration characterizes the transition both from the hypothyroid to the euthyroid and from the euthyroid to the hyperthyroid states.

Since the oxidative processes are carried out primarily in the mitochondria, the administration of thyroid hormones is accompanied by an augmentation in the rate of mitochondrial oxygen consumption. The close association of the mitochondrion with energy metabolism and oxygen consumption has for many years attracted the attention of investigators interested in elucidating the mechanism of thyroid hormone action.[46–48] Thyroid hormone treatment also results in an increase in the number and size of mitochondria and in an enlargement of its inner surface and of the mitochondrial complement of many enzymes involved in energy and intermediary metabolism.

A fundamental problem, however, has consistently faced investigators interested in defining the mechanism by which thyroid hormones increase oxygen consumption and thermogenesis. This issue touches on the quantitative relationship between oxygen consumed and high energy phosphate produced by oxidative phosphorylation. Under normal circumstances, the moles of ATP generated/moles of oxygen consumed (P:O ratio) is fixed at a value of approximately 3 and is considered to be "tightly coupled." Early investigators had suggested that the hyperthyroid state was associated with mitochondrial uncoupling, thus allowing fewer high energy phosphates to be formed for each mole of oxygen consumed. In this fashion, more heat would be generated per erg of useful work performed. Although this hypothesis was attractive, it could not be experimentally verified.[49] Uncoupling of oxidative phosphorylation by thyroid hormone could be demonstrated in mitochondria only in the presence of suprapathologic concentrations of T_3. Furthermore, there appeared to be no difference in respiratory control. The augmentation of mitochondrial respiration produced by ADP was not influenced by the thyroidal status of the animal.

The recognition of normal mitochondrial coupling and respiratory control posed a fundamental conceptual problem in understanding thyroid-induced thermogenesis. The doctrine of tightly coupled oxidative phosphorylation presumes that the respiratory rate of the mitochondrion is determined by the ADP generated by the dephosphorylation of ATP by the tissues. This mechanism would thus keep energy expenditure and utilization in balance. Thus, it follows that the ATP generated by the mitochondrion as a consequence of thyroid hormone must drive definable energy-requiring processes.

Edelman has proposed that an important component of these energy-requiring processes is the maintenance of the Na/K gradient between the cellular and extracellular compartments. (For review, see Guernsey and Edelman.[50]) The gradient is maintained by the Na/K ATPase system. Thyroid hormone is postulated to induce the formation of Na/K ATPase pump units and to increase the formation of ADP, which in turn stimulates mitochondrial metabolism to generate the high energy phosphate required to maintain the substrate for the augmented ATPase activity. This hypothesis was based on the finding that addition of ouabain, a specific inhibitor of Na/K ATPase, to incubated tissue slices of thyroid hormone–treated animals resulted in a substantial reduction in thyroid hormone–augmented oxygen consumption.

Sestoft has challenged this hypothesis on the grounds that the ouabain-inhibitable Na/K ATPase is artifactually high in tissue slices.[51] In perfused liver, ouabain inhibits only about 10% of the thyroid hormone–induced augmentation in oxygen consumption. He suggests that the increased sensitivity of thyroid hormone–induced oxygen consumption to ouabain is related to the leak of Na and K through the cut surfaces of the tissue slices. When oxygen consumption and ouabain sensitivity were measured in primary hepatocyte cultures in which cells were presumably not "leaky," the results of one study showed substantial maintenance of ouabain sensitivity.[52] Such sensitivity could not be confirmed in another study.[53] Sestoft has argued that the "sink" for the increased production of ATP is the futile metabolic cycles that are stimulated by thyroid hormone, cycles in which both the formation and the degradation of substances are stimulated simultaneously.[51] In particular, he has proposed that the major ATP-consuming cycle is the one involved in triglyceride formation and lipolysis. Another futile cycle, fatty acid synthesis and degradation, will be subsequently considered as a potential energy sink.

Regardless of the precise mechanisms involved, there can be little doubt that thyroid hormones play a critical role in the maintenance of constant body temperature in the homeotherm. The constancy of plasma thyroid

hormone levels in the homeotherm may be a reflection of this role.

Carbohydrate Metabolism

In the past, the interrelationship of thyroid hormones and carbohydrate metabolism has been most often perceived in the context of abnormalities in blood glucose concentrations under fasting conditions or in the course of a standardized glucose tolerance test. (For review, see Mariash and Oppenheimer.)[54] Occasional elevations in the fasting values are seen in clinical hyperthyroidism, and fasting hypoglycemia is seen in hypothyroidism. On the one hand, in hyperthyroidism, a brisk rise in blood glucose concentrations following administration of a glucose load indicative of a "deterioration" of the glucose tolerance curve has been attributed to an enhanced rate of gastrointestinal absorption.[55] On the other hand, in hypothyroidism, a diminished rate of absorption may result in a flat glucose tolerance curve. More often than not, however, the glucose tolerance is normal in hyperthyroidism and hypothyroidism. When hyperthyroidism occurs in a diabetic patient, the diabetic state is frequently aggravated with an increased tendency to ketosis, possibly as a consequence of a thyroid hormone–induced augmentation in the rate of beta oxidation of fatty acids.

As indicated, the hyperthyroid state is accompanied by a stimulation of several futile cycles. Both glycolysis and gluconeogenesis appear to be augmented by thyroid hormones. (For review, see Müller and Seitz.[56]) The increase in gluconeogenesis occurs in part as a result of the stimulation of key gluconeogenic enzymes, including phosphoenolpyruvate carboxykinase, pyruvate carboxylase, and glucose-6-D-phosphatase. Furthermore, gluconeogenesis may be assisted by an increase in the level of the mitochondrial enzyme α-glycerophosphate dehydrogenase, which by increasing mitochondrial H^+ import facilitates the mitochondrial export of malate derived from intramitochondrial pyruvate. This arrangement thus represents a mechanism for the conversion of intramitochondrial pyruvate to glucose. The process can also be achieved by an augmented conversion of pyruvate to oxaloacetate and its subsequent transamination to aspartate, which can readily exit the mitochondria. Increased gluconeogenesis also results from enhanced formation of lactate as a consequence of increased glucose metabolism by muscle.

The hyperthyroid state is characterized by a depletion of glycogen content, presumably because of an accelerated rate of degradation and a diminished rate of synthesis.[57, 58] Curiously, however, glycogen content is also decreased in the hypothyroid state.[59–60] The precise biochemical factors that determine glycogen metabolism in altered thyroid states have been only partially elucidated. It appears possible that the effects of thyroid hormones are mediated by altered hepatic glucagon binding, by the levels of α- and β-adrenergic receptors, or by the activities of the enzymes involved in glycogenolysis.

The interaction of thyroid hormone and one or more products of glucose metabolism in the induction of specific mRNA sequences is considered subsequently in connection with the general molecular mechanisms responsible for thyroid hormone action.

Lipid Metabolism

Alterations in lipid metabolism provide other examples of the stimulation of futile cycles by thyroid hormones. (For review, see Müller and Seitz.[61]) From a clinical point of view, perhaps the best recognized are the changes in the level of serum cholesterol and low density lipoprotein (LDL) that occur in hypothyroid and hyperthyroid states.[62–65] A profound increase in total and LDL cholesterol in hypothyroidism may be used as a biologic marker in the treatment of hypothyroidism with thyroid hormone. Conversely, the level of LDL and total cholesterol is depressed in hyperthyroidism.

Isotopic studies have indicated that thyroid hormones increase the rates of cholesterol synthesis and degradation. Since the increase in fractional degradation exceeds the increase in total synthesis, the levels of both LDL and total cholesterol fall. Thyroid hormone induces one of the key enzymes involved in cholesterol biosynthesis, β-hydroxy-methyl-glutaryl-CoA-reductase, which catalyzes conversion of β-hydroxy-methyl-glutaryl-CoA to mevalonate.[66] In addition, thyroid hormone stimulates mevalonic acid incorporation into cholesterol.[66] The augmented fractional disposition of cholesterol is due to several factors. In the hyperthyroid state, there is augmented excretion of cholesterol in bile and feces.[65] In addition, turnover of LDL increases,[67] possibly as a consequence of a T_3-induced increase in LDL receptors[68] and an augmented rate of internalization of the LDL cholesterol complex. Op-

positely directed changes characterize the hypothyroid state with a diminished rate of synthesis and a concomitant decrease in fractional turnover of cholesterol, which is due to diminished biliary excretion and LDL clearance by peripheral tissues.

The capacity of thyroid hormone to lower the level of serum total and LDL cholesterol has prompted the clinical application of thyromimetic substances for the treatment of hyperlipidemia in euthyroid patients. A major problem in the use of such agents is the potential cardiotoxicity associated with therapeutic doses of levothyroxine and L-triiodothyronine, an especially important consideration in hyperlipidemic patients who by virtue of their disease are particularly prone to clinical or subclinical coronary artery disease.[69] For this reason, clinical investigators have exploited the use of analogues reputed to exhibit a selective hepatic effect. The dextro-enantiomer of T_4, especially, has been widely used for this purpose.[70] Further studies, however, have failed to confirm a selective hypolipidemic activity for this agent.[71] Moreover, these and other studies have suggested that in addition to lowering serum LDL cholesterol levels, thyroid hormones also tend to reduce the protective high density lipoprotein (HDL) cholesterol fraction. These findings thus have raised basic questions regarding the therapeutic efficacy of thyromimetic agents in the euthyroid hyperlipidemic state.

One of the most striking biochemical manifestations of thyroid hormone action is an increased rate of fatty acid synthesis and reesterification.[72] Consonant with the enhanced production of fatty acids is a five to sixfold augmentation in free fatty acid levels in the sera of hyperthyroid patients.[73] Conflicting reports, however, have appeared with regard to the concentrations of serum triglycerides in hyperthyroid patients. The increased rate of fatty acid synthesis by T_3 is related to the induction of lipogenic enzymes, including acetyl-CoA carboxylase, fatty acid synthetase, malic enzyme, and diglyceride-acyl-transferase as well as the hexose monophosphate shunt enzymes glucose-6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase, which supply nicotinamide-adenine dinucleotide phosphate (NADPH) for fatty acid synthesis. The detailed mechanism underlying the induction of these enzymes is considered subsequently in connection with the discussion of the molecular basis of thyroid hormone action.

Despite the increase in lipogenesis, thyroid hormones do not increase the production of very low–density lipoprotein (VLDL) triglycerides or the level of intracellular triglycerides.[74] The discrepancy between fatty acid and triglyceride synthesis may be due to a limitation in the availability of glycerol. Further, a normal cellular triacylglycerol content may be due to a thyroid hormone–augmented increase in the activity of lysosomal acid lipase. Lastly, thyroid hormone, by increasing the activity of outer carnitine palmitoyl transferase, facilitates the mitochondrial uptake and oxidation of free fatty acids.[75–78]

The increased levels of plasma-free fatty acids in the hyperthyroid state are probably due not only to an increased rate of lipogenesis but also to an augmented rate of adipose tissue lipolysis that is sensitized by thyroid hormones.[79] The mechanism responsible for the altered sensitivity to thyroid hormone may involve alterations in adrenergic receptor concentration, changes in the coupling between catecholamine binding and adenylate cyclase, and reduced phosphodiesterase activity.

Protein Metabolism

Very little quantitative metabolic and isotopic data are available regarding the overall effects of thyroid hormone on nitrogen and protein metabolism, despite the knowledge that thyroid hormones initiate their function largely by regulating protein synthesis at a pretranslational level. It is obvious that thyroid hormone administered to a young hypothyroid animal results in body growth and hence in a net positive nitrogen balance, and, conversely, that thyroid hormone administered in large quantities to an adult animal results in weight loss and a net negative nitrogen balance. Nevertheless, since it is entirely possible that augmented synthesis and degradation could occur simultaneously, additional balance and tracer studies are needed to quantitate the constitutive processes. The coexistence of increased synthesis and degradation could constitute yet another example of the futile cycles stimulated by this hormone.

In the hypothyroid rat liver, the level of protein, total RNA, and poly (A^+) RNA (the fraction of RNA that contains the predominant share of RNA) are all reduced to approximately half the level that exists in the euthyroid

state.[80] Further, replacement doses of T_4 or T_3 result in normalization of these parameters. Curiously, however, thyroid hormone administered to euthyroid animals results in no further increase in the level of total hepatic protein, total RNA, or poly (A^+) RNA. Thus, changes in total protein content appear to differ markedly from the behavior of a more limited set of proteins that is selectively stimulated or suppressed as a result of thyroid hormone action. Among high or medium abundancy mRNA sequences, Seelig and associates[81] have estimated that approximately 8% are modulated in such a selective fashion by T_3.

It should not be automatically assumed that the generalized alteration in protein content is due directly to hormonally induced variation in the expression of specific genes. Thus, proteolysis may play an important role in determining the overall protein content.[82] A 25% increase in the fractional rate of muscle protein content has been attributed to the action of protein-degrading enzymes, including cathepsin B, leucine amino peptidase, and *N*-acetylglucoaminidase. These enzymes, however, do not appear to play an important role in the heart, where hyperthyroidism leads to hypertrophy with an increase in protein mass. Thyroid hormones may also act either by changing the translational efficiency of the corresponding mRNA or by stabilizing the specific mRNA formed.[83] Such effects of thyroid hormone may represent "downfield" phenomena contingent on the activity of specific genes which are directly regulated by thyroid hormones. As discussed subsequently, the possibility of a direct extranuclear action, though unlikely, has not been ruled out.

Although it is often assumed that thyroid hormones shorten the half-time of various substances, this is certainly not universally the case. The fractional turnover of the hepatic enzymes malic enzyme and α-glycerophosphate dehydrogenase does not appear to depend on thyroidal state.[84] A potentially interesting feature of general hepatic protein metabolism in rat liver is the finding that in euthyroid rats, 40% of total hepatic protein is rapidly depleted by starvation.[85] This "labile pool" is contingent on normal thyroid function, since it appears to be absent in the hypothyroid liver and thus appears to account for the lesser percentage fall in liver protein when animals are subjected to food deprivation.

Effects of Thyroid Hormones on Tissue and Organs

Heart. Among the most striking effects of thyroid hormones are those that are manifested by the cardiovascular system. Tachycardia, cardiac hypertrophy, increased velocity and force of cardiac contraction, and increased cardiac output and peripheral vasodilation are the hallmarks of the hyperthyroid state, whereas bradycardia, diminished contractility, reduced cardiac output, and vasoconstriction are characteristic of the hypothyroid state. It is still not clear to what extent these changes are a direct effect of thyroid hormones on the heart and to what extent they are the indirect consequences of altered metabolic demands by the peripheral tissues. Probably both contribute to the observed effects. Furthermore, it appears probable that the effects of thyroid hormone on the cardiovascular system are also mediated at least in part by changes in the sensitivity to catecholamines produced by the altered thyroid status. These issues serve to complicate the analysis of the underlying mechanisms responsible for the observed phenomena.

A characteristic feature of the hyperthyroid state is the 30 to 50% increase in left ventricular weight and the associated increase in protein content. These increases appear to be due both to a greater rate of protein synthesis and to a diminished rate of protein degradation.[86] Further, treatment of hypothyroid rat heart with T_3 results in an increase in total and poly (A^+) RNA as well as protein. Dillmann and colleagues have analyzed the mRNA changes produced by thyroid hormone administration with the use of two-dimensional mRNA activity profiles.[87] They have shown that of 421 mRNA sequences examined, nine mRNAs increased with T_3 administration, whereas four decreased. Substantial attention has also been directed to the isoenzyme pattern of Ca^{++}-activated myosin ATPase in the various thyroid states. The activity of this enzyme correlates closely with the velocity of cardiac contraction. There are three such isoenzymes in the rat, designated V_1, V_2, and V_3. V_1 has the highest ATPase specific activity, whereas V_3 has the lowest. The normal rat heart is characterized predominantly by V_1, whereas V_3 is the major component in the hypothyroid heart. The expression of each of these enzymes appears to be determined completely by the level of the specific mRNA coding for each of

the forms.[88] Thus, V_1 myosin consists of two α-myosin heavy chains (MHC); V_3, of two β-MHC; and V_2, of one α- and one β-MHC. Further, the α- and β-forms appear to be two members of a multigene family that consists of six isoforms.[89] Further studies have shown that each of these forms appears to be regulated in a highly distinctive fashion, depending on whether atrial, ventricular, or skeletal muscle is analyzed. However, there is considerable species specificity in these isoforms,[90] and no myosin isoforms have so far been demonstrated in human tissue.

As indicated, there continues to be major controversy as to whether these effects are directly mediated by thyroid hormone. In favor of such a view is the existence of cardiac nuclear T_3 receptors, which appear to be indistinguishable from receptors in other tissues. Studies by Mahdavi and colleagues suggest that the direct addition of T_3 to cultured myocytes can mimic many of the effects produced by thyroid hormone in the intact animal.[91] In comparison, Klein and Hong[92] have claimed that when the heart is transplanted to a position where it ceases to pump blood, the T_4 treatment fails to results in cardiac hypertrophy, although the characteristic isoenzyme changes are maintained.

The striking similarity between the cardiovascular effects of thyroid hormone and catecholamines raised the possibility in the past that thyroid hormone effects were mediated by the catecholamines. Whereas such a hypothesis might serve to explain the therapeutic effectiveness of sympatholytic and β-adrenergic blocking agents, it is clearly untenable in view of the fact that the levels of circulating catecholamines in the hyperthyroid state are either normal or low.[93] However, thyroid hormones have been shown to increase the number of β-adrenergic receptors,[94] thus allowing thyroid hormone to sensitize cardiac tissue to the action of catecholamines.

Pituitary. The pituitary is clearly a major target organ of the thyroid. It is the site for negative feedback of TSH secretion, and studies have clearly shown that T_3 inhibits the expression of the genes that code for both the alpha and beta subunits of TSH.[95–98] The nuclear T_3 binding capacity in rat pituitary is high, and approximately 50% of total pituitary T_3 is bound to specific receptor sites.[99] The reduction in circulating TSH is related to nuclear receptor occupancy, and the transcriptional rate of the two genes that code for the alpha and beta subunits is inhibited following T_3 administration.[96] Since the reduction in the transcriptional rate is not blocked by the prior administration of cycloheximide, the effects of T_3 may not be contingent on the formation of an intermediary inhibitory protein as had been postulated.[100]

We have stressed the relationship between pituitary growth hormone production and the thyroidal state of the organism. Many of the growth-promoting functions of thyroid hormone are mediated by increased secretion of pituitary growth hormone. The mechanism by which thyroid hormones govern cellular events has been extensively studied in the rat pituitary tumor cell lines that produce growth hormone and is discussed in further detail subsequently.

Other Tissues and Organs. Although the molecular details of this process remain largely undefined, thyroid hormones play a critical role in regulating the levels of glycosaminoglycans and proteoglycans in connective tissue. In amphibian metamorphosis, thyroid hormone stimulates the activity of specific collagenases as well as that of several acid hydrolases including hyaluronidase, which are involved in the resorption of the tail and presumably other aspects of tissue remodelling. It is possible that an increased activity of these enzymes may be responsible for the decreased tensile strength of wounds in the hyperthyroid state. In hypothyroidism, the characteristic myxedema of the skin is due to the accumulation of glycosaminoglycans with the attendant retention of sodium, water, and protein. Deposition of such mucinous material may also be responsible for the serous effusions of the hypothyroid state and possibly for functional disturbances of other systems. Refetoff and colleagues (see Smith) have reported that deprivation of thyroid hormones stimulates the synthesis of the mRNA for these proteins.[101]

The lung is another target for thyroid hormone, although major abnormalities of pulmonary function are not prominent in most patients with hypothyroidism. However, in severe hypothyroidism, impaired oxygen diffusion has been noted. Nuclear receptors have been demonstrated in lung.[102] Fetal development of this organ may be linked to thyroid hormone regulation. The biosynthesis of the surfactants appears to be under the control of both thyroid hormones and glucocorticoids.[103, 104]

Renal function is clearly influenced by the thyroidal state of the animal. Diminished glomerular filtration is encountered in the hyponatremic state. The hyponatremia is in part due to a diminished fractional excretion of water and an increased distribution volume of total body water, whereas total body Na is actually increased. The effect of thyroid hormone on this organ is reflected at least in part by changes observed in the two-dimensional mRNA activity profiles.[105] The activity of several renal enzymes, including malic enzyme, mitochondrial α-glycerophosphate dehydrogenase, and ornithine aminotransferase, changes with thyroidal status. Ornithine amino-transferase appears to be regulated by T_3 and estrogen at the transcriptional level.[106]

The effect of thyroid hormone on the hematopoietic system has been well described.[107, 108] Most commonly, the anemia of hypothyroidism is normocytic and normochromic; however, because of concomitant pernicious anemia, the anemia is frequently macrocytic and hyperchromic. It is assumed that related immunologic mechanisms produce both disorders.

The effect of thyroid hormone on development of the brain has been emphasized. In the adult animal, CNS manifestations in the hypothyroid and hyperthyroid states are well described, and in humans such manifestations of thyroid hormone deficiency constitute a prominent component of the signs and symptoms that characterize these clinical states. Increased nervousness and agitation with a fine tremor of the outstretched hands are associated with hyperthyroidism, whereas slowness of mentation and drowsiness are features of hypothyroidism. The effects of altered thyroid states are reflected in the electroencephalogram (EEG), but the underlying cellular mechanisms remain obscure. Reports suggest that in hypothyroidism there is a reduction in blood flow and oxygen consumption,[109] whereas there appears to be no change in either parameter in association with hyperthyroidism.[110] The lack of knowledge of the specific biochemical mechanism responsible for the central effects of thyroid hormone is particularly vexing in view of the abundance of T_3 nuclear receptors in both neuronal and glial cells.[43, 44] Using the conventional criteria currently available, these receptors appear to be indistinguishable from those in other tissues.[38]

Similarly, thyroid hormone exerts important influences on the skeletal system with an increase and a decrease in bone turnover in the hyperthyroid and hypothyroid states. In hyperthyroidism, hypercalcemia can result from an increased rate of bone destruction. Thyroid hormones also exert an effect on the gastrointestinal system, with an increase in intestinal motility and an augmented rate of gastrointestinal absorption in the hyperthyroid state, with reverse manifestations in the hypothyroid state. The cellular basis of the effects has not been explored. Lastly, thyroid hormone is associated with an increased rate in the fractional turnover of drugs, metabolites, and other hormones with oppositely directed changes in hypothyroidism. Although these features have been loosely ascribed to the "hypermetabolic" and "hypometabolic" states, the fundamental underlying cellular mechanisms remain undefined.

Interactions of Thyroid Hormones and Catecholamines

Of particular interest have been the interactions of thyroid hormones with catecholamines. In many instances, there appears to be a synergistic relationship between catecholamines and thyroid hormones. Some of the clinical symptomatology of hyperthyroidism may be due to this interaction. As previously pointed out, in the heart this may be explained by a thyroid hormone–induced increase in the number of β-adrenergic receptors and in an increase in the affinity of β-adrenergic receptors.[94] Although measurements of cardiac receptors have been carried out only in animal models, observations with human mononuclear cells also point to an increase in β-adrenergic receptors and their affinity in the hyperthyroid state. Thyroid hormone administration, however, is not universally accompanied by an increase in adrenergic receptors. In contrast to the heart, there appears to be a fall in the number of hepatic β-adrenergic receptors, their affinity, and possibly their coupling to membrane adenylate cyclase.[111, 112] These findings may account for the partial resistance of the liver to glucagon and the isoproterenol effects in the hyperthyroid state. In general, the effects observed in the hypothyroid state are the opposite of those produced by excess thyroid hormone. Further studies of the interaction of thyroid hormone with the catecholamine system emphasize the necessity of examining such interactions in specific tis-

sues. The complexity of the receptor, transducer, and effector components of the system involved in initiating the actions of catecholamine presents many potential sites of interaction with the thyroid hormone system and may be the basis of the diversity of results observed.

MOLECULAR MECHANISMS

During the past two decades, advances in the field of molecular and cellular biology have spurred efforts to understand the molecular basis of thyroid hormone action. In large part, such efforts have centered on the early events that set into motion the series of biochemical events that ultimately result in the cellular phenomena described previously in this chapter. More recently, however, preliminary attention has also been directed towards establishing the nature of the molecular network that connects the early with the late events, as reviewed subsequently.

The Nuclear Initiation Hypothesis

General thought about the early events that characterize thyroid hormone action has been based on what might be designated as the "nuclear initiating hypothesis." This hypothesis has three components as follows: (1) that in any given biologic setting, all thyroid hormone effects can be understood in terms of the fraction of nuclear sites occupied and the duration of such occupancy; (2) that although the number of receptors varies from cell type to cell type, the receptors themselves are identical; and (3) that variation in the occupation of the receptor will change the expression of specific genes as reflected in the levels of the corresponding mRNA sequences and the proteins coded by such mRNAs.

We shall first review the experimental basis for this hypothesis and analyze some of the reservations that have been expressed by investigators. The possibility of extranuclear initiating mechanisms is considered. Current knowledge is summarized regarding the physicochemical and binding characteristics of the nuclear receptor, the relationship of the receptor to chromatin, and the nature of the receptors in other species. The kinetics and energetics of transport of T_3 into the nucleus will be considered. Techniques designed to identify specific mRNA sequences important in thyroid hormone action are discussed. Although only a few examples of thyroid-responsive mRNA sequences have so far been intensively studied, available information with respect to the induction of such mRNAs by T_3 and coordinate regulatory signals are summarized. The broad-based relationships between thyroid hormone and carbohydrate-generated signals at the hepatocellular level are emphasized and related to the phenomenon of thyroid hormone–induced oxygen consumption as discussed previously.

Nuclear Receptors in the Initiation of T_3 Action

Specific limited capacity nuclear binding sites for T_3 were first described in whole animal experiments designed to elucidate the kinetic relationship between iodothyronines in plasma and tissues and their subcellular components.[113] In particular, the ratio of radioactive nuclear T_3 to plasma T_3 was profoundly dependent on the mass of T_3 injected. This approach also allowed quantitation of the mass of plasma-derived T_3 associated with nuclei as well as determination of nuclear binding capacity in individual tissues. The nuclear/plasma ratio was determined at the so-called equilibrium time point, when the specific activity of the nuclear T_3 can be assumed to be identical to that in plasma. This point is identified by the peak value of the nuclear accumulation curve, when the rate of entrance is equal to the rate of exit (Fig. 5–2). Thus, under strict tracer conditions, the product of the nuclear/plasma ratio is corrected for nonspecific binding, and the radioimmunoassayable T_3 in plasma yields the mass of plasma-derived T_3 bound to the specific sites. By progressively increasing the dose of nonradioactive T_3 injected, it is possible to compute the nuclear binding capacity in an individual tissue.

These *in vivo* approaches yielded the following estimates for the nuclear binding capacities in various tissues (ng/mg DNA): liver, 0.6; kidney, 0.4; heart, 0.4; brain 0.3; and pituitary, 0.8.[99] These estimates correspond to approximately 4000 nuclear receptors per diploid hepatic cell. Exceedingly low values were observed in spleen and testis, two classically nonresponsive tissues. These original studies suggested that in the receptor-containing tissues, approximately half of the available nuclear sites were occupied. However, in the

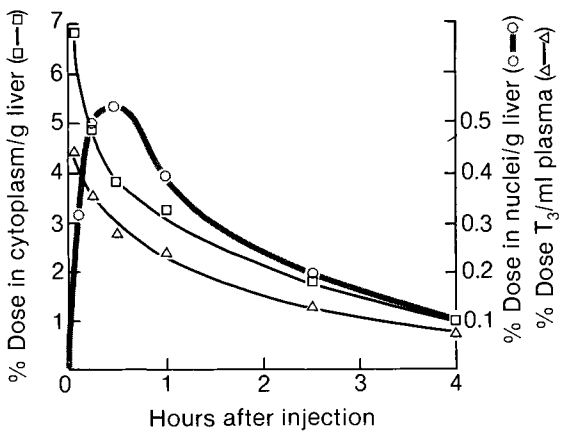


Figure 5–2. Rat plasma, hepatic cytoplasmic and nuclear concentrations of ^{125}I-T_3 following the intravenous injection of a tracer dose. At 30 minutes, the nuclear concentration reaches a peak and the rate of delivery of T_3 equals the rate of exit from the nuclear compartment. At this time, the specific activity of the nuclear T_3 can be presumed to equal that in cytoplasm and plasma. (Redrawn from Oppenheimer, J. H., et al.: Limited binding capacity sites for L-triiodothyronine in rat liver nuclei: Nuclear-cytoplasmic interrelationship, binding constants, and cross-reactivity with L-thyroxine. J. Clin. Invest. 53:768–777, 1974.)

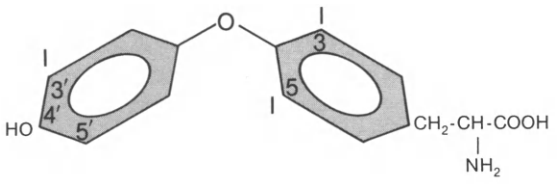
case of the pituitary and brain, this turned out to be an underestimation since the contribution of local tissue monodeiodination was not recognized.[114] Recalculation suggests that in brain, 70 to 90% of the available sites are occupied in the euthyroid state.[115] In liver and kidney, however, the rapid forward rate constant of T_3 from plasma to tissue minimizes the effects of local deiodination, and the original estimates of nuclear T_3 mass in these tissues have been confirmed by direct radioimmunoassay and isotopic equilibration as well as by independent displacement measurements.[116]

Analogous calculations indicate that only approximately 16% of the total cellular T_3 within the liver is specifically bound to nuclear receptors. This accounts for the fact that the ratio of hepatic/plasma radioactive T_3 is not detectably influenced by the mass of T_3 injected.[117] In comparison, however, more than 50% of pituitary T_3 is associated with specific nuclear binding sites, thus explaining the influence of injected T_3 mass on the pituitary/plasma radioactive T_3 ratio.[117]

The kinetics of accumulation of nuclear T_3 in liver have also been examined.[118] Following the intravenous injection of tracer radioactive T_3, the content of rat hepatic nuclear T_3 increases, with maximal levels attained approximately one-half hour subsequently (see Fig. 5–2). As outlined in Chapter 4, the equilibration between plasma and extranuclear T_3 in this tissue is almost instantaneous. In other tissues, such as the brain, equilibration between tissue and plasma T_3 proceeds more slowly, but the kinetics of intracellular mixing between nuclear and extranuclear components show the same general properties as those displayed by the liver. The observed accumulation curve of nuclear T_3 appears to be determined primarily by the rate of dissociation of T_3 from the nucleus. This occurs as a first-order process with a half-time of approximately 15 minutes. Equilibration of nuclear with extranuclear T_3 occurs more rapidly with the injection of larger quantities of T_3, since the forward rate constant is a second-order process.

The initial studies demonstrating specific nuclear sites in rat tissues were soon confirmed and shown to be present in rat cultured pituitary tumor cells (GH and GC cell lines).[119, 120] These observations were particularly important in providing a readily available model for analyzing the relationship between nuclear occupancy and response under controlled culture conditions. The *in vivo* and tissue culture studies were soon supplemented by direct measurement of nuclear binding, using isolated nuclei and solubilized receptors. Important to the demonstration of specific binding by isolated nuclei was the treatment of nuclei with the detergent Triton X-100, which serves to remove the outer nuclear membrane. Apparently, during the process of subcellular fractionation, nonspecific T_3 binding sites are generated on this membrane, a phenomenon that obscures the detection of the specific nuclear receptors. This is the probable reason for the failure of previous efforts to detect specific nuclear binding sites.

Analysis of isolated nuclei incubated in an aqueous buffer of pH 7.4, using standard Scatchard plots, revealed a single binding component with an apparent affinity of $1 \times 10^{10}\ M^{-1}$ and with a binding capacity of 1 picomole/mg DNA, a value very similar to that obtained with saturation analysis in the intact animal.[121] Similar results were obtained when the binding properties of solubilized receptors were analyzed with a G-25 Sephadex column or an anion-exchange resin. It is important to emphasize the predominance of specific binding in isolated nuclei or solubilized preparations. Thus, in general, less than 10% of the total nuclear binding is "nonspecific."



1. Phenolic Ring Requirements
   4′ OH group
   3′ Substitution: halogen or nonhalogen
2. Activity of distal > Prox. conformation
3. Activity of 3′ > 3′, 5′ Substitution
4. Ether linkage: Substitution of O by:
   (a) methylene or
   (b) sulfur results in active compounds
5. Decreased activity after substitution of halogens in inner ring
6. Halogen-free compounds have low but significant activity

Figure 5–3. Summary of the binding characteristics of thyroid hormone analogues to the nuclear receptor. In each instance, the binding relationships are reflected in corresponding relationships of the biologic effect.

The availability of *in vitro* assay systems allowed an extensive analysis of the displacement properties of T_3 analogues (Fig. 5–3) and a fuller characterization of the physicochemical characteristics of the nuclear sites.[122, 123] Labelled thyroxine (T_4) appeared to bind to the same sites: binding analysis yielded the same capacity as that of T_3 but with an affinity some 10- to 20-fold less. Reverse T_3 (3′,5′,3-triiodothyronine) showed only 1/1000 the displacement activity of T_3. This could probably be attributed to contamination of the reverse T_3 preparation with trace quantities of more active analogues. Monoiodothyronine, diiodothyronine, and the iodotyrosines were essentially without displacement effect. Very active displacement was associated with the acetic acid analogue of T_3, triac, and with the isopropyl analogue, 3′isopropyl-3,5,-diiodothyronine. As a generality, compounds substituted with a single iodine substituent in the phenolic iodothyronine ring were more effective in displacing radioactively labelled T_3 from nuclear binding sites than were compounds that possessed iodine substituents in both the 3′ and the 5′ positions. Of interest was the finding that weak but significant displacement activity was associated with compounds completely lacking in iodine substituents, with methyl groups replacing the iodine atoms of T_3. Similarly, a substitution of the oxygen-ether linkage with a disulfide group yielded a compound with measurable displacement activity. An additional requirement for displacement of labelled T_3 was the presence of an undissociated hydroxyl group on the phenolic ring of the iodothyronine molecule. Lastly, compounds constrained in the distal conformation were more potent than compounds constrained in the proximal. In general, the displacement activity corresponded with the known biologic activity. Several exceptions to this correlation are considered next. Also, there appeared to be a general correspondence between displacement activity of thyroid hormone analogues determined in the whole animal and *in vitro* nuclear displacement assays. Moreover, the relative analogue spectra obtained from nuclei from various tissues and from the GH1 cell line appeared identical.[124] The extensive knowledge accumulated from the study of over 40 thyroid hormone analogues has facilitated the projection of the steric configuration of the receptor site.[125]

The solubility properties and the pattern of susceptibility to various proteolytic enzymes facilitated the identification of the receptor as a nuclear nonhistone protein (Table 5–2).[126] There are two types of nuclear proteins, five basic histones, and many hundreds of acidic nonhistone proteins that are believed to be involved in gene regulation. Gel filtration together with sedimentation through a sucrose gradient and an assumed partial specific volume for protein led to the estimation of an M_r of 50,500 daltons, a sedimentation rate of 3.5 S, and a frictional coefficient of 1.4.

Despite the fact that the nuclear T_3 receptor has been recognized since 1972, efforts to isolate the protein have not until recently been successful. A substantial degree of purification (up to 600-fold) was reported in 1981 using a variety of standard separatory approaches including affinity chromatography.[127] Calculations based on the assumption of a single binding site per molecule and the known number of sites per nucleus have suggested that a

Table 5–2. Properties of the Rat Hepatic Receptor*

Molecular weight	50,500
Sedimentation coefficient	3.5 S
Stokes radius	3 nM
Frictional coefficient	1.40
Binding capacity	1 pmol/mg DNA
Association constant	$\sim 1.30 \times 10^9\ M^{-1}$

*Common physicochemical properties of the nuclear T_3 receptor.

20,000- to 40,000-fold purification is necessary for isolation. The apparent instability of the receptor with progressive purification has contributed to the problem. Addition of histone proteins appears to contribute to receptor stabilization, and this may have some importance in maintaining receptor structure and function *in situ*.[123]

The necessity of isolating the receptor by conventional separatory techniques may have been obviated by the recognition that the receptor is related in structure to the superfamily of genes that codes for steroid-binding proteins.[128–130] Two reports have shown that the receptor may be coded by c-erb A proto-oncogenes (Fig. 5–4).[128–129] It should therefore be possible by recombinant DNA techniques to synthesize virtually unlimited quantities of the receptor for use in structural and functional studies. Further, these reports have suggested the possibility that there may be more than one gene coding for T_3 nuclear receptors and that closely related but distinctive forms of the nuclear receptor may serve specific functional roles. If so, this would prompt a reexamination of the widely held assumption of the existence of only a single receptor species.

Studies carried out independently in three laboratories have shown that the receptor is a component of a substantially larger structure with a sedimentation value of 5.5 to 6.0 S.[131–133] The larger receptor-containing complex was demonstrated by digesting chromatin prelabelled with radioactive T_3 with two endonucleases, micrococcal nuclease and pancreatic DNase I. The receptor-containing complex was preferentially liberated compared with the digestion of bulk chromatin, a finding that suggests the receptor is situated in a portion of chromatin, which is accessible to the action of the endonucleases. By analogy to polymerase II, which is involved in the transcription of genes, it is believed that differential susceptibility of a structure to the action of endonucleases connotes that the structure is situated in actively transcribing DNA rather than in supercoiled portions of DNA, which appear to be more protected against enzymatic attack. Further, the finding that micrococcal nuclease readily digests the complex suggests that it is situated in the "linker" region of DNA, i.e., in the region of DNA situated between two adjacent histone core particles. Detailed studies further suggest that in addition to the receptor, the complex contains additional protein as well as a stretch of some 20 base pairs of DNA.

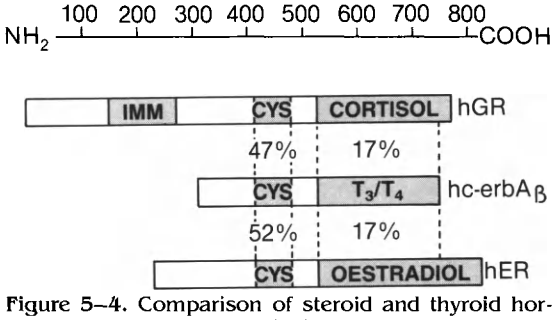


Figure 5–4. Comparison of steroid and thyroid hormone receptors. Three principal domains are recognized for the human glucocorticoid receptor (hGR), the putative thyroid hormone receptor considered to be identical to the hc-erb Aβ protein, and the human estrogen receptor (hER). These include an immunogenic region (IMM), a cystine-rich region (CYS) involved in DNA binding, and a hormone binding region. The percentages of homologous amino acids are indicated. (Adapted from Weinberger, C., et al.: The c-erb-A gene encodes a thyroid hormone receptor. Nature 324:641–646, 1986.)

The early studies showing what appeared to be excellent uptake of labelled T_3 by isolated nuclei incubated in an aqueous medium, which was devoid of an energy-producing system, suggested that active transport was not essential for the accumulation of T_3 by the nuclear binding sites.[134] This conclusion was reinforced by experiments that failed to demonstrate any influence of metabolic inhibitors on the nuclear uptake of T_3 by the incubated nuclei. The inference was therefore drawn that in the intact cell as in the isolated nucleus, an energy-dependent system did not participate in the nuclear accumulation of T_3.

Several subsequent developments, however, suggest that this conclusion was drawn prematurely. Thus, the discrepancy between the biologic potency and nuclear binding affinity of the levo and dextro enantiomers of T_3 has been attributed to the selective accumulation of the L-form by nuclei in the intact animal.[135] The biologic potency of L-T_3 exceeds that of D-T_3 by a factor of 5 to 6, using as an index the level of activity of hepatic and cardiac α-glycerophosphate dehydrogenase. In contrast, *in vitro* binding studies show only a relatively minor increase in the binding of L-T_3 as compared with D-T_3.[135] This, therefore, constitutes an exception to the correlation between *in vitro* nuclear binding and the biologic effect previously discussed. Unlike other discrepancies between nuclear binding and biologic effect, such as those presented by triac, T_4, and 4′-deoxy T_3, the discrepancy could not be

explained by differences in the metabolism of the enantiomers. ^{125}I-L-T_3 and ^{125}I-D-T_3 displayed identical terminal plasma disappearance curves.[135] Moreover, the relative reduced biologic potency of D-T_3 could not be attributed to a failure of the D-enantiomer to penetrate the liver or heart. The discrepancy, however, was explained by the finding that in the intact animal liver and heart nuclei, there was a six to sevenfold preferential accumulation of L-T_3 as compared with D-T_3. This suggested the possibility that in the intact animal an active stereospecific transport system might be responsible for the differential accumulation of L-T_3 by hepatic and cardiac nuclei.

Support for this explanation came from estimates of the ratio of the free hormone concentration in the nucleus and in the cytoplasm.[136] The free hormone concentration in the cytoplasm was calculated on the basis of equilibrium dialysis of cytosol, measurements of the T_3 content by radioimmunoassay, and estimates of cytosol volume. The free T_3 concentration in the nucleus could be calculated by application of the law of mass action, since the fraction of specific sites occupied is known from previous isotopic studies, and the affinity constant of nuclear binding at 37°C could be directly determined from displacement experiments carried out with isolated nuclei incubated in an aqueous medium.

The results of these calculations are summarized in Table 5–3 and show the existence of a major free L-T_3 gradient from the cytosolic to the nuclear compartments, varying from a value of 58 in liver to 251 in brain.[136] In each tissue, the free L-T_3 gradient substantially exceeds the corresponding free D-T_3 ratio, a finding compatible with a stereospecific transport system. The approach taken also allows quantitation of the plasma/cytosolic free hormone ratios. The results of these estimates are also provided in Table 5–2. Unlike the steep gradients observed for free L-T_3 in the transition from cytosol to nucleus, the ratio of cytosolic/plasma free L-T_3 is relatively modest. The highest value is 2.8 for liver, but because of inherent experimental error it is not clear whether this value differs significantly from unity. With respect to D-T_3, however, major free hormone gradients from plasma/cytosol are apparent in liver and kidney. This is simply a reflection of the well-known tendency of these tissues to concentrate D-T_3.[136a] In other words, the high tissue/plasma ratios for total D-T_3 in these tissues are not reflections of increased protein binding by liver and kidney.

Table 5–3. Free Triiodothyronine Concentration Ratios*

Tissue	Cytosol/plasma		Nucleus/cytosol	
	L	D	L	D
Liver	2.80	21.60	58.2	3.70
Kidney	1.17	63.30	55.9	1.54
Heart	1.49	2.07	80.6	24.90
Brain	0.90	0.32	251.0	108.60

*Free T_3 concentrations in plasma, cytosol, and nuclei were determined as described by Oppenheimer, J.H. and Schwartz, H.L.: J. Clin. Invest. 75:147–153, 1985.

Little is currently known about the biochemical basis of the nuclear transport system, nor is it clear whether this system contributes to the variation in the accumulation of T_3 by nuclei under various pathophysiologic settings. Another issue that arises in connection with this system is the possibility that selective analogue accumulation in certain tissues could occur despite the ostensible identity of nuclear binding sites in various tissues. Recent studies have suggested other examples of differences between *in vivo* and *in vitro* binding. The nuclear transport system also appears to operate in freshly isolated hepatocytes[137] and in cultured GH cell lines[138] but with very much reduced cytoplasmic/nuclear gradients compared with what is observed *in situ*. Thus, the nuclear/cytosolic free hormone gradient in the freshly isolated hepatocyte is only approximately six instead of 52.[137] As a consequence, the free hormone concentration in the medium must be higher than in the plasma of the intact animal in order to achieve a similar fractional nuclear occupancy. The transport systems in GH1 cells also provide an explanation of previous observations that intact incubated GH1 cells show poor accumulation of D-T_3 in comparison with L-T_3, whereas the relative binding of the enantiomers by isolated nuclei were approximately the same.[139]

The operation of the nuclear transport system probably also serves to resolve the long-standing discrepancy between the apparent nuclear association constant as determined by *in vivo* techniques, approximately $4 \times 10^1\ M^{-1}$,[118] and the association constant determined at 37°C, using either whole isolated nuclei or solubilized preparations, approximately $1 \times 10^9\ M^{-1}$.[134–136] Although the original explanation of the discrepancy was based on the argument that the ionic conditions *in vitro* failed to simulate adequately the *in situ* situation, this is probably not the case. Under multiple in-

cubation conditions used by many investigators, the association constant has never been reported to equal the value originally estimated *in vivo*. Another way of looking at this problem is to suggest that the mass of T_3 associated with the nuclei *in situ* simply cannot be explained by the association constants commonly derived by accepted *in vitro* saturation techniques. A step-up function from cytosol to nucleus appears to provide a convenient resolution, one which receives additional support from the stereoselective nature of *in vivo* nuclear accumulation.

These considerations thus prompt a reevaluation of the original interpretation of the *in vitro* nuclear accumulation data. Although isolated nuclei incubated *in vitro* can readily accumulate T_3 without the help of an active transport system, the *in vitro* is not a faithful model of the situation as it exists in the intact animal. It appears possible that in the process of subcellular fractionation, there is disruption of an energy-dependent system and perhaps the integrity of the nuclear envelope.

In many physiologic systems, the receptor, in addition to playing an initiating role in hormonal action, also serves to modulate the extent of hormonal expression at the tissue level. For instance, many polypeptide hormone receptors show the phenomenon of "down regulation" in which receptor number varies inversely with the hormonal stimulus. Such a system serves to stabilize the cellular impact of the hormonal stimulus. Whether such a system also operates with respect to thyroid hormones is not clear. In the case of cultured GH1 cells, there does appear to be at least partial down regulation. With increasing concentrations of T_3 in the medium, there is a progressive reduction in the number of nuclear T_3 receptors. With maximal hormonal stimulation, approximately half of the receptors are depleted.[139] The mechanisms underlying this process have been extensively examined by the use of dense amino acid labelling techniques, and the lower receptor concentrations achieved appear to result from both a diminished synthesis and an augmented rate of fractional turnover. In the intact animal, however, there appears to be no major differences in hepatic nuclear receptor concentration in the hypothyroid, euthyroid, and hyperthyroid states.[140] It is possible that in this situation changes in the rate of synthesis match changes in the rate of degradation without alteration in the net receptor number. The average half-time of the hepatic receptor as estimated in euthyroid animals by blocking protein synthesis with cycloheximide is 4 hours.[141, 142] No estimates of turnover rates in hypothyroid or hyperthyroid animals are available.

Several additional factors may be involved in regulating the level of thyroid hormone receptors. Thus, starvation reduces the hepatic nuclear binding capacity by 30%,[143, 144] and a similar degree of reduction has been reported to follow glucagon administration and to accompany partial hepatectomy.[143] The technical limitations in measuring receptor number, however, deserve emphasis. Thus, in starvation, for instance, there appears to be an activation of proteolytic enzymes, the effect of which may still be apparent when the isolated nuclei are incubated with T_3 in order to determine the Scatchard plot. It is possible, therefore, that the 30% reduction may be an overestimation.

Several studies have been directed towards the measurement of lymphocyte T_3 nuclear binding parameters because of the obvious clinical interest attached.[145, 146] In each instance, the number of receptors measured was exceedingly low, generally less than 200/nucleus. These sites do not readily lend themselves to clinical analysis, because of both their paucity and susceptibility to proteolytic digestion. A report suggesting alterations in receptors in obesity[147] could not be confirmed.[146]

Evidence Favoring the Nuclear Site of Initiation of Thyroid Hormone Action

In the preceding discussion, the terms "nuclear binding site" and "receptor" have been used interchangeably, with the tacit assumption that the site of initiation of thyroid hormone action is the specific nuclear site under consideration. The evidence supporting this view is now summarized.

Nature and Location of the Sites. In general, hormone receptors have the properties of limited capacity, high affinity sites, and nuclear binding sites for T_3 that certainly qualify in this regard. However, it should not be assumed that limited capacity, high affinity binding proteins necessarily represent receptors involved in the initiation of hormone action. As an example, the binding proteins discussed in Chapter 4 are high affinity, low capacity sites but are clearly not involved in hormone action

at the cellular level. The evidence previously discussed that the receptors are nonhistone nuclear proteins, which are believed to function in regulating the expression of information encoded in DNA, however, makes the potential role of specific nuclear receptors a particularly attractive hypothesis, especially in view of the data that suggest the receptor-containing complex is situated in the portion of DNA that appears to be actively transcribed.

Widespread Distribution of the Nuclear Receptors Among Thyroid Hormone–Responsive Tissues. Nuclear receptors have now been demonstrated in rat tissues, including liver, heart, kidney, brain, spleen,[99] and lung.[102] Only in testis is there a virtual absence of receptors. Insofar as tests have been carried out, the analogue binding spectrum and the physicochemical properties of the receptors in the various tissues appear to be identical. Although it is readily apparent that all thyroid hormone–responsive tissues should contain receptors, we cannot assume that nonresponsive tissues are necessarily devoid of receptors. Although traditionally spleen, brain, and testis have been classified as nonresponsive tissues, the criteria have been limited to oxygen consumption and the behavior of enzymes commonly used as thyroid hormone indices, α-glycerophosphate dehydrogenase and malic enzyme. As pointed out previously in this chapter, there are excellent reasons for suspecting that the appropriate biochemical parameters of thyroid hormone action in the brain have not as yet been identified. As a consequence, only a limited correlation is possible.

Despite this, the universal distribution of thyroid hormone receptors in all species in which thyroid hormones have been identified speaks to the potential importance of these sites to the mechanism of action of these hormones. Again, using the criteria of analogue spectra and the standard physicochemical properties of molecular weight and sedimentation constants, the sites appear to be identical. Whether more subtle differences in structure or amino acid sequence will be shown with the application of more sophisticated techniques remains to be determined. Similar nuclear binding sites have been demonstrated in a wide variety of species starting with the most primitive vertebrates.[18, 19] The recent description of specific nuclear sites in the lamprey with analogue spectra and physicochemical characteristics identical to those in mammals is particularly pertinent.[18–20]

Occupancy-Response Characteristics. If a given binding site is assumed to be the point of initiation of hormone action, it is reasonable as a first approximation to anticipate that full occupation of such a site would lead to maximal expression of any given hormonal effect. By this criterion, the nuclear binding sites under consideration would appear to qualify as receptors. Injection of a dose of hormone sufficient to saturate the sites for a given period will result, in fact, in maximal levels of activity in the hepatic enzymes α-glycerophosphate dehydrogenase and malic enzyme. Only with more prolonged occupation of these sites will the enzyme level rise further. The relationship between the rate of generation of enzyme and the fractional occupancy of the sites, however, is not linear. Studies in which the instantaneous rate of enzyme appearance is related to the concurrent fractional occupancy as deduced by isotopic methods have shown a nonlinear amplified relationship.[148] Instead of the doubling in the rate of enzyme generation in the transition between the half occupancy of the euthyroid and the full occupancy of the hyperthyroid liver, there is, in fact, a 10- to 15-fold increase. The precise mechanism leading to such amplification remains unclear. An amplified response is characteristic of the response of α-glycerophosphate dehydrogenase and lipogenic enzymes, the precise degree of amplification being determined by the individual enzyme as well as by the dietary status of the animal.[149] Not all thyroid hormone responses, however, are amplified. For instance, the relationship between the pituitary content of growth hormone (GH) and nuclear occupancy is linear[150] in the transition between the hypothyroid and euthyroid states. Similarly, when account is taken of the down regulation of nuclear receptors in GH1 cells by T_3, the relationship between nuclear occupancy and the rate of growth hormone synthesis is also linear.[151]

Temporal Relationship Between Nuclear Occupancy and Response. In assessing the possibility of causal relationships between a set of biologic events, it is essential that an appropriate temporal sequence be established. Thus, it is obvious in the case of thyroid hormone action that nuclear sites must be occupied before the next step in thyroid hormone action

is observed. If an established hormone action were found to precede the occupation of a putative receptor, such a site would automatically be disqualified as a hormone receptor for initiating that action. Using this criterion, nuclear sites are readily compatible with receptor function. As pointed out previously, following the injection of a relatively large dose of T_3, hepatic nuclear sites are almost instantaneously occupied. This finding is due to the fact that the forward rate constant in the binding reaction is a second-order process and that, therefore, the velocity of the forward rate is dependent on mass. Nevertheless, this is still only weak evidence for a receptor role since multiple additional extranuclear binding sites are occupied by T_3 as rapidly as are the nuclear sites.

The plausibility of an initiating function, however, is increased by the demonstration of nuclear events, which rapidly follow the occupation of the nuclear sites. The lapse time between nuclear occupation and nuclear response formerly had been measured in hours. Thus, a lapse of 4 hours intervenes between the injection of T_3 and the first observed increase in the incorporation of labelled orotic acid into new RNA,[152] and a similar lag occurs before an increased rate of poly (A^+) RNA formation is observed.[153] On the basis of a translational assay, the level of the mRNA coding for malic enzymes does not rise until 2 hours following injection of T_3.[154] Further studies, however, have shown that a T_3-responsive, hepatic mRNA, mRNA-S14, rises within 20 minutes following T_3 injection,[155] and its precursor HnRNA increases within 10 minutes.[156] At the same time, *in vitro* nuclear transcriptional assays have shown an almost immediate increase after the addition of T_3 to a cultured rat pituitary cell line.[157] These findings provide strong support for the initiating role of the nuclear sites.

The Correlation Between Thyromimetic Action and Nuclear Binding of Thyroid Hormone Analogues. The strong correlation between hormone action and nuclear binding exhibited by the 40 thyroid hormone analogues studied to date has been discussed. When account is taken of the distribution, metabolism, and nuclear transport of individual analogues, there appear to be no obvious exceptions to the generalization that conventional thyroid hormone action can be understood in terms of nuclear accumulation of the thyroid hormone analogue. Triac, the acetic acid analogue of T_3, shows relatively weak biologic activity in light of the strong nuclear binding that it displays under *in vitro* conditions. However, the rapid metabolism of triac reduces the effective nuclear residence time when administered as a daily pulse.[158, 159] The disproportionately strong biologic activity of T_4 compared with its relatively low affinity for binding to nuclear sites can readily be attributed to the well-known conversion of T_4 to T_3. The 4′-deoxy analogue of T_3 is biologically active despite its apparent inability to bind to nuclear sites, but the paradox is resolved when it is recognized that 4′ hydroxylation is readily carried out *in vivo*. Lastly, the discrepancies between the enantiomers of T_3 have been attributed to the phenomenon of nuclear transport as previously discussed. The cumulative data presented by the analogue studies are perhaps the most important line of evidence that favor a receptor role for the nuclear sites.

Extranuclear T_3 Receptors

Several groups proposed that T_3 receptors can also be found in other subcellular fractions and that such receptors mediate distinctive hormonal actions without the necessity of first stimulating protein synthesis. (For review, see Sterling.[160]) Thus, Sterling and associates have reported specific, T_3-binding, inner mitochondrial membrane sites, which they claim mediate at least some of the mitochondrial effects of thyroid hormone.[161, 162] Two groups have reported finding such sites,[163, 164] but another has been unable to confirm their existence.[165] In one of the confirmatory reports,[163] however, the analogue specificity differed significantly from the accepted activity of thyroid hormone analogues. In the other, the number and affinity of sites were greater on the outer than the inner mitochondrial membrane.[164] There are no published occupancy-response studies to validate their functional role. Both the nature and potential role of mitochondrial sites remain uncertain.

Several groups have also reported the demonstration of membrane-linked, T_3-binding sites.[166–169] Partial purification has been reported.[169] However, there does not appear to be a strong correlation between analogue binding and thyromimetic effects. Segal and Ingbar[170, 171] have proposed that these sites may mediate the transport of amino acids and sug-

ars in thymocytes, but the concentrations of thyroid hormone and analogues, which have been used *in vitro* to demonstrate these effects, greatly exceed the physiologic range.[170, 171] Further, the biologic effects observed do not appear to be limited by saturation of these binding sites. Evidence has been advanced that these sites may be involved in the transport of T_3 rather than its action.[172] A third group has focused on a direct effect of thyroid hormone analogues on the activity of red blood cell Ca ATPase.[173, 174] Although the effective concentrations of thyroid hormone required for these effects are remarkably low, there appears to be only a poor relationship between the activity of a given analogue and the conventional spectrum of thyroid hormone analogue activity.

In essence, there have been no convincing demonstrations so far that the traditional effects produced by thyroid hormones in physiologic concentrations are mediated by extranuclear receptors. However, it is conceivable that future research will in fact demonstrate such actions. The demonstration that the suppression of deiodinase-II by T_4 and reverse T_3 is not mediated by nuclear T_3 receptors and does not require ongoing protein synthesis provides a well-documented extranuclear effect of thyroid hormone.[175] Whether this is an example of thyroid hormone "action" is a semantic problem.

General and Specific Effects of Thyroid Hormone on RNA

If one assumes that the predominant evidence points to a nuclear site of action of thyroid hormone, it is essential that one considers the overall effects of thyroid hormone on RNA. The concept that thyroid hormone functions by stimulating the synthesis of RNA was first advanced in the mid-sixties by Tata and colleagues,[49] who noted a sequence of changes that suggested that thyroid hormone exerted an action on rapidly labelled RNA prior to its effects on protein synthesis. In addition, they reported an increase in total RNA that is composed largely of ribosomal RNA. Subsequent studies indicated that in the transition between the hypothyroid and euthyroid states, the level of poly (A^+) RNA, which contains most of the mRNA, paralleled the twofold increase in total RNA.[176] No further changes in total or poly (A^+) RNA characterized the transition between the euthyroid and hyperthyroid states. Such generalized increases in mRNA, however, could hardly account for the highly specific effects produced by thyroid hormone as previously described in this chapter. In order to understand thyroid hormone action, it was necessary to identify the specific mRNA sequences that are regulated by thyroid hormone. Progress in understanding thyroid hormone action in the past decade has consisted largely of defining those genes that appear to be directly regulated by thyroid.

T_3-Responsive Genes

Pituitary Growth Hormone. Although the enzymes and proteins known to be influenced by thyroid hormones number in the hundreds, only a few of these have been investigated at the gene level (Table 5–4). Some have been mentioned in connection with the survey of the cellular effects of thyroid hormone. Of these, perhaps most attention has been directed towards the gene coding for pituitary growth hormone. In the rat, the expression of the pituitary growth hormone gene is under the tight regulation of T_3, and the level of growth hormone in the athyreotic state is virtually zero.[177] Samuels first recognized the possibility of exploiting the T_3 response of cloned pituitary tumor cells lines for studying GH gene regulation.[178] The response of GH appears to be immediate, direct, and of sufficient extent to allow detailed analysis.

Studies have clearly established that the principal mechanism responsible for the increased level of expression of the GH gene is an augmented rate of gene transcription.[157] This has been demonstrated by measuring the rate at which labelled nucleotides are incorporated into hybridizable transcripts in isolated nuclei incubated under *in vitro* conditions. In this nuclear "run-on" assay, incorporation of radioactive nucleotide is only possible when the polymerase enzyme is already engaged in

Table 5–4. Genes Studied as Models of T_3 Action

Rat pituitary growth hormone
Rat and avian hepatic malic enzyme
Rat hepatic S14 gene
Rat and mouse pituitary growth hormone
alpha and beta subunits (negative regulation)
Rat and rabbit myosin heavy chains
alpha subunit (positive regulation)
beta subunit (negative regulation)

transcription. Since this system does not permit any initiation of transcription, the nucleotide incorporation is essentially a measure of the number of such engaged polymerase molecules. For the growth hormone gene, transcriptional increases occur within minutes after the addition of T_3 to the incubation medium. Thus, in this instance, it is likely that the effect of T_3 is direct and not mediated by an antecedent gene product induced by T_3.

Studies by several investigators have focused attention on the flanking DNA regions as potential control points in the regulation of the transcriptional rate. Casanova and associates[179] have identified a 200 base-pair region "upstream" from the transcriptional starting site, which appears to be indispensable for regulation of the gene. This has been shown by the use of chimeric constructs, consisting of the 5′ flanking region of the growth hormone gene and a "reporter" cholinesterase acetyl transferase (CAT) gene, which is readily monitored. Deletion of a critical upstream region of the growth hormone gene resulted in a loss of T_3 regulation of the reporter gene. Analogous experiments with glucocorticoid-responsive genes have shown that the glucocorticoid receptor appears to bind to those flanking regions that are important in gene regulation. It is therefore tempting to speculate that a similar mechanism may involve the binding of the thyroid hormone receptor to a specific locus in the upstream region of flanking DNA.

Alpha and Beta Subunits of Myosin Heavy Chain. As discussed previously in this chapter, other responsive genes that have been studied include those coding for the alpha and beta myosin heavy chains, the alpha gene being under positive and the beta gene under negative regulatory control.[180, 181] These corresponding mRNAs, however, respond only after a lag time of 2 hours. Although this raises the possibility of indirect regulation, it certainly does not rule out the possibility of a direct effect.

Alpha and Beta Subunits of TSH. The negative regulation of the gene coding for the alpha and beta subunits of TSH has also been examined.[96–98] This occurs rapidly in response to thyroid hormone administration. Although considerable attention has been directed during the past 10 years or so to the gene coding for hepatic α2U globulin as a potential model of thyroid hormone action, the mRNA for this product is probably stimulated by GH, and the response in the hypothyroid animal to T_3 is probably indirect, reflecting the stimulation of GH production by T_3.[182]

Effects of T_3 on Lipogenesis and Lipogenic Enzymes. As discussed previously, thyroid hormones play an important role in the generation of lipogenic enzymes. Such stimulation results from an increase in enzyme mass rather than from an augmentation of the biologic activity of the enzyme. Among the various lipogenic enzymes, malic enzyme shows one of the most marked increments, in the transition both from the hypothyroid to the euthyroid and from the euthyroid to the hyperthyroid states. Translational assays of malic enzyme mRNA activity showed that the enhanced levels of malic enzyme are due to a proportional increase in the level of corresponding mRNA,[183] a finding subsequently confirmed by hybridization assay.[184]

Other studies point to an important synergistic interaction between T_3 and carbohydrate in the induction of lipogenic enzymes, including malic enzyme.[185] High carbohydrate, low fat (lipogenic) diets also induce lipogenesis and lipogenic enzymes. Clearly, the conversion of carbohydrate to fatty acid is a necessary prerequisite to the storage of carbohydrate fuel in the form of triglycerides. An analysis of the simultaneous application of T_3 and dietary stimuli on the induction of malic enzyme; fatty acid synthetase; and the two hexose monophosphate shunt enzymes, glucose-6-P dehydrogenase and 6-P-gluconate dehydrogenase, revealed a marked multiplicative interaction. Studies with malic enzyme revealed that the T_3-carbohydrate interaction was also mirrored by the level of the specific mRNA, thus suggesting that the interaction occurred at a nuclear level.[183] Such an interaction may provide some general insight into precisely how the effect of T_3 at the genomic level is coordinated with other hormonal, dietary, and metabolic stimuli.

The nature of the signal that appears to interact with T_3 in these studies has elicited some interest.[186] Addition of increasing concentrations of glucose to the culture medium in the presence of a constant concentration of insulin resulted in the progressive induction of malic enzyme mRNA. At any given glucose concentration, the induction could be augmented by the addition of T_3 to the medium. As in the animal, there appeared to be a simple

multiplicative interaction between these stimuli. These results indicate that the effects of T_3 and carbohydrate were mediated directly at the hepatocellular level and effectively excluded the possibility that the induction of malic enzymes by either carbohydrate or T_3 was contingent on a rising level of insulin per se. A wide variety of carbohydrate intermediates stimulated the formation of malic enzyme. In each instance, insulin was essential. These findings, and the demonstration that nonmetabolizable analogues of glucose were without effect on malic enzyme levels, suggested that the intracellular metabolism generated a product that served as the proximal trigger responsible for regulating gene expression. Dichloroacetate, a known inducer of pyruvate dehydrogenase, was, however, capable of augmenting the production of malic enzyme in the absence of insulin and even in the presence of glucagon.[187] These findings thus raise the possibility that the putative carbohydrate-inducible factor is a product of pyruvate metabolism. The precise chemical nature of the compound, however, is still not known.

Two-Dimensional mRNA Activity Profiles. A broader perspective of the effect of thyroid hormones on hepatic gene regulation was obtained by generating two-dimensional mRNA activity profiles (Fig. 5–5).[188] With this technique, RNA extracted from the tissues of animals subjected to various experimental manipulations is used to direct the *in vitro* synthesis of protein products by a reticulocyte lysate translational system. The addition of ^{35}S-methionine to the system allows radioactive labelling of the translational products, which are subsequently separated by two-dimensional gel electrophoresis.[189] The first dimension involves isoelectric focusing, thus separating proteins by virtue of their isoelectric point. The second dimension, involving sodium dodecyl sulfate (SDS) gel electrophoresis, separates proteins according to their molecular weight. Radioautographs are obtained, and the intensity of individual spots can be quantitated by videodensitometry.[190] In normal rat liver, approximately 250 discrete spots can be identified, each presumably representing the translational product of one mRNA sequence. By examining such gels, it is possible to analyze a large cross section of the genes, which is expressed in high or medium abundancy in a given tissue. Of the 250 spots demonstrated in the two-dimensional gels, 19 showed selective changes in response to alterations in thyroidal state.[188] Following thyroid hormone administration, the expression of 11 genes increased and seven decreased. One gene showed a biphasic change. Thus, if the behavior of the medium and high frequency mRNAs is typical of the low frequency sequences, one could conclude that, either directly or indirectly, thyroid hormone causes the selective regulation of approximately 8% of the genome.

Messenger RNA activity profiles, such as the ones generated in the various thyroidal states, have several important applications. As a cross section of gene activity in a given tissue, they yield rather distinctive patterns and thus provide a "fingerprint" of the genomic response to the stimulus applied. Thus, the pattern developed in the hyperthyroid state strongly resembles that produced by feeding an animal a high carbohydrate diet, and the pattern of starvation resembles that of experimental diabetes mellitus.[191] Further, an analysis of such profiles helps to exclude the possibility that the effect of a given stimulus on the expression of a gene is mediated by a specific factor. For example, approximately 30% of the genes influenced by thyroid hormone were influenced by virtue of a thyroid hormone–induced increase in pituitary GH.[192] Among the genes indirectly regulated by T_3 in this fashion is that gene coding for α-2U globulin, which as pointed out previously had received considerable attention in the past decade or more as a potential model of thyroid hormone action.

Lastly, mRNA activity profiles are useful in defining specific mRNA sequences, which respond rapidly to a given stimulus and undergo a large excursion. As discussed, such attributes make a given mRNA particularly attractive as a model for investigating hormone action. The rapidity of response can provide reasonable assurance that the hormonal effect is direct. By performing sequential gel analysis after the injection of T_3 into hypothyroid animals, Seelig and coworkers demonstrated an mRNA, designated arbitrarily as mRNA-S14, which responded without an appreciable lag time and displayed a 20- to 30-fold increase above hypothyroid levels within a 4-hour period.[155] This sequence codes for a protein of M_r 17,000 and pI of 4.9. Of special interest is the fact that the administration of a high carbohydrate diet also induces the formation of mRNA-S14.

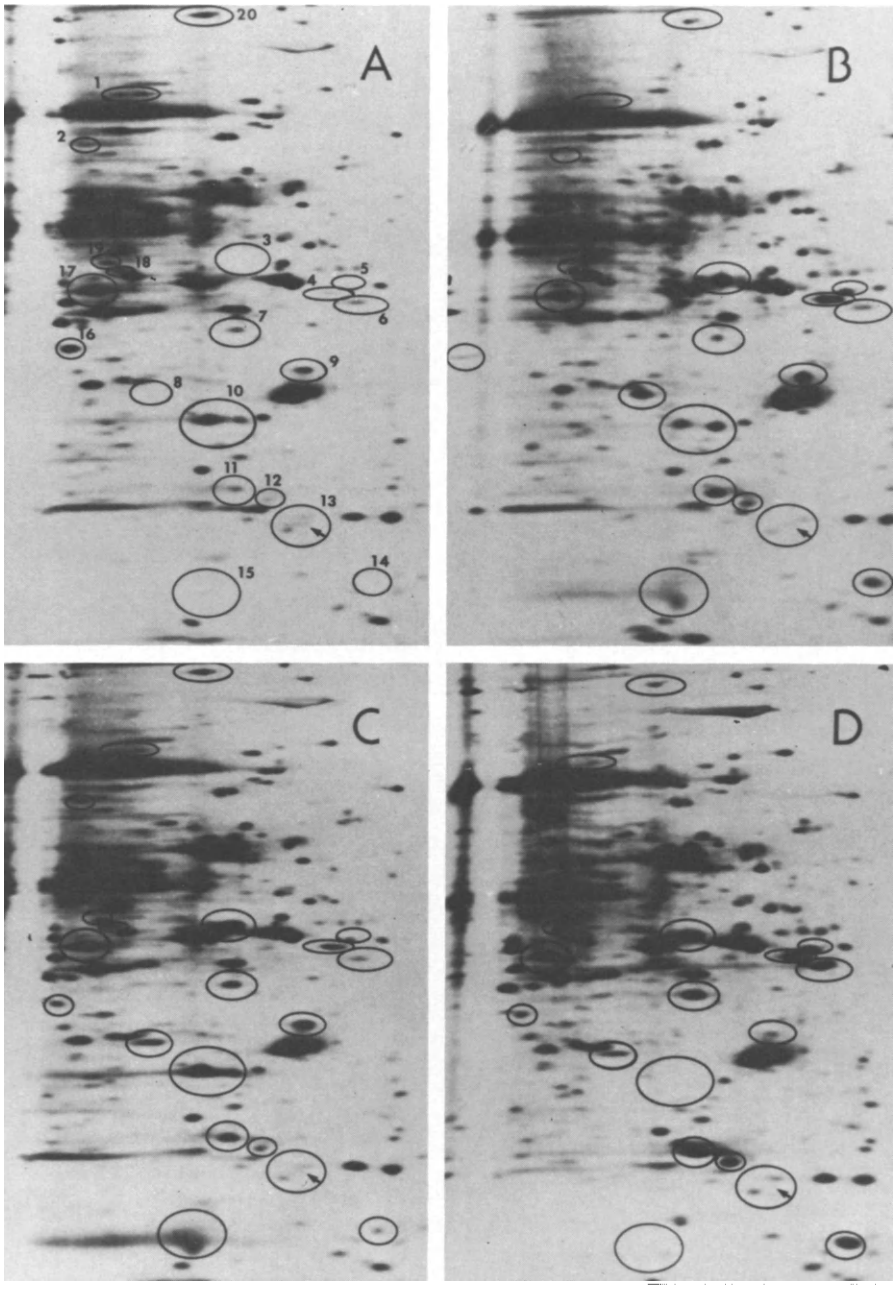


Figure 5–5. Two-dimensional gel representation of the *in vitro* translational products of RNA obtained from the liver of animals subjected to various hormonal manipulations. Each panel represents the fluorograms of 35S-labeled protein separated by the isoelectric point in the horizontal direction (pH 7.9, left; pH 4.5, right) and by molecular weight (M_r 100,000, top to 12,000, bottom). *A*, Euthyroid unmanipulated animal. Each circled spot represents a sequence that is changed as a result of alteration in the thyroidal state. *B*, Thyroidectomized animals treated with a replacement dose of 0.3 μg/day for 12 days. *C*, Thyroidectomized animals. *D*, Thyroidectomized animals treated with 15 μg T_3 for 12 days, rendering the animals metabolically hyperthyroid. Note position of Spot 14, M_r 17,000, pH 4.9. (Redrawn from Mariash, C. N. and Schwartz, H. L.: Effect of dichloroacetic acid on rat hepatic messenger RNA profiles. Metabolism 35:452–456, 1986.)

Messenger RNA-S14 Characterization. Liaw and Towle[193] (Table 5–5) have cloned the cDNA to mRNA-S14 and have sequenced the coding regions of the gene. The gene is 4.5 kilobases (kb) long and consists of only two exons separated by one intervening sequence. The translated region of the gene is located entirely on the first exon, and there are two polyadenylation sites on the second gene, resulting in the formation of two mRNA sequences on Northern blot analysis. A TATA box variant is situated some 27 base pairs upstream from the transcription start site. From the nucleotide sequence, it is possible to infer that the mRNA codes for a protein of 150 amino acids with a molecular weight of 17,010. Carefully updated examination of oligonucleotide and polypeptide sequences encoded in national data banks has so far failed to reveal significant homologies.

Hybridization analysis confirmed the rapid response to T_3 inferred from the previous translational assays. A lag time of only 20 minutes preceded the rise in the mRNA,[194] and only 10 minutes lapsed before the rise in the HnRNA precursor.[195] The rapidity of these events virtually excludes the possibility that the effect of T_3 is contingent on the generation of an antecedent T_3-induced gene product. Further, the findings that the precursor rose before the mature mRNA and that the rise in precursor was proportional to the product pinpointed the site of generation of the mRNA to the nucleus. Studies by Mariash and associates have confirmed a similarly rapid response to the intragastric administration of sucrose.[196]

Another unusual feature of mRNA-S14 is its marked circadian variation, with maximal values in euthyroid chow-fed animals occurring at 8:00 p.m. and minimal values occurring at 8:00 a.m. A threefold variation in levels characterizes the daily cycle.[194, 197] This appears to be entrained to some as yet unidentified neuroendocrine signal, since it is reversed by a 180-degree phase shift in the light-dark cycle but not by a similar shift in the feeding cycle.[197] Further studies have demonstrated hybridization of rat cDNA probes with both human DNA and RNA, thus suggesting that this gene is also expressed in humans.[198]

Table 5–5. S14: Characteristics of the Protein and Its mRNA

Protein	
Molecular weight	17,010
pI	4.9
Subcellular localization	Cytosol
Proposed function	Stimulation of lipogenesis
mRNA	
Tissue Distribution	Liver, white and brown fat, and lactating mammary gland
Response Characteristics	
Hepatic mRNA	
stimulated by	T_3 and carbohydrate
inhibited by	Glucagon, starvation, and diabetes
White fat mRNA	
stimulated by	T_3 and carbohydrate
Brown fat mRNA	
stimulated by	Cold and cafeteria diet
inhibited by	T_3

Molecular Mechanisms of Induction of mRNA-S14

The molecular mechanism by which T_3 increases the expression of the hepatic S14 gene is still poorly understood. Narayan and Towle[199] originally hypothesized that T_3 was instrumental in stabilizing the precursor mRNA of S14. They reached this conclusion on the bases of the parallel increase in the level of the precursor and mature mRNA following T_3 administration and their inability to demonstrate the expected fold increase in *in vitro* transcription as inferred from the nuclear "run-on" assay. However, subsequent studies both by Towle and by Jump using other cDNA probes have demonstrated up to tenfold increases in hepatic S14 transcriptional rate after T_3 administration (personal communications). Further, studies by Kinlaw and coworkers[200, 201] have taken advantage of the capacity of cycloheximide to abrogate the transcription of the hepatic S14 gene, and that T_3 and active thyroid hormone analogues reverse this effect within minutes. These investigators proposed that T_3 works by removing a nuclear inhibitor of precursor mRNA production. Given the variable results of transcription assays it is impossible at this stage to decide whether T_3 regulates transcription of the S14 gene in whole or part or whether T_3 stabilizes either the precursor or mature mRNA. This problem may be equally applicable to the induction of other sequences induced by T_3. Only one third of the increase in the mRNA of hepatic malic enzyme[184, 202] and only a small fraction of the rise in the mRNA for hepatic S11 can be attributed to transcriptional changes as inferred from run-on assays.[203]

Cycloheximide acts promptly to block the induction of several thyroid hormone–responsive hepatic mRNAs[155, 203, 204] and the mRNA coding for GH in the pituitary.[204a] This raises the possibility that the synthesis of a short-lived protein may be essential for hormonal induction since other inhibitors of protein synthesis exert a similar effect. In the case of S14 and pituitary GH, cycloheximide inhibits induction by impairing S14 transcription.[205] Since there is no generalized reduction in poly (A) mRNA,[203] the effect of cycloheximide appears selective for the transcription of certain genes. Since the half-time in the disappearance of the T_3 nuclear receptor has been estimated to be about 4 hr[141] and since the effect of T_3 on the inductive process is essentially complete within half an hour, it is unlikely that the T_3 nuclear receptor itself is the rapidly turning-over cycloheximide-sensitive protein.

Inferences Regarding the Function of S14

The exceedingly rapid effects of thyroid hormone and carbohydrate on mRNA-S14 together with the striking excursion in the response of this sequence raise the possibility that mRNA-S14 may code for a functionally important hepatic protein. As previously indicated, the oligonucleotide sequence of cDNA and the peptide sequence do not appear to exhibit any homology with sequences registered in national data banks. However, a series of functional correlations strongly support the possibility that this sequence codes for a protein involved in lipogenesis. Thus, similar to lipogenic enzymes, mRNA-S14 is coordinately regulated by T_3 and a high carbohydrate diet. Further, there is a synergistic interaction between these two stimuli.[196] A survey of the various tissues of the rat reveals substantial expression only in lipogenic tissues including liver, white and brown adipose tissue, and lactating mammary gland.[194, 206] Experiments by Perez and coworkers[207] have shown that the rise in hepatic lipogenic enzymes that accompanies spontaneous weaning of rat pups from their mother's milk is accompanied by a rise in mRNA-S14. When the weaning process is hastened by weaning the rats prematurely to a laboratory diet, both the rise in lipogenic enzymes and the rise in mRNA-S14 are hastened.

Perhaps the most convincing evidence favoring the role of S14 in lipogenesis is found in studies that involve brown fat by Freake and coworkers.[208] This tissue is known to be responsible for the dissipation of energy in the form of heat both in cold adaptation ("nonshivering thermogenesis") and following the ingestion of surplus calories contained in especially delectable snack foods in a "cafeteria"-diet feeding. The mechanism of heat generation is mediated by a mitochondrial protein, thermogen, which serves to uncouple oxidative phosphorylation.

The content of mRNA-S14 in brown fat is the highest of any tissue so far examined, some six times that of white fat and 20 times the level in euthyroid liver. The response of mRNA-S14 has been measured in animals subjected to several physiologic and hormonal manipulations. The rate of brown fat lipogenesis was determined by the incorporation of ^3H into brown fat fatty acids following the administration of ^3H_2O. Of interest was the finding of increased levels of mRNA-S14 in the hypothyroid state, as compared with the euthyroid baseline state. Accompanying the increase in brown fat mRNA-S14 was an augmentation in the rate of lipogenesis. Thus, despite the apparently paradoxic regulations of the S14 gene compared with liver and white fat, the correlation between mRNA-S14 and lipogenesis was preserved. At the same time, cold exposure and cafeteria-diet feeding both resulted in three to fourfold increases in the content of mRNA-S14/gm of tissue and a roughly proportional increase in the rate of brown fat adipose lipogenesis.

Fatty Acid Synthesis and Degradation: Clues to the Molecular Basis of Thyroid Hormone–Induced Thermogenesis (Figure 5–6)

The results of these studies in brown fat tissue, coupled with previous observations on the effect of thyroid hormones on lipogenesis and beta oxidation of fatty acids, suggest a potential mechanism to explain the well-known thermogenic effects of thyroid hormones. Thus, the augmented brown fat lipogenesis demonstrated in the cold adapted, cafeteria-diet fed, and hypothyroid rat could well represent an adaptation to increase the synthesis of fatty acids for the purpose of sustaining the rate of mitochondrial oxidation of brown fat. In each instance, increased dissipation of energy serves a biologically useful purpose: for maintaining

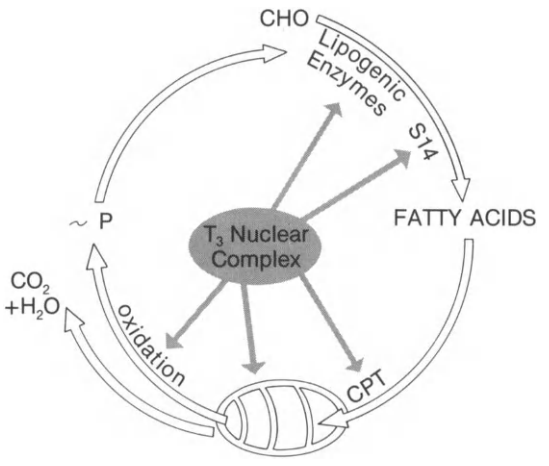


Figure 5–6. Schematic representation of the effect of thyroid hormone on fatty acid synthesis and degradation. Thyroid hormone stimulates the formation of fatty acids from carbohydrates (CHO) by augmenting the induction of S14 and lipogenic enzymes. At the same time, the hormone increases the activity of carnitine palmitoyl transferase (CPT), the rate-limiting enzyme governing the entrance of fatty acids into mitochondria, and stimulates other mitochondrial enzymes involved in fatty acid oxidation. The high energy phosphate (~P) required for fatty acid synthesis is supplied by their intracellular degradation. This appears to result in a futile cycle with increased oxidation but without the generation of "useful" work.

body heat in the cold-exposed animals, for the expenditure of unneeded calories, and for counterbalancing the hypothermic effect of the hypothyroid state.

Similarly, the augmented rate of hepatic lipogenesis associated with the hyperthyroid state appears to depend on the continued synthesis of fatty acids to fuel the mitochondrial oxidative machinery. Increased rates of lipogenesis and increased levels of free fatty acids are uniformly found in the hyperthyroid state. Protein S14 probably plays an important role in this process. At the same time, other studies have shown that thyroid hormone also increases the rate of beta oxidation of fatty acids, both by increasing the formation of the mitochondrial enzymes responsible for such oxidation[209, 210] and by increasing the activity of carnitine palmitoyl transferase, the rate-limiting enzyme controlling the entrance of long chain fatty acids into the mitochondrion.[211] It is possible that thyroid hormone is responsible for the activation of the genes that code for these enzymes and any regulatory proteins required to coordinate the system. Since lipogenesis is a high energy–requiring process, this could serve as a ready sink for the ATP produced in the course of mitochondrial beta oxidation. As a consequence, sufficient ADP would be available to maintain continued mitochondrial oxidation in the tightly coupled oxidative phosphorylation as discussed. In essence, hepatic thyroid hormone–induced thermogenesis would result from a futile cycle of fatty acid synthesis and degradation. Additional experiments are clearly required to assess in a qualitative fashion, the contribution of T_3-induced lipogenesis to T_3-induced thermogenesis and to assess the mechanism that underlies extrahepatic thermogenesis.

A reduction in futile cycling in animals under the stress of catabolic illness might well have adaptive value in a reduction in the expenditure of energy and might explain the function of the lowered serum T_3 observed in such states as well as in starvation. With the limited availability of food to the injured or sick animal, reduction of the fatty synthesis-degradation cycle might be expected to result in the diversion of caloric energy for essential life-preserving and reparative processes. Such a mechanism might well explain the finding of low concentrations of T_3 in sick and calorically deprived patients.

CONCLUSIONS

The multiple effects of thyroid hormones on tissues and cells are surveyed, and some guidelines to understanding these complex phenomena on the basis of developments in the fields of molecular and cellular biology are provided. Several impressions emerge from these efforts. First, it is clear that almost all of the generalities offered are preliminary and tentative and that the vast bulk of phenomenology that has been reported in the past 100 years remains unexplained. The field of thyroid hormone action is replete with the litter of many failed efforts to achieve a unified understanding. Second, despite these reservations, it is clear that the recognition of the apparent universality of the receptor-T_3 interaction has been instrumental in focusing attention on nuclear events in explaining the initiating mechanism of thyroid hormone action. Further studies, however, are required to place into appropriate perspective direct interactions of thyroid hormone with extranuclear events. Third, evolutionary considerations suggest that the thyroid hormone system has been exploited for a wide

range of purposes. In particular, the T_3-receptor complex has played a critical role in the development of homeotherms some 200 million years ago. Thus, it may be appropriate to regard T_3 as a relatively nonspecific chemical regulator of gene expression, the specific systems subserved being dictated by evolutionary factors. Last, the tools of modern recombinant DNA technology should be helpful in defining the specific details of the initiation process and resolving current uncertainties regarding the relative importance of transcription, RNA stabilization, and precursor processing. Of equal importance, however, is the potential application of these techniques to an understanding of the sequential molecular steps past the point of initiation, which lead to the characteristic physiologic and biochemical manifestations of thyroid hormone effects in the organism.

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6
Measurement of Thyroid Function

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IN VITRO THYROID FUNCTION TESTING

Nature of the Circulating Thyroid Hormones

The kinetics, transport, and metabolism of the thyroid hormones are discussed elsewhere in this text. A few points need emphasis in relation to the clinical usefulness of thyroid hormone measurements. The two clinically important and metabolically active thyroid hormones in human circulation are L-3,5,3′,5′-tetraiodothyronine (thyroxine, T_4) and L-3,3′,5-triiodothyronine (triiodothyronine, T_3).[1] T_3 is about 3 to 5 times more potent than T_4 on a weight for weight basis with respect to calorigenesis and suppression of thyrotropin (TSH) production.[1-5] Although thyroidal secretion is the only known source of T_4, T_3 derives from both thyroidal secretion (20 to 30%) and monodeiodination of T_4 to T_3 peripherally in extrathyroidal tissues (70 to 80%).[6,7] T_4 and T_3 are transported in the blood stream bound to three binding proteins, thyroxine binding globulin (TBG), thyroxine binding prealbumin (TBPA) or transthyretin, and albumin.[1-4,8] TBG is the most avid but has the lowest capacity, whereas albumin has the greatest capacity but is the least avid. T_3 has very little avidity for TBPA, binding primarily to TBG and albumin. Approximately 99.96% of T_4 is in the bound form (0.04% in the free form), whereas binding of T_3 to these proteins is ten times less, only 99.5% being bound and 0.5% being free.[9] It is this small free fraction of T_4 and T_3 that is free to diffuse into the tissues and that will then be responsible for thyroid hormone effects. Discussion in previous chapters indicates that the prime burden of thyroid hormone action occurs via T_3.

Most of the biologic activity of T_4 is due to its conversion to T_3.[6] However, 10% of the total iodothyronine specifically bound to the nuclear receptor is in the form of T_4, with the remaining 90% in the form of T_3.[10] Since equimolar amounts of iodothyronine bound specifically to the receptor appear to exert equivalent biologic effects it is probable that 10% of thyroid hormone effects can be attributed to the intrinsic thyromimetic properties of T_4.

In addition to T_4 and T_3, some other iodothyronines have been detected in human plasma.[1-5] These include reverse T_3 (3,3′,5′-T_3, rT_3) and 3,3′-diiodothyronine (3,3′-T_2).

Table 6–1. Thyroid Function Tests

Test	Abbreviation	Synonym	Normal Range		Comments
			Conventional Units	*SI Units*	
Serum thyroxine	T_4		4.6–12 μg/dl	60–155 nmol/L	—
Free thyroxine fraction	FT_4F	Dialysis fraction (DF)	0.03–0.005%	—	Measured by dialysis
Free thyroxine	FT_4		0.7–1.9 ng/dl	8–25 pmol/L	= $T_4 \times FT_4F$
Thyroid hormone binding ratio	THBR	T_3 uptake ratio (T_3RU)	0.9–1.1	—	Normalized to control reference serum
Free thyroxine index	FT_4I		4–11	60–155	= $T_4 \times$ THBR
Serum triiodothyronine	T_3		80–180 ng/dl	1.2–2.8 nmol/L	—
Free triiodothyronine	FT_3		230–619 pg/dl	2–6 pmol/L	= $T_3 \times FT_3F$
Free triiodothyronine index	FT_3I		80–180	1.2–2.8	= $T_3 \times$ THBR
Reverse triiodothyronine	rT_3		30–80 ng/dl	—	—
Radioactive iodine uptake	RAIU		10–36%	—	Range depends on iodine intake
Serum thyrotropin	TSH		0.5–6 μU/ml	0.5–6 mU/L	Range of sensitive TSH assay
Basal metabolic rate	BMR		−10 to +10%	—	Not readily available
Thyroxine-binding globulin capacity	TBG		12–20 ug/dl T_4 + 1.8 μgm	170–300 nmol/L	—
TRH stimulation test			Peak TSH 9–30 μIU/ml at 20–30 min	—	—
Serum thyroglobulin	Tg		0–30 ng/ml	0–45 pmol/L	—
Thyroid microsomal antibody titer	TMAb		Varies with method	—	—
Thyroglobulin antibody titer	TgAb		Varies with method	—	—

More than 90% of the daily production rate of rT_3 is derived from the peripheral deiodination of T_4.[5] Thyroid secretion contributes less than 10%. Reverse T_3 and 3,3′-T_2 have little, if any, calorigenic or TSH suppressive effects. In large doses, however, rT_3 can inhibit the peripheral effects of T_4.[1–5] In normal individuals, secretion of T_4 and T_3 by the thyroid is regulated by TSH from the anterior pituitary.[11] The secretion of TSH in turn is influenced by the level of free T_3 and free T_4 in the plasma and by the thyrotropin releasing hormone (TRH) secreted by the hypothalamus.[11] There is a negative feedback system between circulating thyroid hormones and pituitary TSH that maintains circulating thyroid hormone levels within normal limits.[11] Disruption of this system with changes in circulating hormone levels can occur as a result of hypothalamic, pituitary, or thyroid dysfunction.

The development of radioimmunoassay (RIA) techniques to measure T_4, T_3, and TSH in the plasma and the availability of TRH have certainly simplified the diagnosis of thyroid disease.[12] The speed, specificity, reliability, and sensitivity of these relatively new procedures have made virtually obsolete many previous tests, such as the basal metabolic rate, the serum protein bound iodine (PBI), the butanol extractable iodine (BEI), and the thyroxine iodide by column.[12] Moreover, many other procedures, such as the response of the thyroid to TSH and the T_3 suppression test, are not required nearly as often. Since so many conditions affect thyroid function, or T_4 or T_3 binding in the plasma, no single test can be employed to "screen" for thyroid disease. Selection of appropriate procedures depends upon the particular clinical problem to be investigated as well as an understanding of the significance of each test and the problems encountered in interpreting the results of these procedures (Table 6–1).

Total Serum T_4

The radioimmunoassay for the measurement of serum T_4 has virtually replaced all other techniques.[13] A competitive protein binding (CPB) technique was employed, but it is used rarely now because of the efficiency and ease of the RIA procedures, some of which are highly automated. Moreover, determination of PBI and T_4 iodine by column are no longer utilized. Iodine, iodine-containing drugs, and radiocontrast agents do not interfere with the RIA procedure as they did, of course, with the PBI and T_4 iodine by column.

In most laboratories, the normal range for serum T_4 measured by RIA is about 60 to 155 nmol/L (4.5 to 12.0 μgm/100 ml). Regardless of the procedure utilized, the technique does measure total (free and bound) serum T_4. Since the free form represents only 0.05% of the total, it is negligible in terms of the total RIA value, although very important in its own right. The serum T_4 levels are usually, but not invariably, increased in hyperthyroidism and decreased in hypothyroidism. There is also a perceptible but very minimal decline with advanced age.

It is obvious that changes in the concentration of binding proteins will profoundly affect the results of the serum T_4 determination (Table 6–2). Many agents will elevate TBG, such as estrogen, oral contraceptives, methadone, heroin, and perphenazine (Trilifan).[12, 13] TBG is also elevated in pregnancy owing to increased estrogen, infectious hepatitis, and intermittent porphyria, and may also be elevated on a genetic basis.[1–4, 13] Thus, there will be a consequent increase in the serum T_4, and since 70% of T_3 is bound to TBG, in total serum T_3 as well. Conversely, TBG may be lowered in cirrhosis, nephrotic syndrome, severe illness of any kind, and in treatment with androgens, anabolic steroids, and glucocorticoids. Large doses of diphenylhydantoin (phenytoin, Dilantin) may also result in a decrease in the affinity of TBG although the mechanism underlying the reduction of T_4 in patients treated with this agent appears to be related to an increased fractional rate of metabolism as a consequence of drug stimulation of hepatic hydroxylating enzymes and an impaired compensatory response by the hypothalamic-pituitary-thyroidal axis.[14] Once again, a low TBG may be a result of a genetic abnormality.[1–4] In this situation the total T_4 and total T_3 levels are low. Under these circumstances, however, the person remains euthyroid because the free T_3 and T_4 values remain normal. These elements must be kept in mind when results of the serum T_3 and T_4 are not in accord with clinical status. In acutely moribund euthyroid patients (e.g., patients dying of myocardial infarction) the serum T_4 and free T_4 index are low, but the free thyroxine by equilibrium dialysis is often normal.[15–17] These issues are considered in further detail subsequently.

Table 6–2. Conditions Affecting Thyroxine Plasma Protein Binding

Increased plasma protein binding (resulting in the increased serum T_4 and T_3, but reduced T_3 resin uptake)	Decreased plasma protein binding (with low serum T_4 and T_3, but increased T_3 resin uptake)
Pregnancy	Drug therapy
Estrogen therapy (particularly oral contraceptives)	Androgens
	Corticosteroids
	Diphenylhydantoin
Perphenazine	Salicylates
Infectious hepatitis	Major illness
Acute intermittent porphyria	Nephrotic syndrome
	Congenital hypoTBGemia
Congenital hyperTBGemia	
Dysalbuminemic state (albumin or transthyretin)	

Rare causes of increased T_4 concentrations in the absence of hyperthyroidism include increased peripheral resistance to thyroid hormones (Refetoff's syndrome)[18]; high T_4 values secondary to unusual albumin and prealbumin binding proteins with high affinity to T_4 but not T_3 and often with a familial distribution[19]; and high T_4 values secondary to an antibody against T_4, sometimes observed in patients with high titers of antithyroglobulin.[20, 21] Where such antibodies to T_4 occur, the values for serum T_4 may be either very high or very low, depending on the particular radioimmunoassay procedure employed.[21] With polyethylene glycol radioimmunoassays, the values will be spuriously low, whereas with hormones extracted from serum with a Sephadex G-25 column, the values are increased to their true high values.[21]

Total Serum T_3

The normal range for the total serum T_3 levels determined by radioimmunoassay varies from approximately 1.2 to 3.4 nmol/L (90 to 210 ngm/100 ml), depending upon the laboratory that performs the procedure (Fig. 6–1).[1–5, 12, 13] Although the affinity of binding proteins for

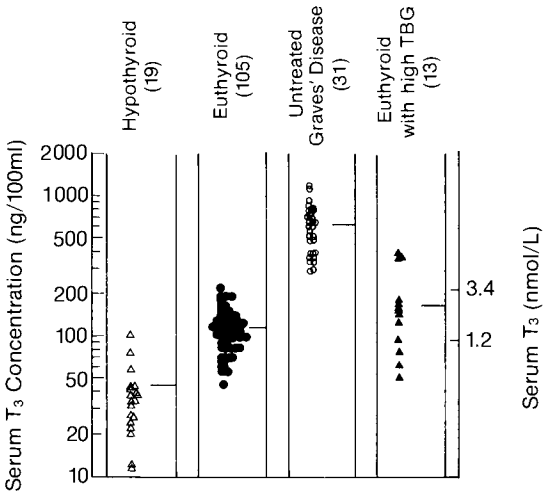

Figure 6–1. Serum T_3 concentration as measured by radioimmunoassay in normal subjects and patients with hypothyroidism and hyperthyroidism. (Redrawn from Chopra, I. J. and Solomon, D. H.: Thyroid function tests and their alterations by drugs. Pharmacol. Ther. 1:367–399, 1976.)

T_3 is much less than that for T_4, about 99.5% of T_3 in the serum is bound to proteins.[1–5] Thus, changes in protein concentration will affect T_3 levels in a manner similar to that described for T_4.

The level of serum T_3 is often disproportionately increased in relationship to that of T_4. Stimulation of the thyroid, whether by TSH or by the thyroid-stimulating antibody (TSAb) of Graves' disease, leads to direct thyroidal secretion of T_3. Thus, in Graves' disease the high level of circulating T_3 derives from peripheral conversion of the excessive T_4 produced as well as augmentation in direct thyroidal secretion of T_3. Graves' disease therefore is characterized by a high T_3/T_4 serum concentration ratio and renders the serum T_3 assay particularly valuable in diagnosis.[22] The concentration of free T_4 may be in the normal range with an elevation of the free T_3 concentration only. The patient is still considered to be hyperthyroid and is often designated as having "T_3-toxicosis."

Increased levels of T_3 will also be encountered in euthyroid persons who have elevations of TBG,[1–5] in those who are resistant to thyroid hormone at the tissue level,[18] and depending on the technique employed in those with antibodies to T_3.[20, 21] Conversely, normal T_3 levels may be present in hyperthyroid patients with low TBG concentrations and in rare instances of "pure T_4 thyrotoxicosis."[1–5] The latter is most often observed in patients with true catabolic disease, which tends to depress the serum T_3 concentration. However, most patients with high serum T_4 with normal or low T_3 levels are not hyperthyroid. Such a condition, which may be called "pseudohyperthyroidism," may be seen in euthyroid persons with other nonthyroidal systemic disorders in whom there is a defect in T_4 to T_3 conversion. As indicated previously, this association may be due to abnormalities of albumin or prealbumin binding.[19] Under these circumstances it is important to measure free thyroxine and the TSH concentration by a sensitive assay to rule out hyperthyroidism.

T_3 determinations may be useful in following patients who are taking thyroxine for either hypothyroidism or goiter suppression, providing they are not ill with "nonthyroidal" disease. Under such circumstances, when patients take thyroxine in dosages of 0.10 to 0.15 mg/day, the serum T_4 level will often be elevated.[21] However, the serum T_3 level will usually be within normal limits.

In a recent study of replacement therapy in patients with primary hypothyroidism,[7] suppression of TSH into the normal range was accompanied by a mean serum T_3 concentration nearly identical to that of an age- and a sex-matched normal control group, whereas the concentration of serum T_4 was elevated considerably over that of the normal controls. Calculations suggested that in normal euthyroid subjects approximately 18% of circulating T_3 is derived from direct thyroidal secretion with the remaining 82% derived from peripheral conversion of T_4 to T_3. In athyreotic patients treated with exogenous T_4 a higher level of circulating T_4 must be achieved in order to compensate for thyroidal section of T_3. The fact that TSH normalization was associated with a normal T_3 and an elevated T_4 prompted the investigators to suggest that the level of pituitary TSH is determined by plasma T_3 rather than by plasma T_4, as had been proposed for the rat.[23] If a patient has an autonomous thyroid or a nonsuppressible nodule, T_4 treatment can also be helpful in diagnosis since under such circumstances the serum T_3 will be definitely elevated.[22]

If the physician elects to follow a hypothyroid patient who is receiving replacement therapy with serum T_3, it is important to be certain of the normal range of serum T_3 used by the laboratory. This range tends to vary from laboratory to laboratory and to depend on the precise techniques and the nature of the anti-

serum used. Further, a fall in serum T_3 level may accompany even minor illness or dietary restrictions.[24] These factors should be taken into account in assessing the meaning of the given serum T_3 obtained in such a patient.

T_3 determinations are not very helpful in the diagnosis of hypothyroidism, since they may continue to be within normal limits or only slightly reduced when the serum T_4 is already very low and the TSH very high.[1-5] T_3 declines well below normal only in very severe hypothyroidism, where the diagnosis is usually very obvious clinically. The tendency to maintain the serum T_3 concentrations within normal limits in the face of hypothyroidism probably reflects selective stimulation thyroidal secretion of T_3 by augmented levels of TSH as well as the effects of increased peripheral conversion of T_4 to T_3 associated with a depressed T_4 concentration.[25]

Conversely, low T_3 values are found in a variety of euthyroid states. Such low values are found in normal newborn infants, as well as in association with a variety of nonthyroidal illnesses, such as malnutrition or starvation, anorexia nervosa, acute and chronic systemic disorders, and major surgery. The "euthyroid sick" syndrome or the "low T_3 syndrome" refers to the findings of such values in these euthyroid ill patients. The low T_3 concentrations in these patients do not reflect the presence of any thyroid disorder and are related only to changes in peripheral generation of T_3.[4, 5, 16] (This problem is discussed subsequently.) In addition, corticosteroids, propylthiouracil, and certain radiographic agents, e.g., sodium ipodate, will also depress the serum T_3.[26] Such low values as occur under these circumstances generally reflect reduced peripheral conversion of T_4 to T_3. The nature of this change in conversion is discussed elsewhere in this text.

Serum Reverse T_3 (rT_3)

Serum rT_3 is a product of monodeiodination of the inner ring of thyroxine.[5] As previously indicated, this iodothyronine has little calorigenic or TSH-suppressive effects, but in high concentration may inhibit the effect of T_4 by preventing the peripheral conversion of T_4 to T_3. A small fraction (about 3%) is derived not from the peripheral conversion of T_4 to T_3, but from direct thyroidal secretion. The mean plasma, rT_3 varies from 0.4 to 0.9 nmol/L (30 to 80 ngm/100 ml) in the human. The serum rT_3/T_4 ratio is elevated in both hypothyroidism and hyperthyroidism, although the precise mechanisms leading to these changes have not been clarified.

There is an increase in the serum rT_3 concentration in normal newborn infants and in amniotic fluid. It has been suggested that amniotic fluid rT_3 measurements may be useful for detecting serious fetal hypothyroidism and that cord blood rT_3 measurements may be useful in the measurement of neonatal hypothyroidism. However, these hopes have not yet been realized.

The rT_3 levels are also elevated in the euthyroid sick syndrome in persons who are ill with a variety of nonthyroidal disorders already discussed.[5, 16] Thus, in the absence of thyroid disease, a low T_3 will be associated with a high rT_3, whereas if both values are low, true hypothyroidism exists. The high rT_3/T_3 ratio in nonthyroidal disease is not merely due to increased conversion of T_4 to rT_3, but rather due to a reduced clearance of rT_3.

Protein Binding of Thyroid Hormones

As pointed out in Chapter 5, the levels of both T_4 and T_3 are determined by the production of the iodothyronines, their distribution volume, and their rates of peripheral metabolism. From a clinical point of view, an important variable that determines both the distribution and the fractional metabolism of the thyroid hormone is the level of plasma protein binding. In pregnancy, for example, high estrogen levels stimulate the production of TBG by the liver. Since there is a rapid exchange of iodothyronines between cellular and plasma protein compartments, a shift of both T_4 and T_3 from the cellular to the extracellular compartments will result as will a decrease in the fractional rate of hormone metabolism. The level of total serum T_4 and T_3 will increase. Since the fraction of T_4 that dissociates from the binding protein is reduced, the level of the free hormone will remain essentially unchanged. Thus, in the steady state in a pregnant woman, we anticipate an elevated level of serum T_4, an expanded total volume of T_4 distribution, a diminished fractional rate of disappearance from plasma, and no change in the effective thyroidal state of the tissues. These apply to T_3 as well as T_4. An oppositely directed sequence of events will follow the administration of large doses of testosterone, since this hormone will reduce the level of circulating TBG.

From a clinical point of view, therefore, it is important to discount the potential effects of plasma protein binding on the level of total circulating hormones. In the steady state, the free hormone concentration will not change in response to an alteration in the level of binding proteins. Neither will there be an alteration in the intracellular hormone pool or the thyroidal state of the patient.

Multiple approaches are available to assess the effect of plasma protein binding on the serum concentration of total hormone measured. The most direct way of performing such correction is to measure the free hormone concentration by equilibrium dialysis as described in Chapter 4. In brief, radioactive T_3 or T_4 is added to dilute serum contained in a semipermeable dialysis bag, which is subsequently dialyzed against a surrounding compartment of aqueous buffer (see Fig. 4–3). Tracer ^{125}I-T_3 is used to measure the binding of T_3, and ^{125}I-T_4, the binding of T_4. After equilibration has been attained, the fraction of tracer in the system that is not bound to protein is determined. From this value and the known dilution of serum it is possible to estimate the fraction of total T_4 or T_3 that is unbound in whole undiluted serum. This is designated as the dialysis fraction (DF). The product of the DF and the total T_4 will provide an approximation of the free hormone concentration. From the foregoing discussion, it follows that with any alterations in binding, the level of total hormone and dialysis fraction will change in a reciprocal fashion so as to leave the product, the free hormone concentration, unchanged.

From a practical point of view, the equilibrium dialysis method has been considered to be tedious by most clinical laboratories, owing to problems in automating the procedure and long time (18 to 24 hours) required in achieving equilibrium. As a consequence, resin uptake techniques have been developed for the purpose of providing a relatively rapid assessment of the strength of plasma protein binding (Fig. 6–2). Radioactive tracer iodothyronine is added to serum and the mixture is exposed to solid matrices that bind thyroid hormones nonspecifically and compete with serum for ^{125}I-T_4 or ^{125}I-T_3 bound to plasma proteins. A partition is achieved between tracer hormone associated with plasma and resin, the latter serving as a neutral index against which the strength of plasma binding can be quantitated. For example, if the serum contains a high concentration of TBG a higher proportion of the added ligand will remain bound to the plasma and a correspondingly lower proportion to the resin. The opposite would be the case for serum with diminished plasma protein binding. A smaller fraction of tracer will associate with plasma and a larger proportion with resin.

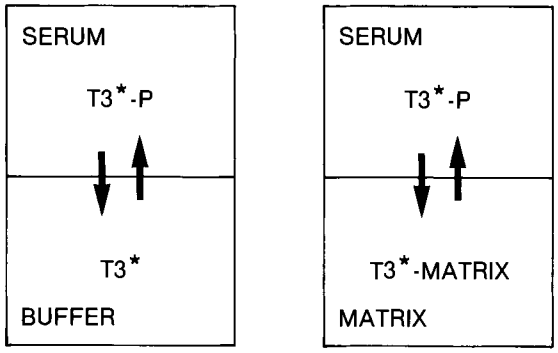

Figure 6–2. Comparison of the dialysis and matrix uptake procedures for assessing plasma protein binding. An equilibrium dialysis procedure can be considered as a mechanism for partitioning iodothyronine between a buffer and serum protein phase, with the final distribution dependent on the net strength of binding of the iodothyronine by plasma proteins and the buffer. In an analogous fashion, the matrix (resin) uptake procedure can be viewed as a mechanism for achieving a partition between the serum and matrix phase, again with the final distribution of the tracer iodothyronine contingent on the relative strength of binding of the matrix and the serum. By dividing the matrix/serum uptake ratio of a test serum by the matrix/serum ratio of pooled normal serum, the role of the matrix binding can be factored out and the relative strength of the plasma binding compared with the normal reference serum assessed. This quotient multiplied by the total T_4 or T_3 yields the free T_4 or free T_3 index, values that are proportional to the free hormone concentrations under many circumstances. By convention, radioactively labelled T_3 is used as the tracer to measure the partition, a fact that accounts for the general designation of this test as the "T_3 resin uptake."

As detailed in Chapter 4, several factors determine the overall strength of plasma protein binding. These include the concentrations of each of the major binding proteins (TBG, TBPA, and albumin); the avidity of each, as represented in the association constant; and the degree of saturation of each binding protein. The last factor deserves some explanation. With excessive production of T_4 by the thyroid an increase in the circulating level of T_4 will result in the occupation of a larger fraction of TBG binding sites and a reduction in the number of unoccupied sites. As a con-

sequence, the DF increases. In hyperthyroidism, diminished protein binding is due to a modest decrease in the concentration of the transport proteins as well as to a reduction in the available binding sites resulting from excess T_4 production. In hypothyroidism, conversely, T_4 production decreases and the fraction of total unoccupied sites increases. The DF falls in part because of an increased TBG protein concentration and in part because of a desaturation of the TBG sites as a consequence of diminished circulating T_4. In hyperthyroidism an increased total T_4 and an increased dialysis fraction will result in a disproportionate elevation in the free hormone concentration; conversely, in hypothyroidism a diminished total T_4 and a diminished resin uptake will result in a disproportionate decline in the calculated free hormone concentration.

A major problem in the interpretation of thyroid function tests results from the diversity of methods for measuring plasma protein binding. In particular, the tradition of using radioactive T_3 to assess the plasma protein binding of both T_4 and T_3 remains in wide use. This practice can be only marginally justified, is totally unnecessary and, as discussed subsequently, can lead to major errors in assessing the free T_4 index in specific sera.

A second problem derives from the fact that resin assays results are frequently expressed as the percent uptake rather than as the resin-to-serum ratio (Fig. 6–3). This practice can lead to conceptual difficulties, as illustrated by the following example. Assume that in a resin uptake a normal serum permits an uptake of 50%. If we could now in some hypothetical fashion remove 99% of the thyroid hormone binding plasma proteins the concentration of free T_4 would increase some 100-fold. However, the percent of T_4 bound to resin would increase by a factor of only 2, from 50 to 100. A more appropriate way of expressing resin uptake binding would be the ratio of labelled iodothyronine bound to plasma bound to iodothyronine bound to plasma. In the example cited, 50% resin uptake would correspond to a resin uptake ratio of 1, and with the removal of 99% of the binding protein the uptake ratio would increase to a correct theoretical ratio of 100.

A convenient method of expressing the free T_4 and free T_3 indices is to normalize the plasma binding ratio of a test serum to that of a pool of normal serum determined concomitantly. The quotient will be unitless, and a

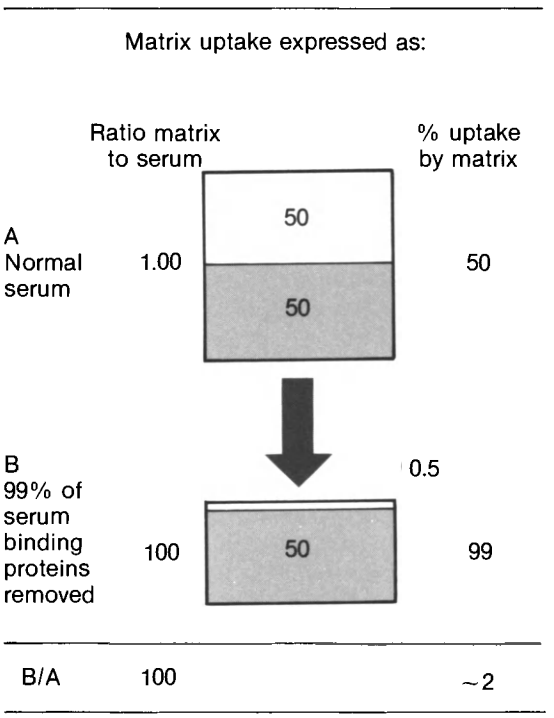

Figure 6–3. Importance of expressing matrix hormone uptake as a ratio rather than percent. In the example illustrated and discussed in the text, 99% of the binding proteins in a normal serum are hypothetically removed. Whereas this clearly should result in a 100-fold reduction in serum binding, the percent matrix uptake is reduced by a factor of only 2.

serum with normal plasma binding should yield a value close to 1. If, however, plasma binding is diminished, the normalized binding ratio will be significantly greater than 1, and conversely increased binding will be accompanied by a ratio significantly less than unity. The product of the normalized binding ratio and the total T_4 or T_3 concentration will result in a free T_4 or T_3 index, each of which will then have approximately the same range as the serum T_4 or T_3.

As pointed out previously, radioactively labelled T_3 is conventionally used to assess plasma protein binding of both T_4 and T_3. Under most circumstances no serious error is introduced, since T_3 and T_4 are predominantly bound by TBG. Only about 15% of T_4 is associated with this protein and only an exceedingly low percentage of T_3 is bound to TBPA. Major problems, however, will be encountered in the aforementioned patients who have abnormal proteins that preferentially bind T_4. This problem may occur in patients with Hashimoto's disease who show specific antibodies to T_4 and in those patients with

familial disorders characterized by abnormal selective T_4-binding albumins or prealbumins. If the level of such proteins is sufficiently high, the total T_4 but not the total T_3 concentration will increase. In such a patient, the free T_4 concentrations calculated from the product of an elevated total T_4 and a diminished DF of labelled T_4 will be normal and will correspond to the euthyroidal state. In contrast, if a resin uptake test is performed with radioactively labelled T_3, the uptake ratio will be normal. Thus, the product of an elevated T_4 concentration and a normal T_3 uptake ratio will result in a falsely high, free T_4 index. For this reason it is clearly preferable to assess the free T_4 index with a radioactively labelled T_4, and the free T_3 index with a radioactively labelled T_3. Although commercial kits for assessing the binding of radioactively labelled T_4 by plasma proteins are not currently available, a report of the Nomenclature Committee of the American Thyroid Association has pointed out the problem of using radioactive T_3 to determine both the free T_3 and the free T_4 index.[27]

An additional recommendation of this Committee was that the term "T_3 uptake" be abandoned because it is so commonly confused with an assessment of total radioimmunoassayable T_3. The committee recommended that the term "thyroid hormone binding ratio" (THBR) be substituted. Until the T_4 resin uptake tests become generally available it will be necessary to substitute THBR values determined on the basis of radioactively labelled T_3. However, the clinician should be alerted to the possibility of abnormal specific T_4 binding proteins by the euthyroid status of these patients and by the detection of normal serum TSH assays. The existence of a dysalbuminemic syndrome can be further supported by a normal level of free T_4 as determined by equilibrium dialysis.

Although measurements of free hormone by equilibrium dialysis remain the generally accepted "gold standard," a variety of methods designed to measure free hormone concentration directly have recently been introduced.[28, 29] It appears doubtful that these methods present a significant practical advantage over free T_4 indices as determined with the appropriate radioactive ligand and calculated with the use of the ratio approach previously discussed. Technical objections to the use of the newer "analogue" methods have been voiced.[27] Even assays of free hormone concentrations by equilibrium dialysis should be regarded largely as approximations of the true concentration of free hormone *in vivo*. Such factors as dilution of the serum, composition of the diluting equilibriating buffer, and difference between incubation temperature and body temperature all contribute to potential variations between the absolute free hormone concentrations determined *in vitro* and the free hormone concentrations that exist in the circulation. The best that one can hope for is to obtain an approximation of the relative changes in binding that occur under a variety of physiologic and pathophysiologic settings.

Most of the currently available methods succeed in separating the effects of increased or decreased TBG. However, when the sera of individual patients with nonthyroidal illness are compared using multiple methods considerable variation is encountered.[30–32] From a practical standpoint, therefore, judgments made regarding the thyroidal status of patients with nonthyroidal disease should not be based primarily on free hormone measurements or free hormone indices. The problem of thyroid function tests in nonthyroidal disease is considered in further detail subsequently.

Serum TSH Assays

Measurements of TSH levels have assumed progressive importance over the past two decades or more (Fig. 6–4). The introduction of the TSH radioimmunoassays by Utiger[33] clearly established the expected increase in serum TSH levels in patients with documented hypothyroidism. The earlier assays, however, did not exhibit the requisite sensitivity to define lower limits, which could effectively separate the results of patients with subnormal TSH values from those of normal subjects.

Conventional TSH radioimmunoassays, which are still widely used at this time, exhibit an upper limit of approximately 6 μU/ml, and the lower limits of detection are from 1 to 2 μU/ml. Approximately 15 to 20% of random samples from normal subjects fail to yield detectable values. In general, patients with primary hypothyroidism display values in excess of 15 μU/ml. Thus, there is a gray, indeterminate zone between 6 and 15 μg/ml. Elderly individuals, especially females, may often show such intermediate TSH values without exhibiting any evidence of overt clinical hypothyroidism or manifesting depressed values of free T_4 or free T_3. If such patients do not have goiter and do not exhibit elevated levels

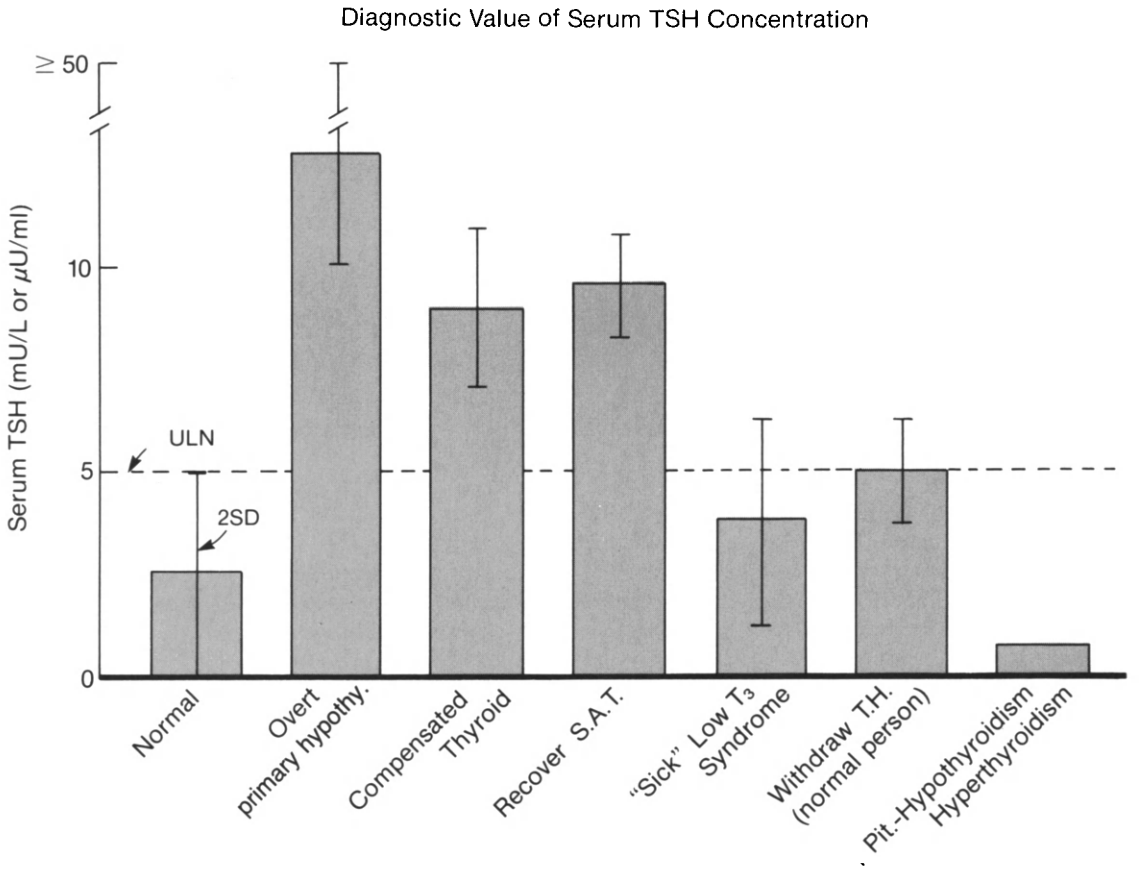

Figure 6–4. Thyroid-stimulating hormone (TSH) concentration in various conditions of health and disease (ULN = upper limit of normal; T.H. = thyroid hormone).

of antimicrosomal and antithyroglobulin antibodies, they can be followed clinically with impunity.[34] However, if such patients do exhibit some manifestations of underlying thyroid disease, such as a goiter or elevated antimicrosomal and antithyroglobulin antibodies, they may well have incipient hypothyroidism and probably deserve treatment with thyroid hormones to forestall the effects of eventual thyroid decompensation.

As mentioned, limitations in the sensitivity of the conventional assays have not allowed use of these tests to detect patients with suppressed TSH concentrations. However, recent technical advances,[35, 36] including the application of monoclonal antibodies and "sandwich" assays, have considerably increased the sensitivity of the TSH assay. It is now possible reliably to detect TSH concentrations below the normal range.

In general, for the newer assays the normal range of TSH lies between 0.4 and 6 μU/ml and values below 0.2 μU/ml are invariably encountered in patients with the common causes of hyperthyroidism and with presumably suppressed TSH values in serum. The "sensitive" or "supersensitive" assays are rapidly replacing the conventional techniques and should be helpful in supporting the diagnosis of hyperthyroidism. Although one might suppose that patients with central hypothyroidism due to pituitary or hypothalamic disease should exhibit low levels of TSH, this is not necessarily the case. The basal TSH concentrations in such patients can be subnormal, normal, or slightly elevated.[37] The reasons for such anomalous behavior are unclear but may well be related to an altered biologic/immunologic potency ratio of TSH in some of these patients.[37, 38]

In essence, the diagnosis of primary hypothyroidism rests on a demonstration of an elevated TSH (> 15 μU/ml). A reduction in the serum T_4 supports such a diagnosis and provides a quantitative index of the severity of the hypothyroidism. As previously pointed

out, measurement of the concentration of serum T_3, however, is rarely justified in the diagnosis of hypothyroidism. The level of serum T_3 is maintained long after the serum T_4 has decreased as a result of the combined effects of increased TSH levels, which stimulate direct thyroidal secereton of T_3, and augmented conversion of T_4 from T_3, which accompanies the hypothyroid state. Further, measurements of serum T_3 in hypothyroidism are likely to lead to confusion in patients with the "low serum T_3 syndrome" who are generally considered to be euthyroid, who most often exhibit normal TSH levels, and who are not suitable candidates for treatment with thyroid hormones.

The diagnosis of central hypothyroidism (due to pituitary or hypothalamic failure) must rest on an assessment of a low free T_4 concentration or index in association with clinical symptoms of hypothyroidism and laboratory findings consistent with hypothyroidism at the tissue level, such as an elevated serum cholesterol or serum creatine phosphokinase level. The basal metabolic rate may also be helpful, but is now rarely performed and not readily available on a routine basis. These tests also obviously lack specificity. Central hypothyroidism is frequently accompanied by other evidence of pituitary trophic hormone failure as well as radiologic or neurologic indications of central nervous system (CNS) disease. Previous reports had suggested that hypothalamic hypothyroidism is characterized by a prolonged elevation of the serum TSH concentration after TRH administration. Further studies, however, have minimized the importance of such patterns.[39]

The increased sensitivity of the TSH assay has led to the proposal that this test also be applied to the diagnosis of hyperthyroidism. Although experience is still limited, it appears unlikely that a depressed TSH by itself will be sufficient to establish a diagnosis of hypothyroidism. Some observers have reported depressed TSH concentrations in patients with nonthyroidal illness[40]; however, most patients with nonthyroidal illness have normal TSH values. Patients receiving levothyroxine replacement treatment may exhibit depressed TSH concentrations without manifesting clinical evidence of hyperthyroidism or elevated T_3 levels.[7] Lastly, patients with apparently nontoxic goiters without serum iodothyronine elevations may show depressed TSH values. In such patients, a minimal increase of T_4 secretion may result in a slight rise in the concentration of serum T_4 and T_3, sufficient to shut off TSH secretion in the patient but insufficient to increase the serum iodothyronine concentrations over the normal population range. Such patients probably do not require treatment for hyperthyroidism.

The principal value of the TSH assay in the diagnosis of hyperthyroidism is to rule out peripheral thyroid hormone resistance (Refetoff's syndrome) or some unsuspected disorder of T_4 binding as discussed. Under almost all clinical situations, the level of TSH is suppressed in patients with hyperthyroidism. The only exceptions are patients with selective pituitary resistance to thyroid hormone or with TSH-producing pituitary tumors, both excessively rare entities. Therefore, any physician who suspects hyperthyroidism and encounters detectable levels of TSH above 0.2 μg/ml in a patient should reconsider the diagnosis.

Frequently, the presence of high normal or supranormal levels of TSH in the face of high free T_4 and T_3 values connotes generalized cellular resistance to thyroid hormone. The molecular basis of such resistance remains unclear. Although defective T_3 receptors have been postulated, defect in intracellular transport or a "postreceptor" defect may be equally responsible. Thyroid hormone resistance at the peripheral level is only partial. With increased concentrations of thyroid hormone the biologic effects of such resistance can be overcome. In the steady state, such patients will exhibit elevated levels of T_4 and T_3, with corresponding increases in free T_4 and free T_3 concentrations; relatively elevated levels of TSH; and goiter. Failure to recognize the syndrome of generalized thyroid hormone resistance may lead to unnecessary treatment for presumed hyperthyroidism either with radioiodine or with surgical removal of the goiter. Normal levels of TSH in patients with elevated total serum T_4 and T_3 concentrations raise the possibility of increased plasma protein binding and point to the necessity of obtaining an appropriate binding assay.

In the past, failure of TRH to elicit the expected increase in TSH (an increase of at least 9 μU/ml, one half hour after 250 μgm TRH) has been used as a criterion for making the diagnosis of hyperthyroidism, where other procedures have yielded borderline or conflicting results. This test, however, is not specific for hyperthyroidism. Elderly patients and patients with nonthyroidal disease may also fail

to respond.[34, 41] With the introduction of the sensitive TSH assay the TRH test may become obsolete.[42] A baseline depression in the TSH level probably has the same diagnostic significance as a subnormal TSH response to TRH. Measurement of thyroidal uptake of ^{123}I is helpful in ruling out silent or DeQuervaine's thyroiditis. Hyperthyroidism associated with thyroiditis is due to the liberation of thyroid hormone as a consequence of the primary inflammatory process of the thyroid gland. Pituitary TSH is reduced, and the thyroidal uptake of radioiodine is severely curtailed. The diagnosis of thyroiditis is important since the clinical management differs markedly from that used in other forms of hyperthyroidism. In general, hyperthyroidism due to thyroiditis is self-limiting and treatment with antithyroid drugs or thyroid ablation is ineffective.

Thyroid Function Testing in Nonthyroidal Disease

Reference has been made to abnormal thyroid function test results in nonthyroid disease. These are exceedingly common and often present perplexing problems in the diagnosis of thyroid disease. The most frequently encountered abnormality is a decrease in the concentration of serum T_3 in a patient with even minimal catabolic disease and one who is undergoing caloric restriction. Thus, the levels of serum T_3 may fall after abdominal surgery or while on weight-reducing diets. Since the concentrations of T_4 as well as the metabolic clearance rates of T_3 and T_4 are unchanged under such conditions,[43] it is clear that a diminished conversion of T_4 to T_3 is the basis of the lowered T_3 concentration.

With advanced neoplastic disease the concentration of T_3 can be depressed to levels as low as 20 to 30 ng/dl. Curiously, such profound depressions are not associated with either increased levels of TSH or clinical features of hypothyroidism. Two studies have failed to demonstrate any benefit of thyroid hormone treatment of patients with the "low T_3 syndrome."[44, 45] The reason why clinical hypothyroidism does not supervene has not been adequately explained. Some investigators have proposed that concomitant metabolic alterations brought about by the primary disease process may somehow compensate at the cellular level for the reduction in plasma T_3 and thus prevent many of the characteristic manifestations of thyroid hormone deficiency.[46] At the pituitary level, these could account for the failure of the TSH rise.

Experimental studies in the rat may shed some light on this paradox. Thus, in starved animals[47] or in animals that bear rapidly growing transplantable tumors[48] the level of serum T_3 also falls, largely because of diminished thyroidal secretion rather than reduction in T_4 to T_3 conversion.[49] In addition, there is a modest reduction in the nuclear T_3 receptor level.[50] Two hepatic enzymes, both of which are characteristically responsive to thyroid hormone under normal conditions, are differentially affected in these two animal models. The level of cytosolic malic enzyme falls to exceedingly low levels, whereas the level of mitochondrial alpha-glycerophosphate dehydrogenase is preserved. Presumably, for alpha-glycerophosphate dehydrogenase the hormonal and metabolic alterations generated by the catabolic stimuli offset the reduction in the hepatic T_3-nuclear receptor complex, which would be expected from a discrete fall in T_3. In contrast, the precipitous fall in malic enzyme appears to reflect a synergism among the fall in serum T_3 and receptor and other metabolic changes, including the development of stress-related insulin resistance. Malic enzyme is one of the lipogenic enzymes involved in the synthesis of fatty acids, and the decrease in this enzyme can be considered to be of adaptive value; the continued synthesis of fatty acids in sick animals would be wasteful of the limited energy reserves available to these animals.

Although the most common abnormality in thyroid function testing among patients with nonthyroidal illness is a reduction in the level of serum T_3, more severe illness can also result in a fall in the level of serum T_4. This decrease appears to be due in large part to a reduction in plasma protein binding of T_4 by a circulating inhibitor.[51, 52] The nature of this inhibitor remains unclear. Claims that the inhibitor is a fatty acid[52] have been contested.[53] Since the putative inhibitor may also prevent the binding of iodothyronines by the cells, the physiologic impact may be more complex than would be anticipated from a simple inhibition of plasma protein binding.

Measurement of plasma protein binding in nonthyroidal disease, using conventional techniques, presents some unanticipated problems. Thus, the circulating inhibitor apparently interferes not only with the binding of T_4 by plasma proteins but also inhibits the uptake of

labelled T_4 by cultured cells and inert resins.[51] As a consequence, if a resin is used to assess plasma protein binding in nonthyroidal disease, the results will show considerably less diminution in binding than would be evident by equilibrium dialysis. The dialyzable fraction of T_4 in a patient so affected is invariably increased. The level of the absolute free thyroxine determined from the product of the dialyzable fraction and the total T_4 by equilibrium is generally normal or slightly elevated.[15]

Because of complex alterations in nonthyroidal illness it is probably wise from a clinical point of view to rely on TSH measurement as the most reliable index of thyroidal status. In general, TSH concentration in such a patient remains in the normal range. Occasionally, however, slight elevations into the gray zone, between 5 and 15 μg, are observed.[24] Such findings may represent a minor decrease in thyroidal secretion of thyroxine. As previously emphasized, it appears unlikely that the administration of thyroid hormone is clinically helpful.

Serum Thyroglobulin

Serum thyroglobulin, which may now be detected by radioimmunoassay[2, 54, 55] and which has a normal range of 0 to 50 pmol/L (0 to 30 ng/ml), may show increased levels in hyperthyroidism, in thyroiditis, in nontoxic goiter, and in thyroid carcinoma.[56, 57] While such elevations of serum thyroglobulin levels are of no diagnostic value with the thyroid gland intact, following removal of the gland for thyroid carcinoma, the test may be very useful in monitoring the presence and activity of metastatic thyroid carcinoma.[57] In recent studies by Pacini and associates[58, 59] of 56 patients' scans with negative findings, 42 had undetectable or very low serum thyroglobulin levels and were considered to be free of metastatic thyroid carcinoma, whereas 14 showed the presence of nonfunctioning metastases in the clinical and/or radiologic examination. In this last group, 11 had elevated serum thyroglobulin levels, while the other three patients had detectable concentrations within the normal range. All 45 patients with positive scan findings in this study, i.e., functioning metastases, had elevated serum thyroglobulin concentrations. These results indicate that serum thyroglobulin measurements correlate very well with scan findings and have the advantage of detecting nonfunctioning metastases, which would not have been detected by scanning.

Another use of this procedure is the detection of persons who are surreptitiously taking thyroid hormone. Since the level of serum thyroglobulin is related to thyroidal secretion, the concentration of thyroglobulin in a patient so affected is low.[60] Lastly, in prolonged subacute or silent thyroiditis, the level of serum thyroglobulin may be the last to revert to normal.[61]

Thyroid Antibodies

Antibodies to a variety of antigens within the thyroid are readily demonstrable by routine procedures.[57] The most useful of these are techniques to detect antibodies against thyroglobulin and microsomal thyroid antigen, which have recently been identified as thyroid peroxidase. Antibodies have also been identified against a colloid component other than thyroglobulin, against the thyroid hormones themselves and against thyroid growth promoting or inhibiting antibodies as well as antibodies to the TSH receptors, which are presumed to represent the proximal thyroidal stimulus in Graves' disease.[62] (These will be discussed in the sections devoted to thyroiditis and hyperthyroidism.)

IN VIVO TESTING

Radioactive Iodine and Technetium Uptake by the Thyroid

Radioactive iodine uptake, a useful and common procedure, has many variations, the most common of which is the 24-hour radioactive iodine uptake.[12, 13, 63] A tracer dose of radioactive iodine is administered orally, and 24 hours later the proportion of radioactive iodine accumulated by the thyroid gland is readily measured by a scintillation counter. Two nuclides of iodine are available for this purpose, ^{131}I and ^{123}I. The latter is currently preferred for routine uptake and imaging since the radiation burden is considerably less than with ^{131}I. However, uptakes at shorter periods—10 minutes, 3 hours, 6 hours—may provide additional information, and technetium can be preferentially employed for such early uptake studies. In fact, in many medical centers, only early uptake studies are performed, but these have the disadvantage of measuring only the initial concentration of free halogen by the

thyroid gland, rather than the subsequent organification of the iodine. Thus, where only early uptake studies are performed, it is possible to overlook the disorders of organification.

The normal range for the 24-hour uptake varies with location, depending largely on the average iodine intake in the diet. Such intakes of iodine have slowly increased in North America, largely because of the varied sources of food, the iodination of bread, and sometimes even the iodination instead of chlorination of water.[64] In North America, the 24-hour ^{131}I uptake normally varies between 10 and 35%. As with all other tests, the results may be misleading. Uptakes may be elevated in conditions other than hyperthyroidism, such as iodine deficiency; rebound phases after subacute thyroiditis, after thyroid, or antithyroid drug administration; or in certain enzymatic defects of the thyroid gland. Nevertheless, it is important to carry out this procedure in patients with hyperthyroidism so as to detect those who are suffering from silent thyroiditis and the typical manifestation of hyperthyroidism. The radioactive iodine uptake will be markedly suppressed in such patients. The uptake also may be suppressed with iodine contamination, antithyroid drugs, thyroid administration, and subacute thyroiditis.[63, 65]

Further tests employing ^{131}I that measure secretion rates or incorporation of ^{131}I into the protein-bound iodine ($PB^{131}I$) suffer from many of the same drawbacks.[63] Radiothyroxine turnover rates, while of interest in studying abnormalities of thyroid function, are not useful as diagnostic procedures.[12, 13, 63]

Thyroid imaging is carried out to determine the anatomic features of the thyroid, to define areas of diminished or increased thyroid function, and to identify metastatic or ectopic thyroid tissue. Thyroid scanning or imaging employs either an isotope of iodine, which is concentrated and bound by the thyroid, or technetium, which is concentrated but not bound by the thyroid.[66, 67] Technetium is used because the isotope is so short-lived that the radiation dose to the thyroid and whole body is reduced in comparison with ^{131}I.

Thyroid scans should be performed in young persons only when really necessary, since the scans involve a significant amount of radiation. In an older person, the test can be employed as required. Scans are most useful in determining gland size in Graves' disease and in determining the presence of "cold" (nonfunctioning) or "hot" (autonomous or hyperfunctioning) thyroid nodules.[68, 69] Scans are also of great importance in discovering metastases from well-differentiated thyroid carcinoma; whole body scans are useful in this respect.[70] Various other isotopes, such as cesium 137, gallium,[67, 75] selenomethionine, and others, have been used in an attempt to illuminate thyroid carcinomatous tissue with limited success.[71–73]

Another form of thyroid imaging, which is now quite popular, is that of ultrasonography, which will determine whether a thyroid nodule is solid or cystic.[74, 75] Ultrasonography has also been successfully used to estimate thyroid size and sometimes used in conjunction with thermography.[76] These aspects are discussed further in relation to the evaluation of a solitary thyroid nodule. In this respect, computerized axial tomographic scanning also has some limited usefulness in determining the extent of substernal goiter formation and malignant masses.[77] Still another means of scanning is the fluorescent scan, in which no isotopes are used.[63] The test determines the quantity and distribution of iodine within the gland. Because the equipment is highly specialized, the procedure has not found wide application.

Other Procedures

The basal metabolic rate (BMR) was historically the first useful test of thyroid function. Used widely until the mid-1950s, it suffered as a precise technique for a variety of reasons.[78] Unfortunately, the BMR was often not "basal," and even when accurately performed, it was often elevated in nonthyroidal conditions, such as leukemia, lymphoma, emphysema, congestive heart failure, and perforated eardrums. Similarly, the BMR was depressed in a variety of diseases that did not affect thyroid function, such as starvation, thiamine deficiency, nephrotic syndrome, Addison's disease, and periodic catatonia. It is evident that the BMR is only an indirect measure of thyroid activity, since other factors affect the consumption of oxygen. Nevertheless, it is one of the few clinical tests that measures a tissue effect of thyroid hormone. For this reason, it is still useful in circumscribed purposes, although it is no longer available in most hospitals.

Another procedure that measures an effect of thyroid hormone on tissues is that of kinemometry, a procedure which measures the

Achilles tendon reflex on electrocardiographic paper.[79, 80] This return phase of this reflex is characteristically slowed in hypothyroidism and is more rapid in hyperthyroidism. The test is simple, but there is considerable overlap between the responses in normal persons and those in patients with hypothyroidism or hyperthyroidism. Thus in problem cases, the test is not definitive. It is, of course, useful in the follow-up of an individual patient. It should perhaps be noted that the relaxation time is altered in other conditions, including hypothermia, local edema, various neurologic disorders, diabetes, and propranolol therapy. The normal range is 240 to 320 milliseconds.

Two readily accessible tests that can be used to monitor tissue response in the treatment of hyperthyroidism and hypothyroidism are serum cholesterol and serum creatine phosphokinase.[81] In hypothyroid patients under treatment with thyroactive preparations there is a progressive fall in both values. Conversely, treatment of patients with hyperthyroidism produces a rise in both parameters. As with the BMR, these tests of thyroid function suffer from lack of specificity.

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PART IV
THYROID DISORDERS

7
Nontoxic Goiter—Diffuse and Nodular

GERARD N. BURROW

In North America, a working definition of a goiter is that of a thyroid gland that is twice larger than normal or about 40 gm in weight. Probably more than 200 million individuals in the world have goiter, most on an endemic basis. Many of these goitrous thyroid glands contain one or more nodules.[21] Endemic goiter has been eliminated as a medical problem in developed countries through the introduction of iodine. However, in developing nations, endemic goiter continues to be a major problem, frequently made worse by geologic positions, e.g., the Andes, predisposing to iodine deficiency.

The classification of goiter to be used here is that of the American Thyroid Association and is based on whether the nontoxic goiter is diffuse or nodular (Table 7–1). Further subdivisions are based on whether the goiter is endemic, sporadic, or compensatory. If the goiter is nodular, it may be uninodular or multinodular and functional or nonfunctional.

Paracelsus, the Swiss-German physician (1493–1541), described endemic cretinism in the region around Salzburg[33] and pointed out that it occurred in association with endemic goiter. Goiter was widely present in this alpine region (Fig. 7–1). Iodine was discovered in 1817, and in 1820 Jean Francois Coindet reported to the Swiss Scientific Society that he had administered iodine to 150 goitrous patients without ill effects. Almost 100 years elapsed before Marine, in 1917, popularized the use of iodized salt in North America for prevention of endemic goiter.[25]

GOITER FORMATION

The classic explanation for goiter formation is that periods of iodide deficiency and subse-

Table 7–1. Classification of Nontoxic Goiter*

I. Nontoxic diffuse goiter
A. Endemic
1. Iodine deficiency
2. Iodine excess
3. Dietary goitrogens
B. Sporadic
1. Congenital defect in thyroid hormone biosynthesis
2. Chemical agents, e.g., lithium, thiocyanate, *p*-aminosalicylic acid
3. Iodine deficiency
C. Compensatory following subtotal thyroidectomy
II. Nontoxic nodular goiter due to causes listed above
A. Uninodular or multinodular
B. Functional, nonfunctional, or both

*Modified from Werner SC: J. Clin. Endocrinol. 29:860, 1969.

Figure 7–1. Goitrous girl. (Reproduced from Merke, F.: Geschichte von Kropf und Kretinismus. Bern, Hans Huber Verlag, 1971, with permission.)

quent repletion result in cyclic hyperplasia and involution of thyroid follicular cells.[25] Iodide deficiency or whatever the specific cause happens to be is thought to result in inadequate thyroid hormone production with a compensatory release of thyroid-stimulating hormone (TSH), leading eventually to goiter formation. Iodide deficiency would be an extremely rare cause of goiter formation in North America. This explanation is predicated on the assumption that TSH is ultimately responsible for all thyroid gland growth, which may not be correct.

Acute reduction in TSH stimulation appears to be responsible for conversion of a hyperplastic goiter into a colloid goiter.[38] Any situation that would result in periodic elevation and cessation of TSH secretion might eventually result in the production of a nodular goiter. Nodularity might also be caused by the shunting of blood to a particular area of the thyroid with a consequent increase in growth. In chronic goiter, the blood supply to the thyroid may be markedly increased.

There has been recent interest in the possibility that growth factors besides TSH play a role in goiter formation.[6, 10, 41] Immunoglobulin fractions have been obtained from patients with nontoxic goiters as well as with Graves' disease that stimulate thyroid growth as measured by DNA.[10] Goiter size correlates with these thyroid-growth immunoglobulins rather than with thyroid hormone concentration. Epidermal growth factor has also been found to stimulate thyroid cell growth and again indicates that factors other than TSH are capable of stimulating thyroid growth.[12, 32, 44]

Evidence that goiter is due to increased TSH stimulation has been derived from studies of patients with endemic goiter. Mean serum TSH concentrations were found to be increased (16 μU/ml) in 285 patients from endemic goiter regions of New Guinea.[8] However, there was no difference in serum TSH concentrations between goitrous and nongoitrous patients. Serum TSH concentrations from goitrous patients who lived in iodine-deficient areas of Brazil were higher than those from goitrous patients who lived in iodine-replete areas.[28] Studies from another endemic goiter area, Greece, suggested that the iodine-deficient thyroid gland was more sensitive to TSH stimulation.[23, 40]

Because of diverse etiologies, ranging from thyroiditis to goitrogen ingestion, studies of sporadic goiter are even more difficult to interpret. In some instances, there have been slight increases in the serum TSH concentrations, often with increased TSH responses to thyrotropin-releasing hormone (TRH).[9] The TSH response to TRH was more often impaired in patients with nodular goiters as compared with diffuse goiters. Whether these findings are indicative of a degree of autonomy in the nodular gland is not clear. Interpretation of various studies may also be complicated by the fact that serum TSH concentrations are higher in early goiters.[45] TSH concentrations also tend to be higher during the early decades with a progressive decrease of TSH secretion and reserve.[3] Actual thyroid growth is more rapid early in life and then regresses (Fig. 7–2), although thyroid nodularity may increase with age (Fig. 7–3). Therefore, failure to find elevated serum TSH concentrations in goitrous individuals may be a function of time. Of course, identification of factors other than TSH capable of stimulating thyroid gland growth offers another possible explanation.[6, 10, 41]

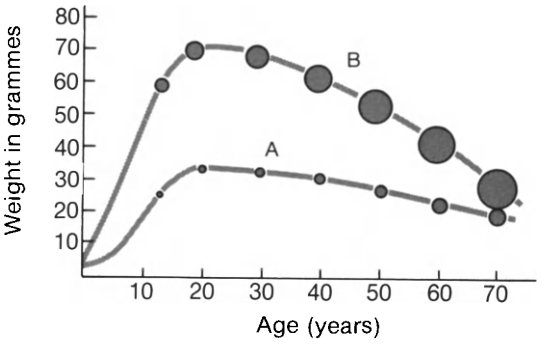

Figure 7–2. Goiter formation as a function of age. Curves depict the weight of the thyroid as a function of age for both nonendemic goiter areas (*A*) and endemic goiter areas (*B*). Circles represent the relative size of thyroid nodules throughout life in both groups. (Redrawn from Burrow, G. N.: The thyroid: Nodules and neoplasia. *In* Felig, P., et al., eds.: Endocrinology and Metabolism. New York, McGraw-Hill Book Co., 1981, with permission.)

ENDEMIC GOITER

Goiter becomes endemic in a population when the incidence increases above 10%. Iodine deficiency is the major cause on a worldwide basis, but other goitrogens have been described which affect entire populations. The development of an endemic goiter presumably follows the general pattern of goiter formation previously described. Growth factors other than TSH would explain why goiter is present in only certain people in some endemic areas. In areas of severe iodine deficiency, virtually everyone is goitrous, regardless of sex or age. Increases in thyroid size can be found even in areas of mild iodine deficiency.[16a] The thyroid may enlarge moderately during childhood, and the incidence is usually highest by puberty. In fact, goiter of puberty is a sensitive indicator of the lack of iodine in a region. After puberty, the goiter tends to persist in the female but decreases in size in the male. In New Guinea, thyroid enlargement correlated with breast enlargement but not with pregnancy, lactation, or parity.[8]

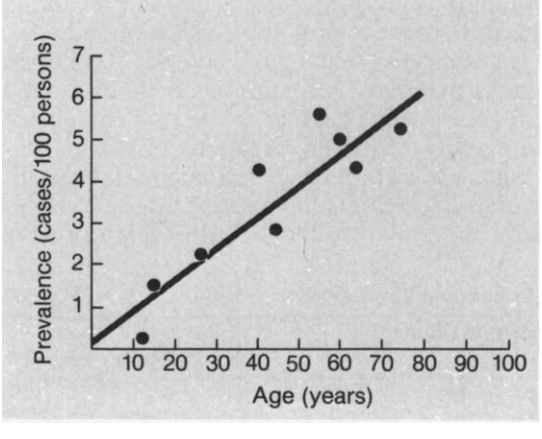

Figure 7–3. Prevalence of thyroid nodules with age. (Redrawn from Maxon, H. R., et al.: Ionizing irradiation and the induction of clinically significant disease in the human thyroid gland. Am. J. Med. 63:967, 1977, with permission.)

In areas of severe iodine deficiency, the human thyroid gland cannot completely compensate for a low plasma inorganic iodide by a proportional increase in the iodide clearance rate.[11] Total iodine stores may actually be normal in the patient with endemic goiter who is euthyroid, but the iodine concentrations in the hormonally active pool are low.[36] Iodine deficiency increases the monoiodotyrosine-diiodotyrosine ratio, and fewer iodothyronines are synthesized. There is also an increased T_3 to T_4 ratio that allows the optimal use of iodine atoms to produce the more potent T_3 molecule.

Cretinism

In areas of severe iodine deficiency, cretinism may occur. Cretinism has been defined as permanent neurologic and skeletal retardation resulting from an inadequate supply of thyroid hormone during gestation.[18, 36] Endemic cretins may present with hypothyroidism and a variety of neurologic defects, including mental retardation, deafness, dwarfism, mutism, and spastic diplegia. The hypothyroidism can be diagnosed clinically by the retarded linear growth and maturation of body proportions, myxedematous skin, and marked delay in sexual development.

Two forms of endemic goitrous cretinism have been described. The "myxedematous type" is characterized by hypothyroidism, dwarfism, and epiphyseal dysgenesis. In contrast, the "nervous type" is characterized by mental retardation and deaf-mutism (Fig. 7–4). The clinical picture of endemic cretinism represents a spectrum of clinical and metabolic signs, which reflect varying impairment of the nervous system and thyroid function (Table 7–2). Iodine prophylaxis will prevent the development of both types of cretinism. The intrauterine period is crucial for the development of cretinism, and pregnant women in endemic goiter areas should receive special medical attention.[39]

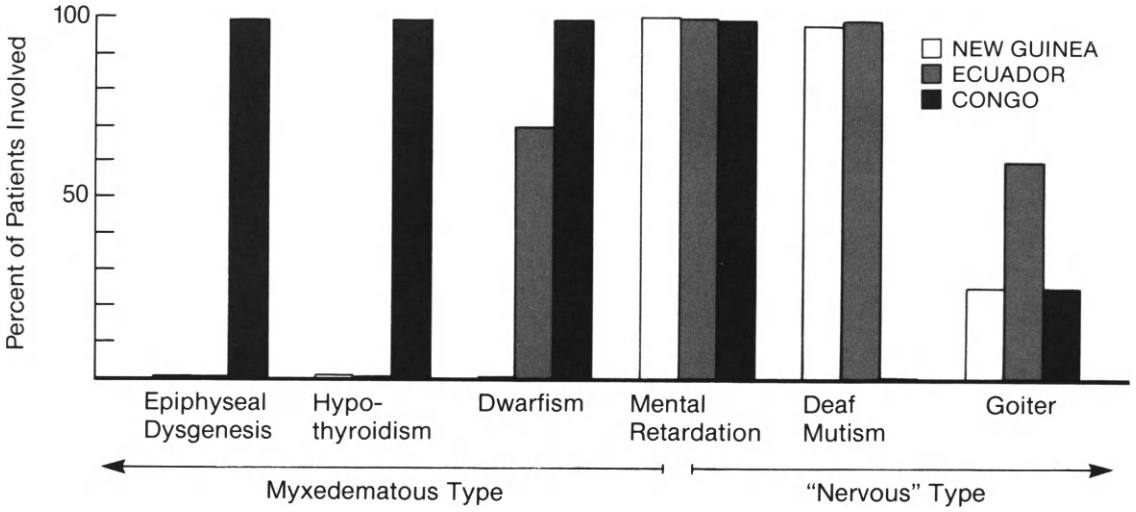

Figure 7–4. Regional variations of the clinical pattern of endemic cretinism. (Modified from Delange, F., et al.: *In* Stanbury, J. D. and Kree, R. L., eds.: Human Development and the Thyroid Gland. New York, Raven Press, 1972, with permission.)

Table 7–2. Types of Cretinism

Myxedematous	Neurologic
Severe mental retardation	Severe mental retardation
Severe growth retardation	Mild growth retardation
Minimal neurologic findings	Severe neurologic findings
Delayed tendon reflexes	Spastic diplegia
Skeletal immaturity	Neuromotor incoordination
	Deaf-mutism (common)

Iodine Prophylaxis

When iodine is added to the diet in an endemic area, cretinism disappears. Most individuals require more than 50 μgm of iodine daily to replace that excreted in the urine (Table 7–3).[18] Individuals on iodine deficient diets most often live in villages in areas where the soil is poor in iodine, and they eat predominantly local food. In areas subjected to intense glaciation or flooding during the last ice age, the iodide has been leached from the soil. Severe iodine deficiency is less likely in the populations of large cities and in upper socioeconomic classes because the diet is more varied and is obtained from a wider area.

Studies have indicated that iodine is a highly effective agent for the prevention of goiter. However, the total impact on the worldwide problem of endemic goiter has been relatively small so far. Part of the difficulty lies in the distribution of iodine in remote, isolated areas. Iodization of salt has been the most satisfactory method developed to supply an entire population, particularly in developed countries with adequate systems of distribution. In remote areas, iodized oil has been successfully used as an injectable form of goiter prophylaxis that lasts for 3 or more years.

Iodine is much more effective in preventing the development of goiter than in suppressing a chronically enlarged thyroid. Enlarged glands may have formed cysts or nodules and are much less likely to involute than glands with early diffuse hyperplasia. Thyroid hormone therapy will ensure an adequate concentration but will not reverse established cretinism, and, in fact, the behavior pattern may

Table 7–3. Urine Iodide Content, Goiter, and Development*

Endemic	Urine Iodide (μgm/gm Creatinine)	Palpable Goiter (%)	Clinical Spectrum
Mild	>50	10–20	Normal
Moderate	25–50	20–50	Occasional deafness and retardation; serum TSH ↑ in 10–20%.
Severe	<25	40–90	Cretinism 1–10%; serum TSH ↑ in 30–50%.

*Modified from Ibbertson, H. K.: Clin. Endocrinol. Metab. 8:97, 1979.

deteriorate with increased activity. If the goiter is extremely large, surgery may be indicated (Fig. 7–5). The major indication for surgery is pressure symptoms. Goiters encircling the trachea or under the sternum are most likely to produce pressure symptoms.

In areas of endemic goiter, thyrotoxicosis appears to be more common after the introduction of iodine therapy (Iod-Basedow phenomenon). Plummer's disease, or toxic nodular goiter, is more common than Graves' disease and may provide the explanation of the Iod-Basedow phenomenon. Hyperfunctioning nodules are avid for iodine and may respond with a marked increase in thyroid hormone production. Alternatively, iodine could have a toxic effect on the thyroid, resulting in the release of thyroid hormone.[4]

After the introduction of iodine prophylaxis, the incidence of both nodular goiter and certain thyroid adenocarcinomas and sarcomas decreased. However, an increase in the incidence of papillary carcinoma of the thyroid was noted, but comparison of the geographic distribution of goiter prevalence with geographic distribution of mortality from thyroid cancer revealed no convincing evidence for the existence of an association between the thyroid cancer and goiter.[31]

CAUSES OTHER THAN IODINE DEFICIENCY

In addition to iodine deficiency, a number of other variables including malnutrition, genetic factors, and environmental goitrogens may play a role in both endemic and sporadic goiter formation.

Protein-Calorie Malnutrition

In addition to iodine deficiency, protein-calorie malnutrition is often present in the same endemic area and may contribute to abnormalities in thyroid function. Malnutrition causes various alterations in the thyroid gland structure and function, including defective hormone iodination that would further deplete iodine stores.[15] Adequate protein-calorie intake corrects these abnormalities. In a study done in Africa, protein-calorie malnutrition resulted in defective formation of the mannosyl-retinol-phosphate complex that is necessary for normal glycosylation of thyroglobulin.[19]

Genetic Influences in Goiter Formation

The suggestion has been made that individuals in endemic areas who develop goiter are genetically distinct from their neighbors who do not. Only certain individuals were goitrous in an endemic region of Greece despite a uniformly high thyroid uptake.[24] The goiters tended to be grouped within families. Goiter prevalence was compared in monozygotic and dizygotic twins of the same sex to estimate genetic factors in goiter formation. The data suggested that a genetically determined propensity for goiter formation exists.

Specific Inherited Genetic Defects. The clearest example of genetic influences on thyroid function are to be found in the small group of

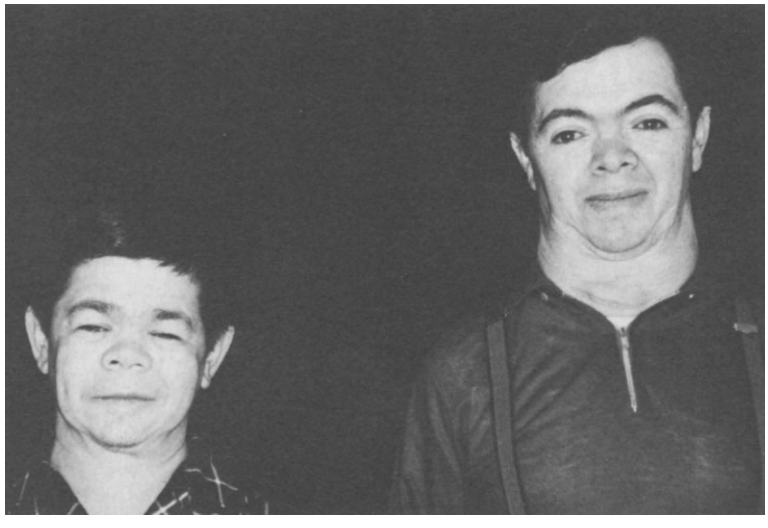

Figure 7–5. Two brothers with goitrous cretinism, secondary to a defect in thyroglobulin. (Reproduced from Burrow, G. N.: The thyroid: Nodules and Neoplasia. *In* Felig, P., et al., eds.: Endocrinology and Metabolism. New York, McGraw-Hill Book Co., 1981, with permission.)

individuals who have autosomal recessive inheritance of specific defects in thyroid hormone synthesis. These defects occur because of genetic defects in enzymes or other proteins that correspond to the various steps of thyroid hormone biosynthesis. Recombinant DNA techniques will permit a more intensive examination of these genetic defects.[42] (The biochemical defects are described in detail in Chapter 2.) Whatever the specific defect, genetic influences lead to inadequate thyroid hormone production and goiter formation. Whether similar but less well-defined genetic influences play a role in sporadic goiter remains to be determined.

Goitrogens

A goitrogen can be defined as any substance that causes the thyroid gland to enlarge, usually by interfering with thyroid hormone biosynthesis. Many of the dietary goitrogens have not been specifically identified, but a number of specific chemical compounds are known that inhibit specific steps of thyroid hormone biosynthesis (Table 7–4). A significant difference in the prevalence of goiter occurred among individuals who live in different parts of an isolated island on Kivu Lake in Zaire.[39] Studies revealed that the differences in goiter prevalence were due to different methods of preparing cassava. Cassava under a variety of names, e.g., manioc and tapioca, is a major world source of carbohydrate, particularly in developing countries. The root contains compounds that release free cyanide on hydrolysis. After ingestion of improperly prepared cassava, the cyanide is converted to thiocyanate, which blocks thyroid function. The iodine content of the diet in Zaire is low, and the thiocyanate in the cassava lowers it still further. Data from Vietnam indicate that administration of adequate iodine will prevent the goitrogenic action of cassava.[17]

Table 7–4. Chemical Goitrogens*

I.	Effect within the thyroid
A.	Iodine transport
1.	Complex anions: technetium perchlorate, thiocyanate (active principle of *Brassica* genus and cassava)
B.	Iodination of thyroglobulin
1.	Thioamides: propylthiouracil, methimazole, carbimazole
2.	Thiocyanate
3.	Aniline derivatives: sulfonamides, *p*-aminosalicylic acid, amphenone, aminoglutethimide, phenylbutazone
C.	Iodothyronine formation
1.	Thioamides
2.	Sulfonamides
D.	Secretion of thyroid hormone
1.	Iodide
2.	Lithium
II.	Effect on peripheral disposal of thyroid hormone
A.	Hormone deiodination: propylthiouracil, iopanoate, ipodate
B.	Intestinal absorption of hormones: soy flour, resins (e.g., cholestyramine)
C.	Hormone inactivation: inducers of hepatic drug-metabolizing enzymes (e.g., phenobarbital)

*From Burrow, G. N.: The thyroid: nodules and neoplasia. In: Felig, P., et al. (eds.), Endocrinology and Metabolism. McGraw-Hill, New York, 1981.

The observation that rabbits fed cabbage developed goiters and further studies in which rats fed the seeds of *Brassica* (e.g., cabbage, turnips, brussel sprouts, and rutabagas)[7] developed goiters, identified thiourea compounds as the specific goitrogen. This finding was pursued and led to the use of thiourea derivatives (i.e., thioamides) as valuable therapeutic agents in thyrotoxicosis. Soybean milk has also been observed to produce goiter in infants. The goitrogenic substance in soybean flour has not been identified, but iodine supplementation eliminates the problem.

Various goitrogens, ranging from salts and minerals to complex aminoheterocyclic compounds, interfere with thyroid hormone synthesis. Lithium is a salt of particular interest because it is a cation but has an effect similar to iodine in blocking the release of thyroid hormone from the gland. More complex chemical compounds with common structural features interfere with thyroid function by blocking iodination and the coupling reaction. None of the aniline derivatives which are aminoheterocyclic are as potent as thioamides but act in the same manner.

The Coho salmon in Lake Erie have recently been found to be goitrous again (Fig. 7–6). Marine's original observations on iodine deficiency were made on Coho salmon in Lake Erie. There is plenty of iodine in the water, and chemical contamination is thought to be the cause. Contamination of the water supply has been recognized as goitrogenic in low-iodine areas.[16] McGarrison noted that goiter prevalence increased down-stream from the water supply in a group of villages in the Himalayan foothills. The incidence of goiter increased from 12% at the source to 45% at the terminus of the river which served as both a source for drinking water and an open sewer for the villages along the banks. McGarrison[26]

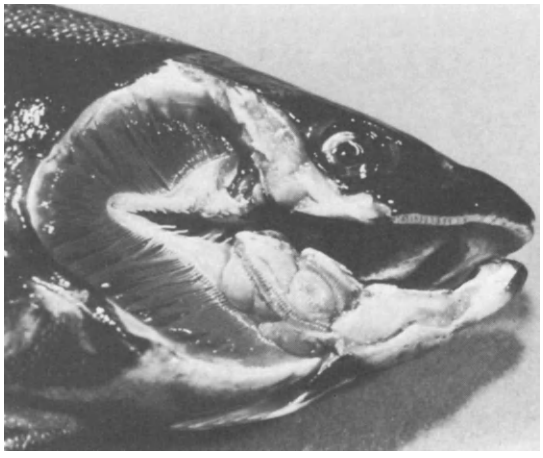

Figure 7–6. Goiter in a Coho salmon. (Courtesy of Dr. John Leatherland.)

was able to produce goiter in himself and his colleagues by the ingestion of suspended matter from river water. If the water were boiled first, the goiter did not occur. Subsequently, the residents in this area were found to be markedly iodine deficient with a urinary iodine excretion of 4 to 6 μg/L. However, equally low iodine values were found in both goitrous and nongoitrous individuals. In Colombia, sulfur-containing compounds found in the water supply derived from sedimentary rock have been thought to be goitrogenic.[16]

SPORADIC GOITER

Goiters that occur in a nonendemic goiter region are called sporadic. The majority of these goiters have no known etiology. Population studies have indicated that about 4 to 5% of the population in a nonendemic area have goiter.[43] Whatever the etiology, the pathophysiology of sporadic goiter formation is presumably similar to endemic goiter.

Sporadic goiter does not usually occur before puberty. The peak incidence of endemic goiter occurs between the ages of 10 and 50 years, with a decrease after 50. Sporadic goiter in contrast has no peak age incidence. The suggestion has been made that goiter may occur during puberty and subsequently disappear without therapy. However, sporadic goiters of this type may represent thyroiditis rather than a "physiologic goiter." The "goiters of puberty" are said to occur more commonly in females. In fact, thyroid dysfunction of all kinds is about five times more common in females than in males. An obvious source for the difference would be estrogens, but there is little evidence to support the contention. In a systematic comparison of the development of the pituitary-thyroid axis in male and female rats, differences were found. Whether they are pertinent to sex differences in human thyroid dysfunction is not clear.[22]

NONTOXIC GOITER

Patients with sporadic and endemic goiters, which may be either diffuse or nodular, usually present clinically as those with nontoxic goiters, regardless of the exact etiology and depending on the duration and perhaps other factors. The incidence of sporadic nontoxic goiter has been estimated in both autopsy and clinical studies in North America and has been found to be about 5%. In a large unselected autopsy series of 1000 patients over 20 years old, half the thyroid glands contained nodules on section that were at least 1 cm and would have been palpable if anterior and superficial.[30]

Clinical Presentation

Aside from endemic goiter areas, nontoxic goiter is usually found during a routine physical examination. Alternatively, the patient or a friend may have noticed a lump in the neck.

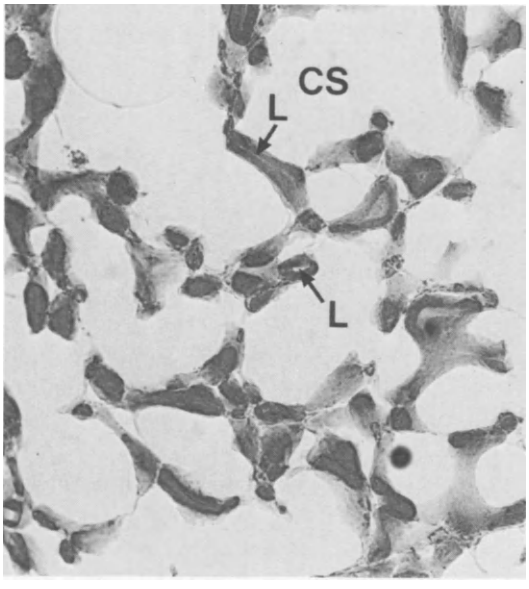

Figure 7–7. Thyroid-stimulating hormone (TSH) stimulation of vascular spaces in the thyroid. Follicles are widely spaced and lumens (L) are generally narrowed. The space between follicles is the lumen of a cavernous capillary system (CS). (Courtesy of Dr. S. H. Wollman.)

Table 7–5. Response of Nontoxic Goiter to Therapy with a Usual Dose of 180 μg of Desiccated Thyroid Hormone for Periods Ranging from 1 to >24 Months*

Type of goiter	No. of patients	Type of response		
		Complete (%)	*Moderate (%)*	*None (%)*
Diffuse	115	33	34	23
Nodular	78	24	52	24
Solitary nodule	37	27	27	46

*From Astwood, E. B., Cassidy, C. E., Aurbach, G. D.: J.A.M.A. 174:459, 1960.

Rarely, the goiter may be symptomatic and cause pressure symptoms, such as wheezing, coughing, dysphagia, or hoarseness. Presentation in this manner is uncommon, and carcinoma or an unrelated condition should be ruled out. When these symptoms occur with benign lesions, the goiter is usually low in the neck, growing around behind the trachea or compressed under the sternum. Radiographs of the trachea commonly show deviation with goiter and may show some compression. Computed tomographic scanning of the thyroid may be helpful but delivers more radiation than conventional x-rays or thyroid scans. Peak inspiratory and expiratory flows should be determined to document a decrease in air flow. Upper airway obstruction may occur more commonly than realized in goitrous patients.[20]

The obstruction is potentially dangerous because the development of tracheitis with edema could result in severe narrowing of the airway. Occasionally, the patient complains of the development of a sudden increase in the size of the thyroid, accompanied by pain and tenderness. This sudden change is often indicative of hemorrhage into a cystic area of the thyroid but usually subsides within several weeks.

Occasionally, the physician encounters the difficult management problem of a patient with a nontoxic goiter who also has globus hystericus and who complains of a "lump in the throat."

Patients sometimes describe variation in goiter size, often related to periods of emotional stress or to the menstrual cycle, but these changes are difficult to document. The irregular shape and position of the gland have made the estimation of thyroid size difficult. There is a rich blood supply to the thyroid, and changes in blood flow might alter the volume of the thyroid. The ancient Romans are reported to have believed that the thyroid swelled with sexual excitement and measured the bride's neck as evidence that the marriage had been consummated.

Table 7–6. Response of Nontoxic Goiter to Therapy with L-Thyroxine and L-Triiodothyronine*

Thyroid hormone	Decrease in goiter size (%)	
	12 weeks	*28 weeks*
T_3	55	73
T_4	39	49

*From Shimaoka, I. K., Sokal, J. E.: Am. J. Med. 57:576, 1974.

Laboratory Evaluation

A patient presenting with a goiter needs to be classified according to whether the goiter is toxic or nontoxic. If the goiter is nontoxic, is thyroiditis present? Is the goiter diffuse or nodular and, if nodular, solitary or multiple? If the gland is nodular, are the nodules autonomous? (Evaluation of thyroid function has been described in Chapter 6.) A patient with goiter should undergo at least a serum T_4 determination and measurement of thyroid binding protein and thyroid antibodies to determine the functional thyroid state and the presence of thyroiditis. A radioisotopic thyroid scan is helpful in identifying nodularity and autonomy.

Therapy

I believe that with some exceptions, all goiters should be suppressed with thyroid hormone therapy. Exceptions include multinodular goiters in elderly patients at risk for heart disease and self-limited conditions, such as goiter of pregnancy. A "caveat" would be the presence of autonomous areas of goiter, which would not be suppressed and would result in too much circulating thyroid hormone. For this reason, thyroid function tests should be obtained 3 or 4 weeks after initiation of therapy. A radioisotopic thyroid scan is not necessary. Treatment of a long-standing goiter may not

result in a decrease in the size of the thyroid because of the presence of fibrosis. Should long-term thyroid hormone therapy be recommended to a patient if the goiter is not large or unlikely to decrease in size? Prolonged TSH stimulation results in dilated capillaries, which may rupture and result in a hemorrhagic cyst. (Fig. 7–7). Additionally, prolonged TSH stimulation may lead to nodule formation. The presence of a nodule in the thyroid can lead to increased anxiety levels in the patient, which in turn leads the physician to the operating table.

The philosophy behind the tendency to treat all goiters with thyroid hormone is predicated on the fact that thyroid hormone is relatively nontoxic and relatively inexpensive. The philosophy is also predicated on the dogma that TSH is responsible for the goiter formation. The possibility that other factors stimulate thyroid gland growth is raised in Chapter 2. Thyroid malignancy can be induced in rats by prolonged TSH stimulation but there is no convincing evidence that TSH stimulation is carcinogenic in normal individuals.[1, 29] Although malignancy has been reported in intensely hyperplastic goiters, the differentiation can be difficult.[13, 27]

Thyroid Hormone Therapy. Sufficient thyroid hormone must be administered to suppress TSH secretion without inducing thyrotoxicosis. L-thyroxine is preferred since the hormone is slowly converted to L-triiodothyronine in the periphery and elevated serum concentrations of T_3 do not occur. Administration of L-triiodothyronine results in markedly elevated serum levels of the hormone for several hours following ingestion. Studies of TSH concentrations in hypothyroid women receiving L-thyroxine indicated that 90% of patients were optimally managed by the daily administration of between 100 and 200 μgm of L-thyroxine.[37] Older patients require less thyroid hormone, between 100 and 125 μgm of L-thyroxine daily.[34] Administration of 300 μgm of L-thyroxine daily has resulted in metabolic alterations suggestive of subclinical hyperthyroidism in some patients.[5]

Evaluation of Therapy. Despite the enormous number of patients who have received thyroid hormone suppression, there is a paucity of large, well-controlled studies that have examined the efficacy of such treatment.[2, 35] In one study, 230 patients with nontoxic goiter were treated with 180 mg of desiccated thyroid and followed at 1- to 3-month intervals. The goiter was regarded as diffuse when the entire gland was enlarged, even if the two lobes differed in size or considerable irregularity was present. The gland was defined as nodular when conspicuous nodules were present or when a single nodule was found in an enlarged thyroid. Solitary nodule was diagnosed when there was an isolated nodule in an otherwise normal gland. Regression of the goiter was considered complete when the thyroid gland decreased to normal size in response to thyroid hormone therapy. The response was termed moderate if there was unequivocal regression without a complete return to normal. About 25% of the patients with nodular goiter had complete regression and another 25% had moderate regression (Table 7–5).

In another study, 114 patients with goiter were randomly assigned to T_3 (50 μgm/day) or T_4 (200 μgm/day).[35] Five patients had diffuse goiters, 38 had multinodular goiters, and 71 had uninodular goiters on palpation. Patients who had significant reduction in goiter size (greater than 20%) at 12 weeks continued to receive the same dose of thyroid hormone for 16 weeks. If the goiter's size did not change, patients were either continued at the same dose or the suppressive dose of the drug was doubled, i.e., 100 μgm/day of T_3 or 400 μgm/day of T_4. The results are shown in Table 7–6. The major determinant of a decrease in goiter size was the duration of treatment rather than the dose of thyroid hormone. Patients who continued to receive the same dose of thyroid hormone had the same response rates as those receiving the higher dose. Studies of the non-nodular thyroid remnant after surgery for nontoxic goiter suggest that tissue with abnormal hormone production is widely distributed within the gland.[44a] Therefore, thyroid hormone suppression may not be helpful.

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8
Nodular Goiter and Thyroid Cancer

GERARD N. BURROW

Since as many as 50% of adult thyroid glands may harbor nodules 1 cm or greater in size, the question arises as to when surgery should be considered. Thyroid carcinoma has been found in approximately 3% of the patients in two different unselected autopsy series.[55, 94] When serial sections of the thyroid are carefully examined, the incidence of histologic malignancies rises to 13%.[72] The clinical incidence of thyroid cancer is about 39 cases/1 million population or 0.0004%.[17] The reason for the marked discrepancy between clinical presentation and autopsy findings is not clear. The findings are similar to prostatic carcinoma in which the incidence at autopsy is higher than clinically apparent disease.

Does the presence of a multinodular goiter increase the likelihood of clinically significant thyroid cancer? The mortality rate for thyroid carcinoma has been reported to be ten times higher in Switzerland, where goiter is endemic, than in England,[91] and the incidence decreased in Switzerland concomitant with a decrease in goiter after the introduction of iodized salt. However, as mentioned previously, careful pathologic examination will result in an apparently higher incidence of thyroid carcinoma. Another way to study the problem is to compare a decrease in goiter prevalence to a possible decrease in the incidence of thyroid carcinoma. Goiter prevalence declined between the two World Wars with the introduction of iodized salt. Changes in goiter prevalence based on medical examinations of draftees could be compared with the incidence of thyrotoxicosis and thyroid carcinoma during the same time period.[64] Despite the marked decrease in goiter, the death rate for thyroid carcinoma changed very little during this time. Immediately after the introduction of iodized salt, mortality rates from thyrotoxicosis increased, perhaps because of the Iod-Basedow phenomenon, and then decreased in parallel with the decline in goiter. These findings strongly supported the importance of endemic goiter in the pathogenesis of hyperthyroidism. In contrast, the comparison of thyroid cancer and goiter failed to provide convincing evidence for the existence of an association between the two. The presence of goiter alone does not appear to increase the incidence of thyroid carcinoma.

SOLITARY THYROID NODULES

In contrast to a multinodular goiter, the risk of thyroid carcinoma is increased wih a solitary

nodule. In the same unselected autopsy series as noted previously, 19 single thyroid nodules or 7% of the 300 cases were found after sectioning of the gland.[83] The incidence of thyroid carcinoma in these single nodules was almost four times the incidence in the entire series. The chance that a solitary thyroid nodule in this series would be neoplastic, benign, or malignant was almost three times that of the entire group. Although the separation of single from multiple thyroid nodules is difficult clinically, thyroid cancer has been found more frequently in the solitary thyroid nodule. Malignant thyroid nodules usually do not concentrate radioiodine as well as normal thyroid tissue, and they usually appear as nonfunctioning or hypofunctioning areas on a radioiodine scan of the thyroid.[79] However, thyroid carcinoma may also occur in functioning nodules and the presence of a functioning thyroid nodule does not eliminate the possibility of thyroid malignancy. In one series an overall incidence of thyroid carcinoma was 29%. If "cold" solitary nodules had been the sole criterion for operative selection thyroid carcinoma would have been missed in 40% of the 202 patients.

To eliminate any bias in case selection, one would have to operate on all patients with solitary thyroid nodules, which is obviously not possible. An attempt was made to eliminate some of the bias by studying all patients who were referred for radioisotopic scan of the thyroid (Fig. 8–1).[31] The supposition was that a physician in the community would ordinarily obtain such a scan before making any therapeutic decision about a solitary thyroid nodule. Solitary "cold" thyroid nodules were present in 130 patients and 68 of these patients underwent surgery. The factors that determined those patients who were referred for surgery were not clear despite the study design. Thyroid carcinoma was found in 18% of the 68 patients who came to surgery. Even if there were no malignancies in the group who did not undergo surgery, the incidence of thyroid carcinoma in these presumably unselected patients with solitary cold thyroid nodules would have been about 9%.

Risk Factors for Thyroid Cancer

The possibility of malignancy is increased in solitary thyroid nodules. The question is whether or not we can select those patients that are at particular risk.

External Radiation to the Head and Neck. The risk of malignancy in a thyroid nodule is significantly increased by previous irradiation of the head and neck. This association was first highlighted in 1950 when the observation was made that a third of a group of 28 children with thyroid carcinoma had received therapeutic doses of radiation to the thymus during infancy. Most of the radiation-associated thyroid malignancies had been reported about 10 years after exposure. With the discontinuance of this type of therapy, physicians then developed a degree of complacency in regard to this entity. However, although radiation therapy for benign diseases of infancy and childhood has been discontinued for many years, the occurrence of radiation-associated thyroid carcinoma has apparently not declined.[22, 69]

The exact magnitude of the increase in thyroid carcinoma after irradiation to the neck is unknown because appropriate age-matched control data are sparse. Estimates have ranged from a twofold to a 100-fold increase in the frequency of thyroid cancer. About a third of patients with a history of head and neck irradiation will have abnormal findings in the thyroid on careful examination.[22, 77] Carcinoma was found in 37% of patients who had single thyroid nodules found on physical examination in one study[22] and in 35% of patients who underwent surgery in another. Whether radioisotopic scans of the thyroid improve the yield as intended of significant thyroid nodules is not clear.[76, 77] Decision analysis suggested that not doing a radioisotopic thyroid scan with periodic reevaluation was the preferred strategy.[90]

The time required for the development of clinically evident thyroid carcinoma after external radiation ranges from 3½ years to at least 30 years and perhaps longer.[22, 77] In one study, the cumulative frequency of thyroid carcinoma after exposure to external radiation showed an initial increase at 20 to 25 years after exposure and then an apparent but not statistically significant decline.[22] These investigators hypothesized that if the progression of the thyroid disease involved hyperplasia, adenoma formation, and eventually malignancy, a progression should have occurred with age. There was actually a reversal in progression, suggesting that irradiation induced malignant change right away that took 20 years to become clinically evident. They further hypothesized that thyroid hormone suppression would not be helpful 20 years after exposure, and in fact they did not find that prophylactic thyroid

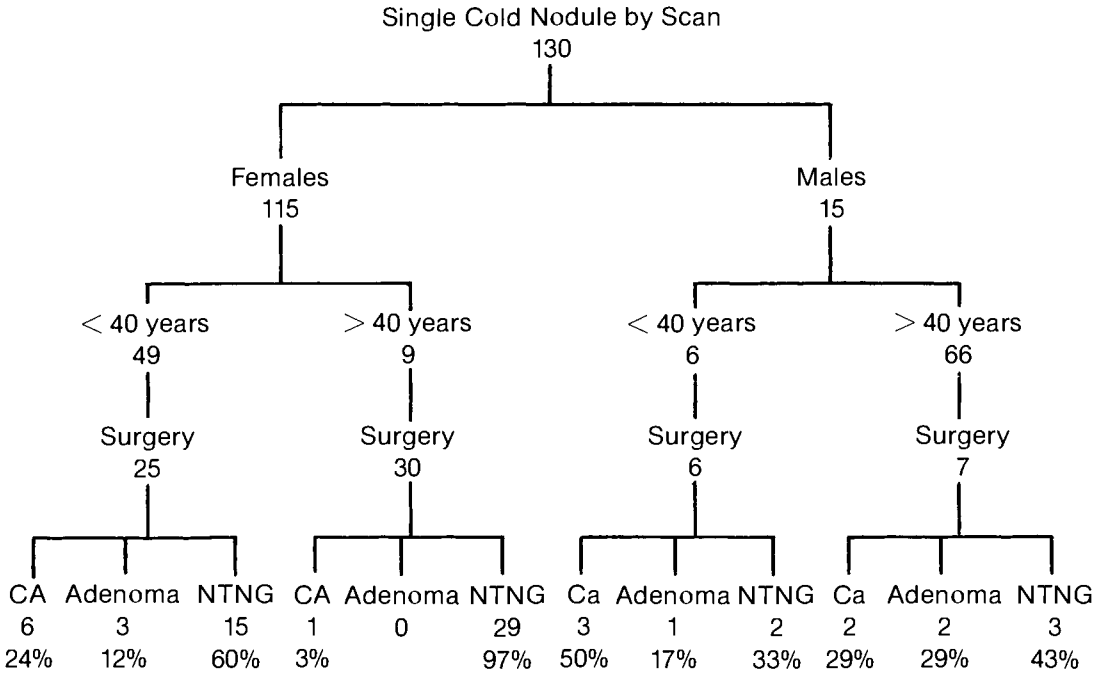

Figure 8–1. Solitary "cold" thyroid nodule by radioisotopic scan. The numbers of individuals in each subgroup are shown (CA carcinoma; NTNG-nontoxic nodular goiter). (Redrawn from Burrow, G. N., et al.: Yale J. Biol. Med. 51:13, 1978, with permission.)

hormone suppression was effective in preventing new nodularity in previously examined glands. However, another prospective study of 1108 patients who received irradiation for benign conditions of the head and neck during childhood indicated that thyroid nodules were continuing to occur at a constant rate.[77] Until further information is available, prophylactic thyroid hormone suppression with attention to possible problems is reasonable in patients previously exposed to head and neck irradiation, unless there are other contraindications.

Radiation-associated thyroid carcinoma has almost always been well differentiated, and the prognosis has been good when detected in patients under 40.[71a, 77a] The absolute risk for thyroid cancer was found to be 1.5 cases/million/rad/year when data from several studies were combined.[51] The relationship between estimated dose to the thyroid and thyroid cancer is shown in Figure 8–2. Carcinogenesis is uncommon after radiation doses above 2000 rads, which suggests an upper limit for the induction of thyroid carcinoma by external radiation to the head and neck. One explanation for the "protective" effect of large doses of radiation has been that the complete destruction of the thyroid cell prevents abnormal growth and neoplasm formation. There is no proof for this hypothesis but it would also explain the failure of therapeutic doses of ^{131}I to be a risk for thyroid carcinoma. Therapeutic doses of ^{131}I for thyrotoxicosis result in delivery of 5000 to 8000 rads to the thyroid. Whether external radiation has a different radiobiologic effectiveness than the same dose of radioisotope is not clear. Doses of external radiation as low as 50 rads have been associated with risk of thyroid carcinoma. The use of ^{131}I for thyroid scans, which may deliver radiation in the carcinogenic range (100 to 200 rads), should be reevaluated. No clear relation exists between the radiation dose and the interval between exposure and detection.

The great majority of tumors have been papillary thyroid carcinoma. These malignancies found at the time of surgery are not simply minimal papillary carcinoma. In one study, two thirds of the carcinomas were greater than 1 cm in size and 59% were multifocal with lymph node metastases.[22] Nonthyroidal tumors are also increased by previous irradiation to the head and neck, particularly neural, salivary gland, and probably parathyroid tumors.[67, 77]

Age. As many as half the thyroid nodules appearing in children may be malignant. Thyroid nodules are uncommon in children so that

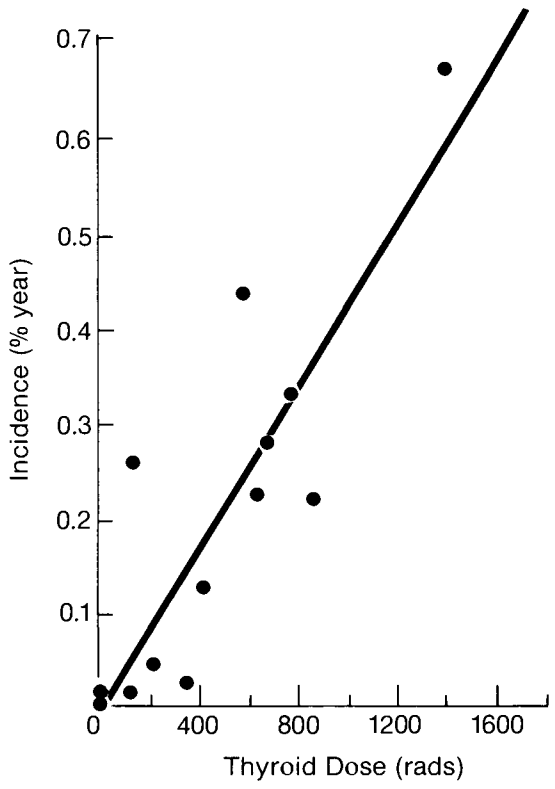

Figure 8–2. The incidence of thyroid cancer as a function of the dose of external radiation. (Modified from Maxon, H. R., et al.: Ionizing irradiation and the induction of clinically significant disease in the thyroid gland. Am. J. Med. 63:967–978, 1977.)

the presence of a nodule should raise the suspicion of malignancy. Much of the data to support this contention were generated in studies of children with past histories of external irradiation to the head and neck.[36, 101] The increased risk for thyroid malignancy needs to be reappraised in children who have not been irradiated. In a larger study of 5179 school children in grades 6 through 12, only two thyroid cancers were found although nodularity of the thyroid was present in 93 or 1.8%.[45] The thyroid nodularity represented thyroiditis in 31 children and adolescent goiter in another 34. The nodules that proved to be neoplasms were usually solitary and firm without changes in the rest of the gland. These findings raise the question of whether all solitary thyroid nodules in children require surgical removal.

However, thyroid carcinoma may continue to be a significant risk in children with solitary thyroid nodules. Only four children had histories of irradiation to the head and neck of 30 children with solitary thyroid nodules.[65] Nevertheless, there was still a 40% incidence of thyroid carcinoma, which included the four children exposed to irradiation. In a 10-year review of solitary thyroid nodules removed from children without history of external radiation, five of 14 revealed thyroid malignancy.[84] Despite possible bias in selection, solitary thyroid nodules in children remain a matter of concern.

Sex. Thyroid malignancy similar to other forms of thyroid disease is more common in females. Nontoxic, nodular goiter also occurs much more frequently in females. As a consequence, a male with a solitary thyroid nodule is three times more likely to have a malignant nodule than is a female.

Physical Characteristics. Certain findings on clinical examination of the thyroid should raise the suspicion of underlying malignancy. Palpation of the thyroid should be done carefully with particular attention to the regularity and hardness of the nodule, whether the gland is fixed to surrounding structures and whether there are palpable regional lymph nodes. In a group of patients first classified clinically followed by surgical corroboration in all cases, thyroid cancer was found in 76% of those thought to be malignant clinically.[9] In thyroid nodules thought to be benign clinically but symptomatic as indicated by pain, recent change in goiter size, or dysphagia, 12% of cases were found to be malignant. Of nodules thought to be benign that were asymptomatic, carcinoma was found in 3%.

The most significant physical sign of malignancy in the thyroid is the presence of a hard, irregular nodule. However, extreme hardness may occur with hemorrhage into a cyst and subsequent calcification. If the cancer has spread beyond the capsule and invaded surrounding structures, the clinical diagnosis of thyroid cancer becomes comparatively simple, although Riedel's struma can also extend beyond the capsule. Fixation to the strap muscles, trachea, or larynx is easily detected on physical examination. Recurrent laryngeal nerve paralysis is not a common presenting symptom in thyroid cancer but does occur.

Diagnosis

Once suspicion of thyroid malignancy is raised by the presence of risk factors or by physical examination, the diagnosis must be confirmed through tests.

Thyroid Biopsy. Although various procedures may heighten the suspicion that the nodule is malignant, only a tissue diagnosis will be confirmatory. Concern that cancer might be disseminated along the track delayed acceptance of needle biopsy of the thyroid. Seeding has not proven to be a problem, and fine needle aspiration of the thyroid has gained wide acceptance.[49] Follicular carcinomas of the thyroid are particularly difficult to diagnose by fine needle biopsy since identification often rests on the demonstration of vascular and capsular invasion. On the one hand, such areas will be missed on fine needle biopsy, which is limited to individual cells. On the other hand, because of unique histologic characteristics, papillary carcinoma is easily identified. Lymphomas may be confused with thyroiditis.[10, 87] This relationship is further confused by the greatly increased risk of malignant thyroid lymphoma occurring in patients with chronic lymphocytic thyroiditis.[41]

Comparison of the fine needle aspiration specimen with the rest of the thyroid gland is frequently not possible because the majority of patients do not undergo thyroid surgery. In one series of 81 cases where 93% of needle biopsy–based diagnoses were confirmed by subsequent thyroid surgery, errors were due to lymphoma complicating Hashimoto's disease, undifferentiated thyroid carcinoma misinterpreted as nonspecific thyroiditis, and follicular carcinoma misinterpreted as follicular adenoma.[97] No evidence of tumor implantation along the needle track was found in any of the primary thyroid tumors, and no serious complications occurred. A renal cell carcinoma metastatic to the thyroid did seed along the needle track. Thyroid biopsy with a cutting needle results in more tissue for examination but also has the potential for more serious complications, which has made many physicians cautious about the procedure. With fine needle aspiration biopsy, 1000 cells may be obtained from perhaps a billion cells that constitute the thyroid gland. With this kind of sampling error, negative findings determined by needle biopsy should not deter immediate referral to surgery if a significant clinical suspicion of thyroid malignancy exists.[66]

Fine needle aspiration biopsy has become the determining factor in the management of the solitary thyroid nodule (Fig. 8–3).[13, 95] In a review of 1330 patients who had surgery for thyroid nodules, 22 patients with negative findings determined by aspiration biopsies had thyroid carcinoma—a false-negative rate of 1.7%.[1] The false-positive rate was 0.5% in the same study. Fine needle aspiration biopsy specimens read as malignant had a specificity of 99% and a sensitivity of 73%.[95] Specificity measures the fraction of patients correctly identified as having no thyroid malignancy. Sensitivity measures the fraction of patients with thyroid cancer that will be detected by biopsy. The results of the various tests to detect thyroid cancer can be expressed according to Bayes' theorem, which gives a predictive value for the diagnostic test (Fig. 8–4).[56]

Three different approaches for the diagnosis of thyroid nodules were examined. The radioisotopic thyroid scan was the first in two approaches, followed by either fine needle aspiration biopsy or ultrasonography if the original scan showed a "cold" nodule.[95] The third approach began with a fine needle aspiration biopsy followed by a radioisotopic scan of the thyroid and was the most cost effective of the three. Neither radioisotopic scan nor ultrasonography of the thyroid had high specificity, and combination of the two procedures yielded the highest sensitivity but did not improve the specificity. Fine needle aspiration biopsy of the thyroid was most cost effective of the available tests when applied alone. Whether cost effectiveness should be the only factor considered in the treatment of individual patients has been questioned.

The number of patients with thyroid nodules referred to surgery decreased by half while the number followed clinically doubled with the diagnostic use of fine needle aspiration of the thyroid in one series of 455 patients with thyroid nodules.[59] Surgery for benign thyroid disease decreased 70%, whereas the diagnosis of thyroid malignancy at surgery increased by 75%. Approximately 15 to 30% of patients with thyroid nodules who have fine needle aspiration biopsies will have suspicious cytologic findings, and 10 to 15% of these patients will have thyroid malignancies at surgery.[29] The accuracy of the procedure depends both on the sampling and the cytopathologic interpretation (Table 8–1).

Radioisotopic Thyroid Imaging. The administration of a tracer dose of radioactive iodine or technetium (^{99m}Tc), which is trapped like iodine but not organified, permits the delineation of functioning and nonfunctioning areas of the thyroid (Fig. 8–5). Hypofunctioning or "cold" thyroid nodules have been considered

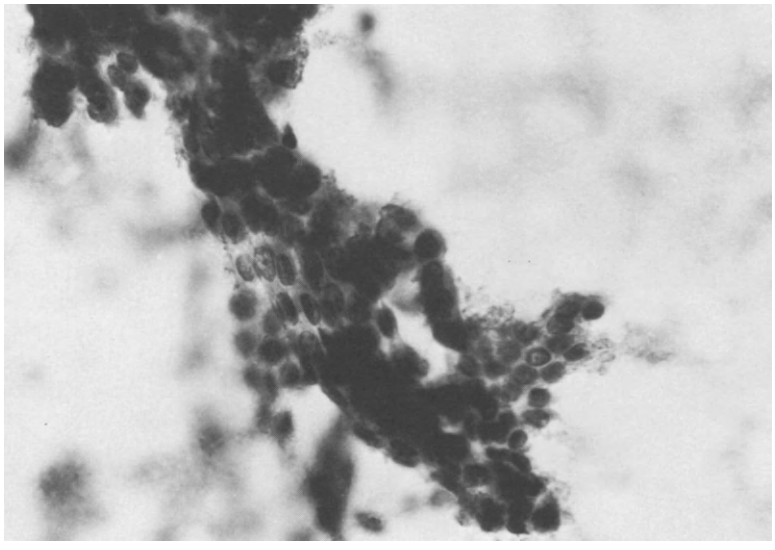

Figure 8–3. Fine needle biopsy aspirate of papillary carcinoma of the thyroid, demonstrating papillary configuration.

Table 8–1. Critical Factors in Assessing Fine Needle Aspiration Biopsy of the Thyroid*

Factor	Per Cent
Inadequate study	3 to 11
Incidence of indeterminable reports	17 to 30
Incidence of carcinoma in indeterminable reports	20 to 60
False-negative reports	2 to 4
False-positive reports	0 to 3

*Reproduced from Block, M. A.: Surgery of thyroid nodules and malignancy. Curr. Prob. Surg. 20:135, 1983, with permission.

to be a greater risk for malignancy since even well-differentiated thyroid carcinomas do not concentrate iodine as efficiently as normal thyroid. The amount of radioactivity taken up by the nodule relative to extranodular uptake is compared. A "cold" nodule must be at least 1 cm in size to be detectable. Very large thyroid nodules can be difficult to interpret because they may show locally reduced parenchymal activity, marginal indentation, or a well-defined focal defect. Demonstration of a "cold" thyroid nodule is helpful, but the majority of clinically significant thyroid nodules, benign and malignant, do not take up radioisotope as well as the extranodular tissue. The presence of a "hot" thyroid nodule that produces enough thyroid hormone to suppress TSH and inhibit radioisotope uptake in extranodular tissue can be helpful. Malignancy is extremely rare in "hot" thyroid nodules, which account for about a quarter of solitary thyroid nodules.

The radionuclide most widely used for imaging of the thyroid has been iodine 131,

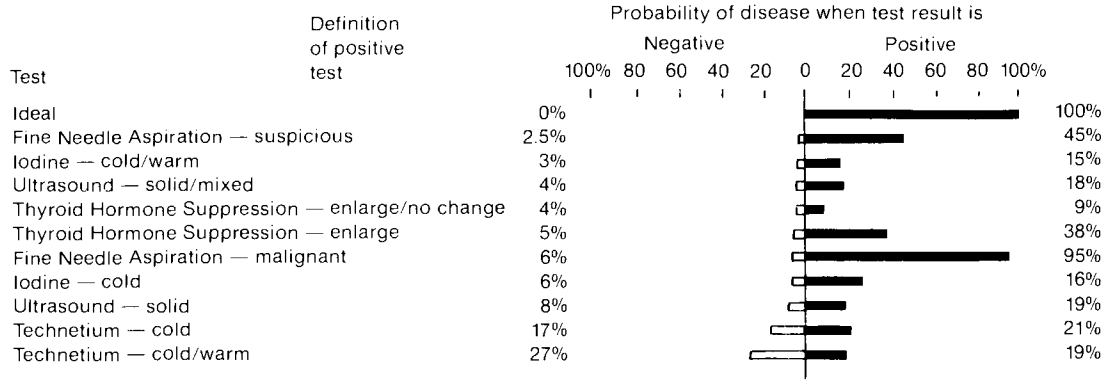

Figure 8–4. Probability of disease based on test results calculated according to Bayes' theory. Open bars represent missed malignancies, and solid bars represent percentage of operations at which malignancies were found. (Modified from Van Herle, A. J., et al.: The thyroid nodule. Ann. Intern. Med. 96:221–232, 1982, with permission.)

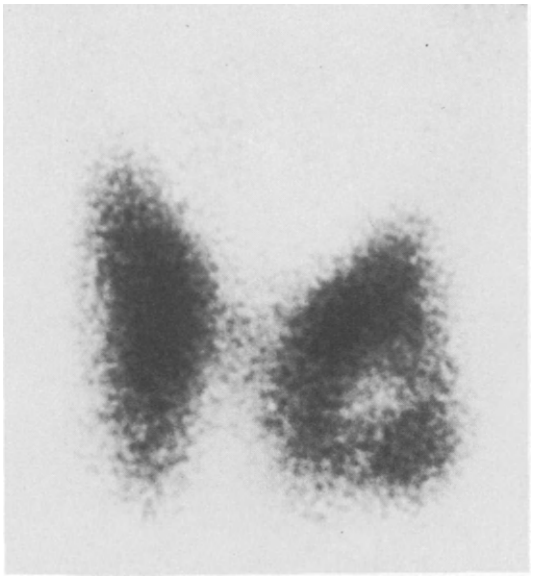

Figure 8–5. Radioisotope scan shows a nonfunctioning solitary nodule in the lower pole of the right lobe of the thyroid.

although there was concern about the radiation dose delivered during the imaging. With the recurrence of interest in the delayed effects of head and neck irradiation, this concern has been rekindled.[19] The thyroid gland receives a dose of 80 rads with the administration of 100 μCi of I^{131} for a scan. Alternative methods of imaging have become highly desirable with the realization that several such scans would deliver a potentially carcinogenic dose to the thyroid, if radionuclides are comparable to external radiation to the head and neck.

For diagnostic studies of thyroid structure and function, I^{123} is potentially the ideal isotope. The half-life of 13.3 hours is long enough to do routine uptakes. Scans superior in resolution to ^{99m}Tc or ^{131} I can be obtained with a radiation dose 1/85 of that for a comparable I^{131} study. ^{99m}Tc delivers about 0.6 rads to the thyroid during a scanning procedure. The pertechnetate ion($^{99m}TcO_4$) is trapped by the thyroid-like iodine but is not organically bound and is rapidly discharged from the gland. This difference between the two isotopes has clinical significance because some thyroid malignancies appear functional with pertechnetate since they trap iodine but are "cold" with ^{131} I as they do not organify iodine.

No patient with a clinically solitary thyroid nodule should be selected or not selected for surgery because of the presence or absence of a "cold" nodule on thyroid scan. However, the presence of a "hot" nodule that is suppressing the extranodular tissue would exclude serious consideration of malignancy in most cases.

Ultrasonic Thyroid Imaging. The presence of a "cold" nodule on thyroid scan does not distinguish between a nonfunctioning nodule and a cyst. An ultrasonic beam directed into the thyroid nodule may permit the differentiation of the nonfunctioning nodule from the cyst. By passing ultrasound frequencies into the thyroid nodule and by analyzing the reflected echoes, one can distinguish soft tissue. The ability to discriminate is based on the partial reflection of the ultrasound at the tissue interfaces or boundaries. Multiple echoes are generated in solid thyroid nodules but not in cysts. Usually, a number of beams is incorporated to form a two-dimensional image. Tracheal cartilage represents the most acoustically dense structure in the area, whereas thyroid gland is less acoustically dense than the surrounding tissue (Fig. 8–6). Thyroid volume also can be estimated by ultrasound.[33] Enlargement of the gland due to tumor can be distinguished from cysts or hemorrhagic degeneration.[8]

Cysts occur in about 20% of solitary thyroid nodules.[60] The incidence of malignancy in thyroid cysts is less than in solid lesions, although it may occur particularly in a mixed (solid and cystic) lesion. Unfortunately, with the increased sensitivity of newer ultrasound equipment, the diagnosis of mixed lesion is made more frequently and is less helpful. Thyroid hormone suppression has not been found to be helpful in preventing recurrence of benign cysts after initial aspiration.[54] Successful sclerosis of a recurrent thyroid cyst has been reported with tetracycline but is not generally indicated.[93]

The use of ultrasound to evaluate solitary nodules over 4 cm is limited because both solid and cystic lesions will generate heterogenous echoes. Small nodules are also difficult to identify, and cystic lesions less than 1 cm in diameter may not be identified. Substernal goiters are difficult to distinguish because the sternum reflects the sound. Therefore, ultrasonic study of thyroid nodules provides useful information about the consistency of the nodule but does not differentiate between benign and malignant ones.

Other Imaging Techniques. In addition to the

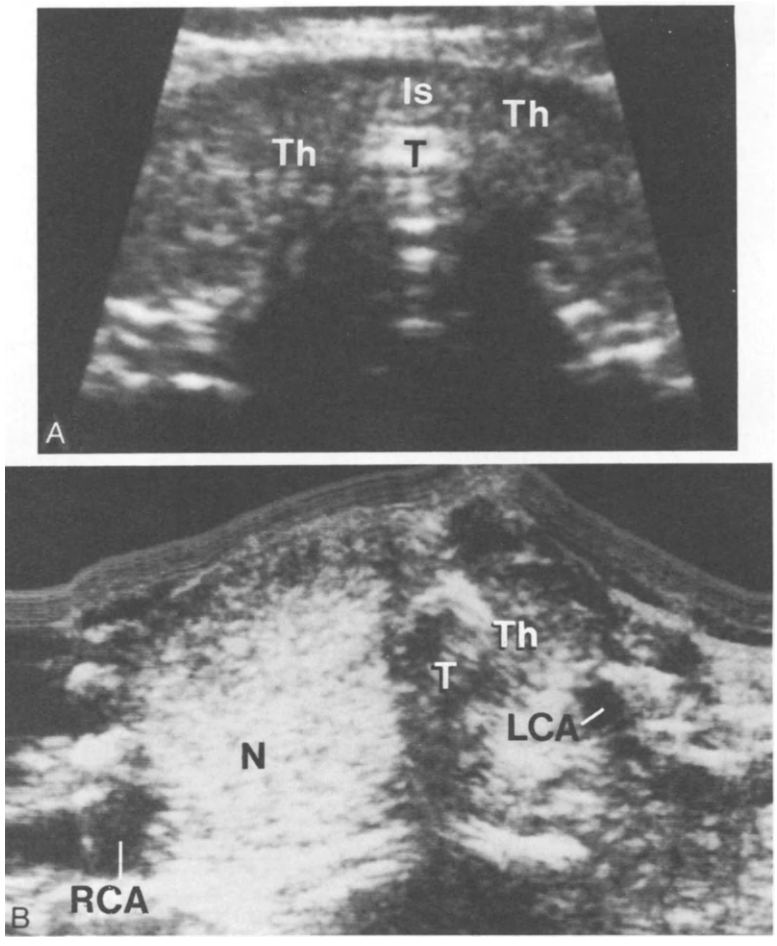

Figure 8–6. Transverse ultrasound scans. *A*, Normal thyroid gland. *B*, A right lobe focal echogenic nodule (Is = isthmus; N = nodule; T = trachea; Th = thyroid; and LCA and RCA = left and right carotid arteries).

radioisotopic scan and ultrasound, other imaging modalities are available to visualize the thyroid. The thyroid gland is relatively dense because of the presence of iodine, which has facilitated the use of the computed tomography (CT) scan with or without prior loading with stable iodine.[68] However, the process results in more radiation of the thyroid gland, and the resolution of thyroid nodules is probably no better than ultrasound.

Radiothallium(^{201}Tl) can substitute for potassium in its intracellular metabolism with preferential uptake in a more cellular lesion and an increased probability of malignancy. In a study of the use of ^{201}Tl scans in 117 patients with solitary thyroid nodules, the predictive value of malignancy was 67% and the predictive value of negative findings was 90%.[42] The procedure may have a useful supportive role in the investigation of nonfunctioning solitary thyroid nodules by heightening the suspicion of malignancy or by indicating a low probability of thyroid malignancy.

Since the thyroid gland has a rich blood supply and thyroid cancers in particular have increased vascularity, angiography findings can also be helpful in conjunction with other findings.[61] In a study of 114 patients who subsequently underwent thyroid surgery, the combined use of thyroid scans with pertechnetate and radionuclide angiography increased the yield of carcinoma in solitary "cold" thyroid nodules from 26 to 60%. The documentation of a hypervascular "cold" thyroid nodule is helpful but not generally indicated.

Nuclear magnetic resonance (NMR) techniques can be used to image the thyroid gland.[18] The spatial resolution of NMR was about as good as CT and allowed better differentiation of thyroid nodules, thyroid cysts, and parathyroid tumors. With further experience, NMR imaging of the thyroid may become a more useful technique for the evaluation of the thyroid.

Therapy of the Solitary Thyroid Nodule

The management of the solitary thyroid nodule is outlined in Figure 8–7. From a practical

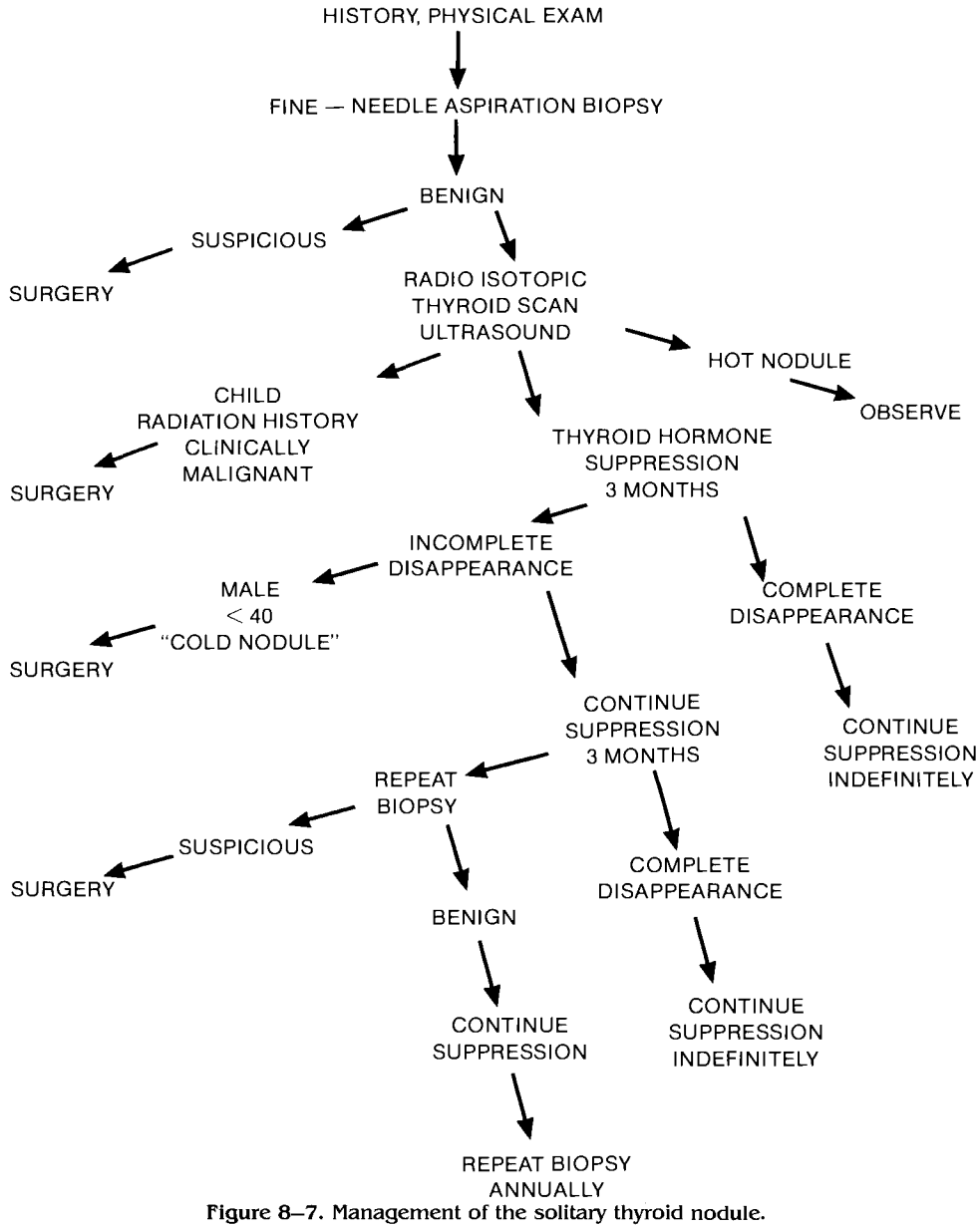

Figure 8–7. Management of the solitary thyroid nodule.

standpoint, it is easier to do the fine needle aspiration biopsy while the patient is in the office for the initial visit and then schedule a radioisotopic thyroid scan. If the biopsy findings raise suspicion, surgery is indicated; if the nodule is "hot," observation is indicated in the euthyroid patient. A solitary thyroid nodule occurring in a child or in an individual with previous radiation to the head and neck should be operated upon as should a thyroid nodule that appears clinically malignant on examination. In the absence of these immediate indications, the thyroid gland may be suppressed with thyroid hormone for 3 months. Disappearance of the nodule is an indication for continued thyroid hormone suppression. An occasional patient may have an autonomous nodule, and thyroid function tests should be repeated 3 weeks after thyroid hormone suppressive therapy to look for elevated values. If suppression of the nodule is incomplete and the patient is a young male or the nodule is "cold," surgery is probably indicated. Otherwise, thyroid hormone suppression can be continued for another 3-month trial period.

The role of ultrasound is to determine

whether a nonfunctioning nodule on thyroid scan is solid or cystic. Aspiration biopsy will often determine the same information, and the exact role for ultrasound is still a matter for discussion.[7]

If the nodule disappears completely with thyroid hormone suppression, suppression should be continued indefinitely. All solitary nodules should be biopsied including those in patients in whom the decision to operate has been made because of mitigating factors, e.g., previous history of external radiation to the head and neck. Knowledge beforehand that the thyroid nodule is malignant is helpful to the surgeon. The degree of function, i.e., "cold" or "warm" in the thyroid nodule is used to separate patients into two branches, but the patients are treated in a similar manner. The presence of a "cold" nodule might influence the decision whether to continue suppression in a patient with an incomplete response to thyroid hormone suppressive therapy.

A study that applied decision analysis to three different therapies for the solitary "cold" thyroid nodule, including (1) immediate subtotal thyroidectomy, (2) thyroid suppression for 6 months followed by surgery in the nonsuppressible lesion, and (3) fine needle biopsy with surgery or suppression, concluded that all were essentially equal in terms of morbidity and mortality.[62] Whether following the approach outline in Figure 8–7 will have a significant effect on morbidity and mortality is not clear but provides a rationale for therapy.

In elderly patients, should the management of thyroid nodules be altered? In response, two distinct groups could be identified in a study of 100 patients who were above the age of 60 years.[15] Sixty-six patients were in the high risk group, e.g., with solitary "cold" nodule and hoarseness; eleven of these patients had thyroid cancer, of which six were poorly differentiated. There was no operative mortality. Surgery is indicated for elderly patients with thyroid nodules who are at risk.

Suppression with Thyroid Hormone. If the decision is made to delay surgery, a course of thyroid hormone suppressive therapy is indicated in an attempt to decrease nodule size. The solitary thyroid nodule should be suppressed more vigorously than the multinodular goiter because failure of suppression may lead to surgery. Complete suppression of thyroid-stimulating hormone (TSH) secretion is probably impossible to achieve. Whether low circulating levels of TSH stimulate the thyroid is not clear. As mentioned previously, growth factors other than TSH may play a role in thyroid gland stimulation. An adequate suppressive dose of thyroxine would result in thyroid function test results in the high normal range.

Since a "cold" thyroid nodule implies inactivity, should it also be suppressed with thyroid hormone? "Cold" nodules that contain thyroid tissue are frequently more active biochemically than "warm" functioning nodules.[26] They may have a defect in iodine trapping but may have levels of glucose oxidation and cAMP that are increased.[81] Half the "cold" nodules in one study decreased with thyroid hormone suppression.

Suppressive Dose. The amount of thyroxine to suppress a solitary thyroid nodule can be adjusted to a dose that is just short of causing thyrotoxicosis with symptoms and signs of sleep disturbance, palpitations, and excess sweating. However, subclinical hyperthyroidism may be undesirable over the long term. The sensitive TSH assay or the thyrotropin-releasing hormone (TRH) test may be helpful in this situation.[40] TRH usually causes a temporary increase in the serum TSH concentration, but effective thyroid hormone suppression should block the TSH response to TRH completely.[63] Although complete suppression is desirable, most patients are placed on 150 to 200 μg of L-thyroxine, and serum T_4 and T_3 concentrations are monitored to keep them within the high normal range unless there are extenuating circumstances, such as heart disease.

Duration of Suppressive Thyroid Hormone Therapy Trial. Some patients have not had regression in the goiter until they had thyroid hormone suppression for a year or more.[2] Half the patients with nontoxic goiter who were treated with thyroid hormone suppression had a decrease in nodular size after 3 months. Of the patients who did not respond initially, a third had a decrease in nodule size after another 4 months of therapy.[80] These data suggest a course of therapy for at least 3 months and preferrably 6 months before the thyroid nodule is considered not to respond. Other data, however, suggest that no further suppression of the thyroid nodule occurs after 3 months of therapy.[33]

Complete disappearance of the thyroid nodule is an indication for continued thyroid hormone suppression. Regression of the nodule does not eliminate the possibility of thyroid cancer but does indicate that the nodule is responsive to TSH suppression. Immediate surgery should be advised if the thyroid nodule enlarges during suppressive therapy in the absence of hemorrhage. Lack of regression of the nodule on thyroid hormone suppression may lead to surgery, depending on the risk factors outlined.

Surgery. At the time of surgery, both thyroid lobes should be totally exposed even though preoperative palpation reveals only involvement in one lobe. At the time of surgery, as many as 60% of thyroids are found to have multiple nodules in cases that were diagnosed preoperatively as a single nodule by careful palpation and scan. If multinodular goiter is found at the time of surgery, the risk of carcinoma lessens dramatically but a subtotal thyroidectomy is indicated.

Confirmation of a solitary nodule at the time of surgery without evidence of extrathyroidal involvement is an indication for an extracapsular lobectomy with removal of the isthmus. If review of permanent sections indicates that the diagnosis is minimal papillary thyroid carcinoma despite a benign appearance on frozen section, adequate surgery has been performed and reoperation is not required (see Treatment of Thyroid Carcinoma).

THYROID MALIGNANCY

Thyroid malignancy has a wide biologic variation. Minimal papillary thyroid carcinoma is considered to have a negligible risk in terms of morbidity and mortality. In comparison, anaplastic carcinoma of the thyroid can be one of the most malignant of all human tumors. The mortality of thyroid carcinoma is low in the young, but the prognosis worsens with age. The disease is found in all ages from infants to octogenarians. Most thyroid carcinoma in younger individuals is well-differentiated, whereas anaplastic carcinoma predominates in the elderly. Highly malignant anaplastic carcinoma seldom occurs before the age of 40. After 40 years of age, both mortality and metastatic thyroid carcinoma increase sharply. Similar to all thyroid disease, carcinoma of the thyroid is three times more common in females than in males.[73]

Incidence

Confusion over the true incidence of thyroid carcinoma is partially due to the frequency of minimal papillary thyroid carcinoma, which has been reported to occur in as many as 13% of the population in the United States.[72] Since the morbidity and mortality connected with these small (< 1.5 cm) thyroid tumors is negligible, they are largely responsible for the disparity between the prevalence and the mortality of thyroid cancer in some surgical series. The reported prevalence varies widely because the diagnosis of thyroid malignancy is exquisitely sensitive to the intensity of the pathologic examination of the thyroid gland. The incidence of 13% was found by carefully examining 300 to 900 sections per gland. If all thyroid glands were subjected to this intensity of examination as many as 10 to 30 million North Americans might receive the diagnosis of thyroid malignancy.[72] From the patient's point of view, the diagnosis has little biologic importance, although from the physician's point of view, it is difficult to talk meaningfully about incidence. A firm endpoint, death from thyroid cancer, is uncommon and approximates 0.8 deaths in females per 1000,000 population per year and 0.4 deaths per 100,000 population per year in males.

Classification

Virtually all thyroid tumors arise from follicular or parafollicular cells and are classified in Table 8–2. Thyroid carcinoma may be follicular, with recognizable thyroid follicles, or the follicular cells may form papillary structures either pure or mixed with follicles. The follicular cells may be largely undifferentiated and appear as large spindle cells, or they may grow as squamous cells. The parafollicular cells may give rise to carcinomas with different histologic subtypes, but they are all labelled medullary.

Histologic classification of these thyroid tumors has biologic significance. In a 5- to 30-year follow-up, 11% of patients with papillary carcinoma of the thyroid died as compared with 25% of patients with follicular carcinoma, 50% of patients with sporadic medullary carcinoma, and 90% of patients with undifferentiated carcinoma. The biologic behavior of thyroid malignancies can also be used for classification (Table 8–3). Papillary thyroid cancers tend to metastasize to cervical lymph nodes and occur in all age groups. Follicular

Table 8–2. Histologic Classification of Epithelial Thyroid Tumors According to the World Health Organization*

I. Epithelial tumors
  A. Benign
    1. Follicular adenoma
    2. Others
  B. Malignant
    1. Follicular carcinoma
    2. Papillary carcinoma
    3. Squamous cell carcinoma
    4. Undifferentiated (anaplastic) carcinoma
      a. Spindle cell type
      b. Giant cell type
      c. Small cell type
    5. Medullary carcinoma
II. Nonepithelial tumors
  A. Benign
  B. Malignant
    1. Fibrosarcoma
    2. Others
III. Miscellaneous tumors
  A. Carcinosarcoma
  B. Malignant hemangioendothelioma
  C. Malignant lymphoma
  D. Teratomas
IV. Secondary tumors

*Reproduced from *Histological Typing of Thyroid Tumors*, International Histological Classification of Tumors, no 11. World Health Organization, Geneva, 1974, with permission.

metastases tend to be blood borne and occur in older age groups. Undifferentiated thyroid cancers usually kill by local invasion and are found predominantly in older age groups.

Although at present there is no completely satisfactory system for staging thyroid cancer, certain characteristics relate to therapy and prognosis. The following factors are thought to be important in the prognosis and therapy of papillary and follicular carcinoma of the thyroid: (1) histologic type, (2) age of patient, (3) extent of primary tumor, (4) distant metastases, (5) size of thyroid, (6) blood vessel invasion, (7) multiple foci, and (8) sex of patient.[57] An attempt has also been made to classify thyroid carcinoma by clinical stages (Table 8–4).

Papillary Carcinoma of the Thyroid. Papillary thyroid carcinoma represents the great majority of childhood thyroid cancer and is three times more common in females. Although more common, the disease also has a better prognosis in women and children as compared with men. The overall prognosis for papillary thyroid carcinoma is generally good in any case, and 80% or more of patients are alive at 10 years.

The histologic pattern of the tumor is definitely papillary (Fig. 8–8). There is a peculiar appearance of the tumor cell nuclei, which has been designated as "ground glass," "optically clear," and "Orphan-Annie eye."[69a] Although the tumor may frequently contain colloid-filled vesicles, the follicular component does not alter biologic behavior of the tumor, which is still classified as papillary.

Psammoma bodies, which are laminated calcific spherules, are present in about 40% of cases of papillary carcinoma. The psammoma bodies can sometimes be identified as finely stippled calcification on a radiograph of the neck.[50] Xerography may be particularly helpful. The tumor is slow growing and usually of low grade malignancy so that long follow-up periods are necessary. Spread occurs to the lymph nodes characteristically, and a patient, particularly a child, may actually present with enlarged cervical nodes rather than a solitary thyroid nodule. Interestingly, for reasons that are not clear, the presence of cervical lymph node metastases seems to have relatively little deleterious effect on mortality.[52, 53] Intraglandular metastases, both in the same lobe as the primary and in the contralateral lobe are not uncommon. Metastases to bones and lungs are much less common. Despite widespread metastases, papillary thyroid carcinoma may remain indolent for long periods and then suddenly assume more aggressive properties.

Follicular Carcinoma. Follicular carcinoma,

Table 8–3. Histologic Classification and Biologic Behavior*

Tumor	Age	Growth Rate	Lymph Node Metastases	Distant Metastases
Papillary	All	Slow	Common	Uncommon
Follicular	Middle-aged to old	Slow	Uncommon	Common
Medullary	All	Moderate	Common	Common
Undifferentiated	Older	Rapid	Extensive	Common and local growth

*Reproduced from Meissner, W. A.: In DeGroot, L. J. et al. (eds), *Radiation Associated Carcinoma*. Grune & Stratton, New York, 1977, with permission.

Table 8–4. Clinical Stages of Thyroid Carcinoma*

Stage 1	Intrathyroidal lesions only
Stage 2	Nonfixed cervical metastases
Stage 3	Fixed lymph node metastases or invasion into the neck outside the thyroid
Stage 4	Thyroid tumors with metastatic disease outside the neck

*Reproduced from Smedal, M.L., Salzman, F.A., Meissner W.A.: *Am. J. Roentgenol.* 99:352, 1967, with permission.

similar to papillary carcinoma, is three times more common in women but occurs less frequently in childen and is more a disease of middle and later years. Histologically, the tumor is characterized by the complete absence of papillary elements and the formation of follicular structures with varying colloid content. Follicular carcinoma can be very difficult to distinguish from follicular adenomas because of the normal appearing morphology, and usually invasion of the capsule, adjacent thyroid (Fig. 8–9), or blood vessels must be demonstrated. The tumor may appear initially as a distant blood-borne metastasis with no

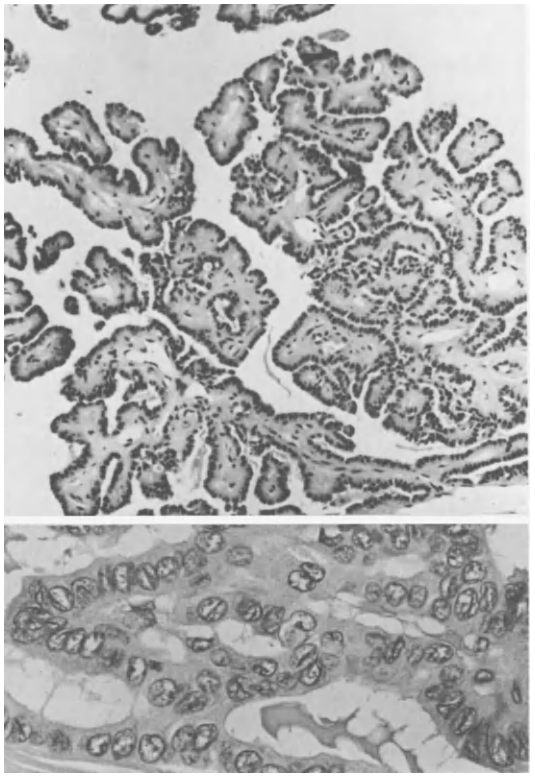

Figure 8–8. Papillary carcinoma of the thyroid. The papillary bandlike structure is lined by low columnar cells. In the lower panel optically clear "Orphan Annie nuclei" are seen.

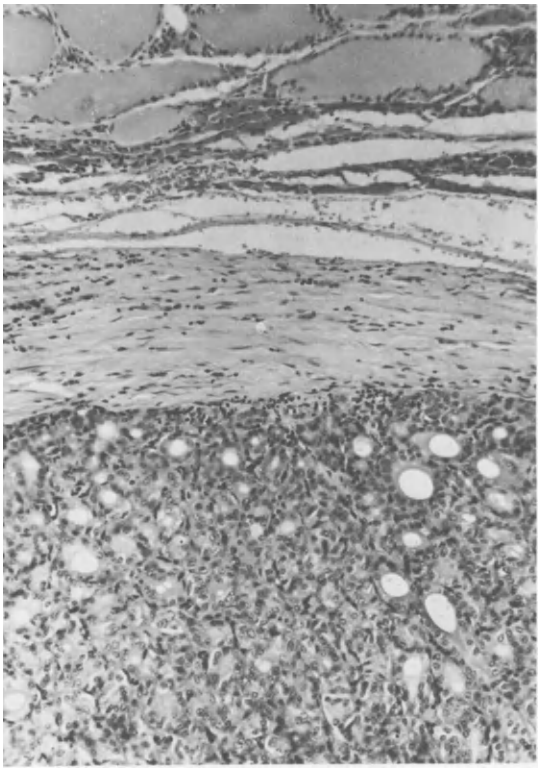

Figure 8–9. Follicular carcinoma of the thyroid. The follicles are of uniform size and are distinctly different from the normal follicles in the upper part of the micrograph. The carcinoma is surrounded by a well-defined capsule without signs of invasion in this instance.

thyroid lesion evident clinically. Hürthle's cell tumors are probably variants of follicular carcinoma. They have been considered a more malignant variant, but this has been questioned.[1a] Well-encapulated, benign-appearing Hürthle's cell tumors may be treated with lobectomy and careful follow-up. The chance that they will later exhibit malignant behavior is small.

Prognosis depends on the extent of tumor spread at the time of surgery. Follicular carcinomas that show minimal invasion and appear benign histologically have a very high cure rate. The prognosis becomes progressively worse with more extensive spread. These lesions may not metastasize for as long as 10 to 20 years. In a study of 18 deaths from follicular carcinomas, 56% had metastases; 89% had lymph node metastases; and 17% had skeletal, liver, or brain metastases.[82]

Anaplastic Carcinomas of the Thyroid. Anaplastic carcinomas of the thyroid do not occur

as frequently in women as do other thyroid tumors and occur in the older age groups (mean age 57 years). These tumors tend to be strikingly pleomorphic and devoid of any special cellular arrangement. Bizarre, multinucleated giant cells are encountered frequently (Fig. 8–10). Fibrosarcoma may be suggested when a spindle-cell growth pattern predominates. Diffuse small-cell carcinoma of the thyroid forms a special group, although the great majority of these tumors may actually be lymphosarcomas.[35]

Anaplastic thyroid carcinomas may grow very rapidly, infiltrate neck structures, and spread to regional lymph nodes, lungs, bones, and liver. Before distant metastases appear, deaths can occur as a result of rapid local invasion and asphyxiation. The prognosis is grim with a 10-year survival rate of about only 1%.

Treatment of Thyroid Carcinoma

The extent of surgery necessary in the treatment of thyroid carcinoma remains controversial in view of the wide behavioral variation of differentiated tumors of the thyroid. The risk of surgical treatment must be weighed carefully against the prognosis for each individual. Where the prognosis is reasonable, radical procedures should be used with restraint because of the disability risk, e.g., recurrent laryngeal nerve and hypoparathyroidism.

There is a unique treatment for thyroid cancer since radioactive iodine is directly targeted to the thyroid gland. The isotope may be of real benefit in treating surgically inaccessible metastases without damaging surrounding tissue. Whether radioactive iodine should be used to ablate the remaining thyroid gland after partial thyroidectomy for well-differentiated papillary carcinoma is a continuing subject for debate. Long-term follow-up of patients treated with radioactive iodine for thyroid cancer revealed a relatively low risk, although there did appear to be an increased risk of cancer of the bladder.[23a] Certain well-differentiated thyroid carcinomas are TSH-dependent, and no clear evidence exists that induction of hypothyroidism with concomitant elevated serum TSH levels has resulted in growth of thyroid metastases. Continued TSH suppression by thyroid hormone and avoidance of hypothyroidism if possible are part of the general therapy for thyroid carcinoma.

Treatment of Papillary Carcinoma. The surgical procedure of choice for minimal papillary carcinoma with lesions less than 1 cm would be a thyroid lobectomy on the side of the nodule and removal of the isthmus.[53] For larger lesions, the evidence favors a near total thyroidectomy with only enough gland left to preserve the recurrent laryngeal nerve and the parathyroid glands. Removal of only the apparently affected lobes would have resulted in residual cancer persisting in two thirds of patients in one study.[14] A significant inverse correlation occurred between the extent of surgery and the recurrence of tumor in a study of 576 patients with cancer of the thyroid (Table 8–5).[52] Papillary thyroid carcinoma re-

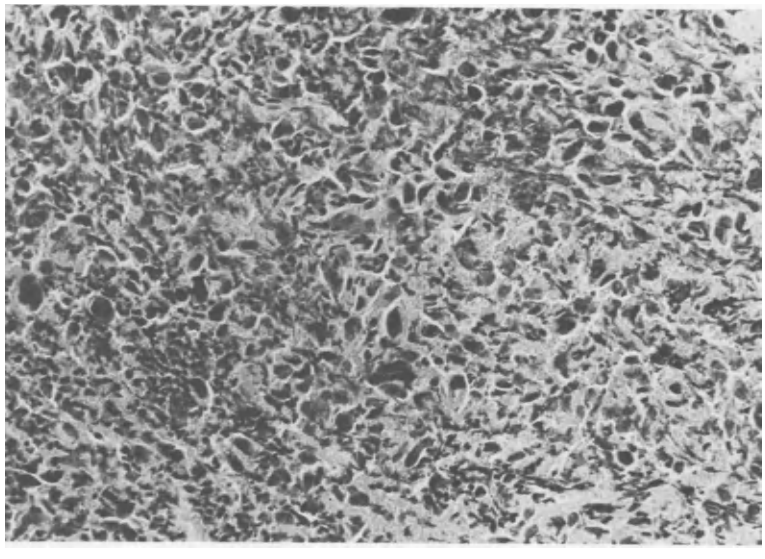

Figure 8–10. Anaplastic carcinoma of the thyroid. The cells are bizarre and pleomorphic. The mitotic rate is high.

curred twice as often after subtotal thyroidectomy as compared with near total thyroidectomy, and the proportion of thyroid cancer deaths was also greater in this group. Interestingly, cervical lymph node metastases at the time of original surgery were correlated with higher recurrence but not high mortality in the gland.

If the posterior capsule of the thyroid on the contralateral side from the original nodule is left intact, the risk of hypoparathyroidism and recurrent laryngeal nerve involvement can be reduced to as low as 5%.[92] Hypoparathyroidism occurred in 13.5% of patients after total thyroidectomy and was clearly related to the extent of the surgery in one series.[52, 53] In the same series, recurrent laryngeal nerve paralysis occurred in 1.2% of patients and was also related to the extent of surgery. Importantly, there was no evidence that radical neck dissection in addition to the near total thyroidectomy lessened recurrence or improved survival. Furthermore, major postoperative complications were reported in 44% of patients treated in this manner in contrast to 13% of patients in whom near total thyroidectomy was combined with selective regional lymph node excision.

Medical therapy, both thyroid hormone suppression and radioactive iodine, significantly influenced survival and recurrence in the treatment of papillary carcinoma of the thyroid (Fig. 8–11).[52] Both treatment modalities resulted in significantly less recurrence than thyroid hormone therapy alone. The indication for therapy with radioactive iodine

Table 8–5. Influence of Initial Treatment on Recurrence and Survival in Thyroid Papillary Carcinoma*

Surgical Therapy	Recurrence (%)	Deaths (%)
Thyroidectomy		
Subtotal	10.9†	0.6
Total	19.2	1.5
Lymphadenectomy		
None	12.3	0.8
Simple	17.9	1.7
Excision	14.7	0.7
Medical therapy after surgery		
None	40.0	10.0
Thyroid	13.1‡	0.2
^{131}I and thyroid hormone	6.4	0.9

*Mazzaferri, E.L. and Young, R.L.: Am. J. Med. 70:511, 1981, with permission.
†$p < 0.01$
‡$p < 0.001$

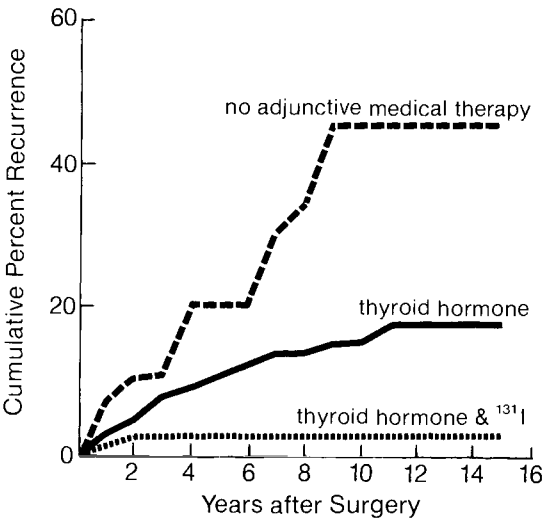

Figure 8–11. Recurrence of papillary thyroid carcinoma after surgery with and without adjunctive medical therapy. (Redrawn after Mazzaferri, E. L. and Young, R. L.: Papillary thyroid carcinoma: A 10-year follow-up report of the impact of therapy in 576 patients. Am. J. Med. 70:511–518, 1981, with permission.)

was residual uptake in the neck, particularly in the presence of a large primary lesion of > 2.5 cm, lymph node metastases, or local invasion.[52] The mean dose of radioactive iodine was 140 mCi and was less than 200 mCi in 87% of patients.

Although at least some radioactive iodine is taken up by as many as 80% of differentiated thyroid carcinomas, the benefits from such therapy are not clear, particularly in patients with small metastases confined to the neck. For patients with papillary tumors of < 1.5 cm, less aggressive therapy such as subtotal thyroidectomy and thyroid hormone suppression may be adequate.[52]

Thyroid hormone administration to 19 patients with inoperable, metastatic papillary carcinoma of the thyroid resulted in regression of the lesions in 12 patients for periods of time averaging 11 years.[16] The aim of thyroid hormone therapy in patients with thyroid carcinoma is suppression of serum TSH secretion rather than replacement. The TRH test may prove helpful as an indicator of TSH suppression by thyroid hormone.[47] The patient should be started on 0.15 mg of L-thyroxine daily. If there is a TSH response to the TRH bolus after 3 weeks of therapy, the thyroid dose could be raised to 0.20 mg and the test repeated until no response to TRH occurs. External radiation in moderate dosages can erad-

icate microscopic papillary and follicular carcinoma. Larger amounts of tumor may also respond favorably but the regression rate is slow.[86]

Treatment of Follicular Carcinoma of the Thyroid. Follicular carcinoma of the thyroid is a more aggressive tumor than papillary carcinoma and should be treated more aggressively. Blood-borne metastases outside the neck are more common, requiring radionuclide therapy. Aggressive therapy is justified since patients freed of well-differentiated metastases outside the neck had a survival rate three times that of patients not freed of such metastases.[16] For these reasons, near total thyroidectomy is indicated even if the diagnosis is not made until the permanent sections are reviewed, necessitating reoperation within 4 to 5 days of the initial surgery. The normal thyroid gland is so avid for iodine that the gland must be ablated before significant uptake into metastases will occur.

Reoperation would not seem indicated if subtotal thyroidectomy was done for noninvasive follicular carcinoma in the past and the patient in this situation could be followed expectantly. Radioactive iodine can be used to ablate the normal remnant but a large dose may be required, which would contribute to the radiation burden. However, further studies have suggested that 30 mCi may be sufficient in such cases.[21]

If the decision is made to treat the thyroid carcinoma with radioactive iodine following surgery, the patient should be allowed to become hypothyroid which would take about 3 to 4 weeks after a near total thyroidectomy.[5] However, the immediate postoperative period is often difficult, and induction of hypothyroidism accentuates the problem. We have preferred to institute postoperative L-thyroxine suppressive therapy immediately, and after 3 months change to triiodothyronine 50 μgm a day for 3 weeks. Two weeks after discontinuing the triiodothyronine, the patient is admitted for radioiodine therapy, usually at 100 mCi.

This regimen results in a serum TSH concentration in the range of 50 μU/ml and appears to produce optimal radioactive iodine thyroid uptake.[30, 39] If the procedure is being done after the initial surgery, a thyroid remnant is virtually always present and a preliminary scan is not necessary. However, in follow-up studies, 2 mCi of ^{131}I can be given and a whole body scan performed. The use of ^{123}I, if available, would lower the radiation dose for diagnostic purposes. In either case, if uptake occurs over the thyroid or elsewhere, 100 mCi of ^{131}I should be given. Measures to maximize the thyroid uptake and to minimize the total body radiation burden include a low iodine diet for 4 weeks previous to therapy and the administration of diuretics to decrease the iodide pool.[32] Lithium can also be given to block the release of iodide from metastases but is rarely indicated.[28, 48]

The patient should be placed on full suppressive doses of L-thyroxine following therapy. Prolonged hypothyroidism with elevated serum TSH concentrations is undesirable because of the possibility of stimulating thyroid tumor growth. Patients can be kept on suppressive therapy and thyroid uptake stimulated with intramuscular bovine TSH, 10 U, daily for 3 days. Unfortunately, antibodies and toxic reactions may result from the administration of the bovine TSH.[58] Whether thyroid stimulation is as great with exogenous TSH stimulation has also been questioned.[37] Certainly, the patient should not be allowed to remain hypothyroid any longer than necessary.

Treatment of Anaplastic Carcinoma. In the majority of patients, anaplastic carcinoma is incurable at presentation. Partial excision of the tumor as well as tracheostomy may be required to reduce the tumor burden and relieve tracheal compression. A needle biopsy may be sufficient to establish the diagnosis. Radical surgical approaches have no place in the treatment of anaplastic carcinoma of the thyroid. Surgical procedures have failed to improve the prognosis, and radiotherapy probably provides the best palliation. External radiotherapy may be given through a variety of ports to the neck and upper mediastinum for a total dose of 4000 to 5000 rads. Shrinkage of the tumor often occurs rapidly but subsequently recurs. Hyperfractionation of the radiation dose, e.g., 400 rads daily at 3-hour intervals, caused complete tumor regression in 6 of 14 patients.[85] Since radioiodine represents a specific chemotherapeutic agent for the thyroid, a radioiodine uptake and scan should be done before other therapy is attempted. Although anaplastic carcinomas of the thyroid rarely pick up sufficient iodine, occasionally radioiodine therapy is possible.[74]

Chemotherapy is an alternative to radiation but has not been helpful in the treatment of these aggressive tumors.[78] Doxorubicin (Adri-

amycin) caused partial remissions associated with subjective improvement in 5 of 19 patients with anaplastic carcinomas of the thyroid.[31] Although spindle and giant cell types appeared to be less responsive, some responsiveness was observed in all the histologic cell types. Cardiomyopathy was the most significant effect of drug toxicity encountered. Bleomycin has also been suggested to be effective in thyroid cancer therapy.[34]

Follow-up

After radioiodine ablation of the thyroid remnant, the patient should be scanned again in 6 months. Further radioiodine scans following withdrawal of thyroid hormone suppression are not indicated in my view, unless there is evidence of recurrence or metastases. Yearly chest films may be helpful in this regard, along with bone scans if indicated, particularly in follicular carcinoma of the thyroid.[23]

Serum Thyroglobulin Determinations. Determination of serum thyroglobulin concentrations is helpful in the follow-up of thyroid carcinoma.[11, 27, 96] The assay is not specific in the diagnosis of malignancy since circulating thyroglobulin concentrations may be increased in a number of benign thyroid conditions including Graves' disease, subacute thyroiditis, and goiter. The major use of the thyroglobulin assay is probably in the follow-up of a patient whose thyroid carcinoma has been removed and whose postoperative serum thyroglobulin concentrations are very low or undetectable. Persistence of elevated serum thyroglobulin levels or an increase in previously low concentrations would indicate that residual tumor or thyroid is present.[6, 12] Determinations of serum thyroglobulin values should be used only if the assay is sufficiently sensitive (< 10 to 15 ng/ml) and no antithyroglobulin antibody has been demonstrated. The serum thyroglobulin determination may be enhanced by thyroid hormone withdrawal and concomitant TSH stimulation.[75] Thyroglobulin concentrations below 10 ng/ml are rarely associated with significant disease, and thyroid hormone withdrawal is not indicated in the routine follow-up of patients with thyroid cancer.

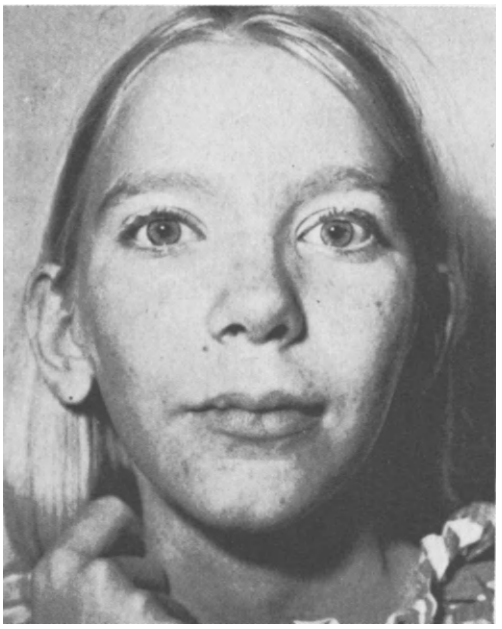

Figure 8–12. Typical facies of a patient with the MEN type III syndrome. (Reproduced from Khairi, M. R. A., et al.: Mucosal neuroma, pheochromocytoma, and medullary thyroid carcinoma: Multiple endocrine neoplasia type III. Medicine 54:89–112, 1975, with permission.)

Other tests have been used to screen patients with previous irradiation to the head and neck for the possible presence of thyroid carcinoma. Positive results for a battery of tests, including serum thyroglobulin, thyroid antibodies, and carcinoembryonic antigen, were more common in the irradiated population than in controls.[20] The tests did not clearly differentiate patients with benign or malignant lesions with the exception of markedly elevated levels of thyroglobulin in which the clinical diagnosis was usually obvious. Papillary and follicular carcinoma of the thyroid has been successfully located by radiolabelling IgG antibody to human thyroglobulin.[25] Results suggest that scanning with ^{131}I-antithyroglobulin may be more sensitive than conventional scanning with radioactive iodine.

MEDULLARY CARCINOMA OF THE THYROID

Medullary carcinoma differs from other thyroid carcinomas in that the tumor originates from parafollicular or C-cells which are of neural crest origin. As a consequence, these carcinomas, which represent only 5 to 10% of all thyroid malignancies, have endocrine and biochemical properties that provide a distinctive means for early detection, follow-up, and possible prevention.

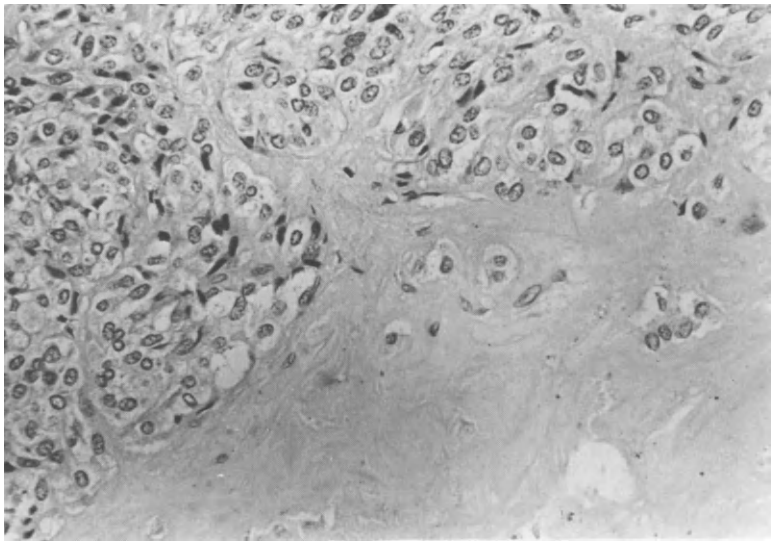

Figure 8–13. Medullary carcinoma of the thyroid. Pleomorphic cells are in clumps separated by amyloid.

Incidence and Classification

Medullary carcinoma has no particular predilection for age or sex and occurs from 5 to 80 years of age. The majority of these malignancies are sporadic and usually unicentric in origin, although in some cases, a third of the patients with apparently sporadic disease actually have multifocal tumors.[71] In about 20% of patients, the disease is familial and transmitted as an autosomal dominant one, with high penetrance and variable expression. Familial medullary carcinoma of the thyroid occurs as part of multiple endocrine neoplasia, type IIA (MEN-IIA) with pheochromocytoma and hyperparathyroidism or as part of MEN-IIB with pheochromocytoma, mucosal neuroma, intestinal ganglioneuroma, marfanoid habitus, and skeletal deformities (Fig. 8–12).[44, 89]

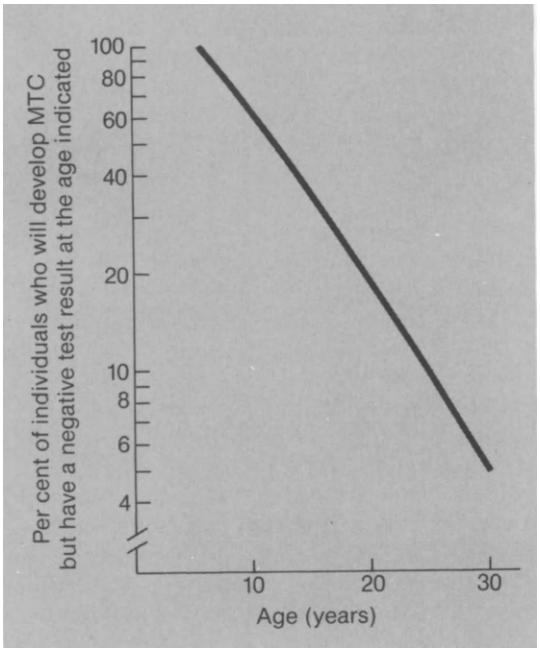

Figure 8–14. Semilogarithmic plot of the per cent of converters from negative to positive results in the calcitonin stimulation test as a function of age. (Modified from Gagel, R. F., et al.: J. Pediatrics, 101:944, 1982.)

Medullary carcinoma with a familial pattern has been described that appears to be the least aggressive form.[25a] Individuals studied had no extrathyroidal manifestations, and tumors either developed at a later age or grew more slowly.

The different characteristics of the hereditary and sporadic varieties of medullary carcinoma may be explained by the two-mutational event theory proposed by Knudson.[46] The first event was postulated to be a germinal mutation, resulting in many susceptible cells. The earliest pathologic change in medullary carcinoma is a progressive multifocal increase in the number of C-cells. A subsequent somatic mutation would lead to the transformation of hyperplastic to malignant cells. Comparison of the ages of onset of hereditary and sporadic medullary carcinoma have been consistent with this theory.[43] Further evidence was provided by studies with glucose-6-phosphate dehydrogenase that the initial inherited mutation, which was thought to occur in the fetal neural

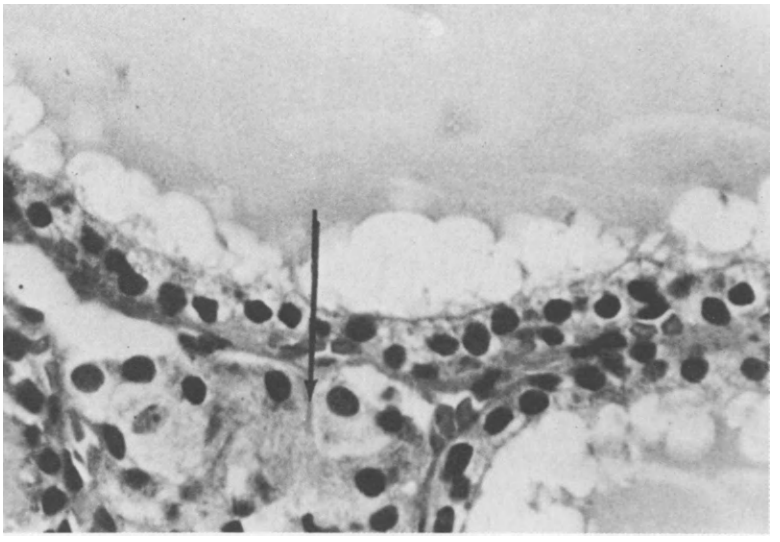

Figure 8–15. C cell hyperplasia. Clusters of polygonal to spindle-shaped C cells in a parafollicular location. (Reproduced from Tashjian, A. H., Wolfe, H. J., and Voelkel, E. F.: Human calcitonin. Am. J. Med. 56:847, 1974, with permission.)

crest, produced multiple clones of cells susceptible to neoplastic change.[3] The subsequent development of medullary carcinoma of the thyroid was the result of one or more mutational changes involving one clone of susceptible cells.

Medullary carcinoma is intermediate in malignancy between follicular and anaplastic carcinoma with a 5-year survival of 70 to 80%.[38] The tumor tends to run a slow, progressive course with both local invasion of structures in the neck as well as metastases to both cervical nodes and more distant sites. The tumor may be sharply demarcated from surrounding thyroid or, alternatively, may infiltrate adjacent thyroid tissue. Small tumors are almost invariably located in the upper posterior portion of thyroid lobes.

Medullary carcinoma of the thyroid is distinguished by clusters of polyhedral, neoplastic cells (Fig. 8–13). Together with the absence of neoplastic follicular elements, this compartmentalized group of cells separated by amyloid-containing stroma is a distinctive pathologic feature. The presence of amyloid, which is formed from calcitonin, is another distinctive feature.[88]

Diagnosis of Medullary Carcinoma

Virtually all patients with medullary carcinoma have elevated blood calcitonin levels, although 30% or more of patients with medullary carcinoma of the thyroid may have normal basal calcitonin levels. Therefore, provocative tests must be used to stimulate calcitonin secretion, e.g., calcium, glucagon, and pentagastrin. The combination of pentagastrin and calcium appears superior to either agent alone.[100]

The calcitonin stimulation test has been used for the early diagnosis of medullary carcinoma of the thyroid. The long-range outcome for patients identified early in the course of the disease must await follow-up studies. Although calcitonin is a sensitive and specific tumor marker, the hormone should not be measured in every patient with a thyroid nodule. The test should be done only if the clinical picture is suggestive, and the main diagnostic value is screening kindred with the familial form of the disease.[99, 102] Screening should begin about the age of 5 years and continue yearly until the age of 30 years.

After age 30, less frequent testing would be adequate based on sequential testing of 445 members of 11 kindreds to determine the age-related probability for developing familial medullary carcinoma (Fig. 8–14). Individuals have been identified with this screening with early changes in the C-cell mass, which could represent preinvasive hyperplasia of the cells. In these patients, microscopic clusters of C-cells occur focally in the middle to upper third of the lateral lobe of grossly normal thyroids (Fig. 8–15). The data suggest that the calcitonin stimulation test should lead to recognition and treatment of the tumor at a potentially curable stage. Basal calcitonin levels can be elevated in other malignancies, such as in the breast, lung, and pancreas, as well as in some benign conditions, such as renal failure.[4] Other markers, e.g., carcinoembryonic antigen, have

also been studied in medullary carcinoma of the thyroid but are both less sensitive and less specific than the calcitonin stimulation test.[70]

Therapy of Medullary Carcinoma

In the sporadic form of medullary carcinoma, metastases have already occurred in more than half the patients at the time of diagnosis, making complete excision of the cancer difficult. In familial medullary carcinoma recognized early, the lesion will be less difficult to excise.

Multicentricity is common, and near total thyroidectomy is the procedure of choice. The lymph nodes in the central compartment of the neck, extending as far substernally as possible, should be dissected.[38] The extent of the thyroid surgery should not be at the expense of the recurrent laryngeal nerve and parathyroid glands. Radical neck dissection should not be done under usual circumstances. Since pheochromocytoma is a distinct possibility in a patient with familial medullary carcinoma of the thyroid, appropriate tests, e.g., urinary catecholamine determination, should be done before surgery.

Radioactive iodine is not picked up well by medullary carcinomas and, therefore, is not recommended. Thyroid hormone is not nearly as effective as in differentiated thyroid tumors.[98] No effective therapy for medullary carcinoma currently exists, and a vigorous screening program offers the best chance for long-term survival, at least in the familial form.

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9 Infective Thyroiditis

ROBERT VOLPÉ

THYROIDITIS (PREAMBLE)

The term thyroiditis implies that the disorders to be categorized herein are inflammatory processes involving the thyroid gland. However, for some of the entities listed in Table 9–1, it is not at all clear that inflammatory process is an appropriate designation, and some are included only for the sake of convenience. The etiology and pathogenesis of the diseases listed in Table 9–1 vary considerably and have little or no relationship with one another.

Traumatic thyroiditis, thyroiditis associated with neoplasms, focal thyroiditis associated with nontoxic nodular goiter, amyloidosis of the thyroid, and radiation thyroiditis are not discussed. (For a review of these entities refer to the monograph by LiVolsi and LoGerfo.[5])

INFECTIVE THYROIDITIS (SUPPURATIVE THYROIDITIS)

These conditions are caused by infective microorganisms, excepting viruses. (Viral thyroiditis is discussed in Chapter 10.)

Infections of the thyroid gland by bacteria are rare; parasitic or fungal infections of the thyroid are so extremely uncommon as to be medical oddities. These infections may be sup-

Table 9–1. Classification of Thyroiditis

Infectious thyroiditis
Bacterial, fungal, parasitic, and viral
Subacute thyroiditis
Subacute pseudogranulomatous (DeQuervain's) thyroiditis
Subacute lymphocytic (painless) thyroiditis
Autoimmune thyroiditis
Hashimoto's thyroiditis
Lymphocytic thyroiditis of childhood and adolescence
Postpartum thyroiditis
Chronic thyroiditis (fibrous variant)
Idiopathic myxedema (atrophic thyroiditis)
Atrophic asymptomatic thyroiditis
Thyroiditis associated with other thyroid disorders
Focal thyroiditis in nontoxic goiter
Focal thyroiditis surrounding neoplasms
Graves' disease
Radiation thyroiditis
Traumatic thyroiditis
Including palpation thyroiditis
Riedel's stroma
Miscellaneous
Sarcoid
Amyloid
Drug-associated thyroiditis

purative or nonsuppurative.[2, 6] Generally, the illness is acute, but it may be chronic or indolent, particularly in the case of fungal thyroiditis. Rare causes of infective thyroiditis, such as parasites or fungi, are dealt with elsewhere.[2, 4] In this account, emphasis is on the bacterial infections of the thyroid.

Bauchet[1] first described acute inflammation of the thyroid in five patients in 1857. My experience now comprises 26 patients with suppurative thyroiditis seen over the past 25 years. In the review of this experience, the disease was more common in women than in men, and there was no particular age incidence. Infection usually remained confined to one lobe but did become diffuse on occasion. The review of Berger and associates[2] in 1983 comprised 224 reported cases culled from the literature between 1900 and 1980.

Etiology and Pathogenesis

The organisms most commonly cultured from these lesions include *Streptococcus hemolyticus, Staphylococcus aureus, Pneumococcus*, and coliform bacilli.[2–4] A variety of other organisms has also been reported, including *Salmonella, Mycobacterium* (tuberculosis), *Treponema* (syphilis), various fungi, *Actinomyces*, and various parasites.[2, 3] Acute suppurative thyroiditis may occur as a complication of nearby bacterial infections by extension or may occasionally result from bacteremia secondary to a distant focus. The special routes by which infection may reach the thyroid gland include the blood stream, direct invasion from adjacent structures, direct trauma, lymphatics, and persistent thyroglossal duct. Often, some abnormality of the thyroid gland is found to be the focus upon which the infection develops. While the gland has shown itself to be resistant to infection by the hematogenous route, it is exceedingly difficult to implicate any other mechanism in many cases. In some patients, no source of infection can be demonstrated that might have been the cause of the thyroid infection.[2–4, 6]

Pathology

Pathologic examination reveals the characteristic changes of acute inflammation with a heavy polymorphonuclear and lymphocytic cellular infiltrate in the initial phase often associated with necrosis and abscess formation. Once this happens, the pus usually dissects anteriorly through the ribbon muscles towards the skin. Occasionally, the abscess may move into the mediastinum or even rupture into the trachea or esophagus. Fibrosis is prominent as healing occurs.[3, 4]

In other instances, no abscess develops and spontaneous subsidence has been reported. Thrombophlebitis of the internal jugular vein may also occur.

As mentioned previously, the process may involve the normal gland or may arise in a cyst, especially in a long-standing multinodular goiter.[2–4, 6]

Symptoms and Signs

The dominant symptom is pain in the region of the thyroid gland, which may become enlarged, warm, and tender (Table 9–2). The patient is usually unable to extend the neck backwards and sits with the neck flexed in order to avoid pressure on the thyroid gland. There is considerable dysphagia in association with the lesion because of the painful swelling. The pain is often referred to the ear, the mandible, or the occiput on the side of the lesion. Moving the head also elicits severe pain. There are frequently signs of infection in structures adjacent to the thyroid, and lymphadenopathy in adjacent lymph nodes is common.

These local symptoms may have been preceded by the sudden onset of malaise, fever, tachycardia, and chills. On examination, temperatures often vary from 38°C to 40.5°C. The pulse rate is constantly elevated. There is

Table 9–2. Signs and Symptoms of Acute Bacterial Thyroiditis*

Sign or Symptom	No. of Patients (%)†	
Pain	66/67	(100)
Tenderness	76/81	(94)
Fever	106/115	(92)
Dysphagia	62/68	(91)
Erythema	56/68	(82)
Dysphonia	27/33	(82)
Local warmth	14/20	(70)
Concurrent pharyngitis	35/51	(69)
Cough		

*Reproduced from Berger, S. A., et al. (1983). Infectious diseases of the thyroid gland. Rev. Infect. Dis. 5:108–122, with permission.

†From a literature review spanning 1900 to 1980.

swelling in the region of the thyroid, usually confined to one lobe and associated with extreme tenderness. On occasion, the whole gland may be involved. In later stages there may be redness over the involved area, and lymphadenopathy can be demonstrated. Fluctuation generally cannot be elicited because of the induration of the overlying tissues.[2-4, 6]

Laboratory Findings

There is usually a polymorphonuclear leukocytosis of moderate or marked degree. The patient usually remains euthyroid, with normal values of thyroxine and total serum triiodothyronine throughout the illness, although occasionally there may be a mild increase in serum thyroid hormone concentrations consequent to the release of stored hormone from the inflamed region into the systemic circulation. While the radioactive iodine uptake is generally within normal limits, the involved area may not pick up the radionuclide well and thus appears as a "cold" area on scanning. An ultrasonographic examination may show what appears to be a cystic lesion or may appear "complex." Thyroid autoantibodies do not appear during the course of the illness and were not present in any of our patients.[2-4, 6]

Diagnosis

The diagnosis requires a high index of suspicion. It is not difficult to establish when all of the aforementioned manifestations are present. Certainly, if the signs of inflammation of the thyroid are associated with local redness, lymph node enlargement, a flexed neck, marked hyperpyrexia, and leukocytosis, acute suppurative thyroiditis must be excluded before any other diagnosis can be considered. The condition must be differentiated from subacute thyroiditis, which does not involve the neck structures and is usually associated with less pain. Moreover, thyroid function is characteristically altered in subacute thyroiditis. Nevertheless, these conditions may be difficult to differentiate from one another prior to the development of acute suppuration.

At times, malignant neoplasms may develop focal necrosis and can mimic quite closely a primary pyogenic infection. When the manifestations of infectious thyroiditis are more insidious, conversely it in turn can mimic thyroid carcinoma. Infectious thyroiditis also can be mistaken for hemorrhage into a cyst of thyroid or chronic thyroiditis. Usually, the passage of time serves to make the correct diagnosis obvious, particularly with the progression of pain, the appearance of or advance in swelling and redness in the thyroid region, and the onset of persistence of fever and leukocytosis. A lateral roentgenogram may also be useful in localizing the area of inflammation to the thyroid, and an ultrasonogram is also useful from this viewpoint.[3, 4, 6]

Course and Management

The course is generally a progressive one, with complications of rupture of the abscess, septicemia, and thrombophlebitis as mentioned apt to develop unless adequate treatment is instituted. Occasionally, the infection may subside spontaneously.

Conservative measures include rest, local heat, and antibiotics. These may be successful but much depends on the identification of the infecting microorganism. Gram-positive cocci are common offenders, and appropriate antibiotics have often proved to be effective.

If an abscess develops and prompt response to antibiotics does not occur, incision and drainage are necessary. Sometimes partial lobectomy must be performed for recurrent disease.

Unlike formerly, the prognosis is usually excellent when a patient is treated appropriately with antibiotic therapy as well as surgery when required. The lesions generally heal with reasonable speed, and recurrences are uncommon. Function of the thyroid remains intact if treatment is adequate and begun promptly.[2-4, 6]

CHRONIC INFECTION

Chronic infection of the thyroid may result from a variety of organisms, although this event is exceedingly rare. Chronic pyogenic thyroiditis due to *Salmonella typhosa* has been reported, but generally chronic infections of the thyroid do not suppurate. Cases of syphilis, tuberculosis, and echinococcosis fall into this category. *Actinomyces* has also been reported several times as the organism responsible for an indolent and a frequently suppurative form

of thyroiditis, and these cases have generally responded to drainage and antibiotic therapy.

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10 Subacute Thyroiditis

ROBERT VOLPÉ

The term "subacute thyroiditis" has traditionally been utilized to describe those cases of primarily painful inflammation of the thyroid gland that are of probable viral etiology. In the following account, however, this term will be applied to *two* rather distinct forms of thyroid gland inflammation, both of which run courses lasting several weeks to several months. It is obvious that the term subacute thyroiditis connotes a temporal quality only and could conceivably apply to any inflammatory process of intermediate severity and duration. However, current usage excludes several forms of infectious thyroiditis (e.g., bacterial and fungal) from this category. Alternatively, it specifically includes a painful form of thyroiditis of probable viral etiology, referred to as DeQuervain's or pseudogranulomatous thyroiditis as well as a painless form of thyroiditis, which may be called "subacute lymphocytic thyroiditis." The last term does not necessarily imply that there is always a relationship to chronic lymphocytic thyroiditis, as discussed subsequently. These disorders may sometimes be seen in males but are overwhelmingly seen in females.

SUBACUTE PSEUDOGRANULOMATOUS THYROIDITIS

This form of thyroiditis appears to have been first described by Mygind, who described 18 cases of "thyroiditis akuta simplex" in 1895.[48] However, DeQuervain's name has been traditionally associated with this disorder; he actually described the pathology of this condition twice, in 1904 and again in 1936.[10, 11] The disorder has many synonyms, some based on misconceptions and thus rather misleading. These include acute simple thyroiditis, noninfectious thyroiditis, viral thyroiditis, acute or subacute diffuse thyroiditis, granulomatous thyroiditis, struma granulomatosa, giant cell thyroiditis, pseudogiant cell thyroiditis, migratory "creeping" thyroiditis, and pseudotubercular thyroiditis.[76] As pointed out subsequently, the "giant cells" or "granulomata," which may appear to be present if the pathologic lesions are examined superficially, are not true giant cells or granulomata but only simulate such structures.

Incidence

The true frequency of this disorder has been difficult to determine. It certainly seems to be

not uncommon in parts of the temperate zone; it has been observed frequently in Canada, the northern regions of the United States, some parts of Great Britain, Japan, Israel, Argentina, Finland, and Sweden.[76] In other parts of the world, however, it seems to be very rarely recognized. It occurs most commonly between the second and fifth decade and is rare in children, and females predominate in a ratio of about 6:1.

Etiology

The etiology of this disorder has yet to be finally settled. In past generations, the importance of the prior occurrence of tonsillitis, pharyngitis, influenza, and other infectious diseases was stressed. Fraser and Harrison,[19] however, after excluding bacteria as causative factors in 1952, first proposed the theory that viruses might be the cause of subacute thyroiditis. However, the proof of this theory has remained elusive.

A number of clinical aspects of the disease tend to resemble benign viral disease, as follows[76]:

1. The disorder is often preceded by infection of the upper respiratory tract.
2. There is often a prodromal phase characterized by muscular aches and pains, malaise, and fatigue.
3. The illness may occur at the time of an outbreak of a specific viral disease.
4. After some weeks or months, complete recovery is the rule.
5. In many instances, there is no leukocytosis. (In some patients a moderate leukocytosis is indeed encountered, as in some acute viral infections.)

During the course of a mumps epidemic in Israel, 11 patients suffering subacute thyroiditis were discovered, who, despite the lack of clinical evidence of mumps, had circulating antibodies to the mumps virus.[15] In two of these patients, the mumps virus was grown from thyroid tissue obtained at biopsy.[15] Moreover, subacute thyroiditis has been recorded in association with mumps in four further reports.[17, 30, 44, 65] Subacute thyroiditis has additionally been reported in association with measles,[5, 45, 57, 59] influenza,[59] the common cold,[29] adenovirus,[46] infectious mononucleosis,[18, 29, 76] Coxsackie virus,[41] myocarditis,[62] cat scratch fever,[63] and St. Louis encephalitis.[20] A virologic study in 28 patients with subacute thyroiditis in 1975 resulted in the isolation of a cytopathic virus in five patients that might have been of pathogenic significance.[66–68] It is curious, however, that viral inclusion bodies have never been observed in sections of the thyroid glands obtained during the course of this disorder.[49, 56]

Studies of viral antibodies in patients with subacute thyroiditis who have no evidence of specific viral disease have shown various common viral antibodies, including Coxsackie, adenovirus, influenza, and mumps.[81] In one study, Coxsackie viral antibodies were most commonly found, and the changes in their titers mostly approximated to the course of the disease.[81] The presence of significant titers of these viral antibodies may, however, indicate merely an anamnestic response to the inflammatory thyroid lesion, rather than a specific viral infection. Alternatively, they may reflect the possibility that any one of a variety of viruses may prove to be an etiologic agent. Subacute thyroiditis, therefore, may represent a stereotyped thyroidal inflammatory response to any one of a variety of viruses.

The malady has also been related to certain nonviral infections, such as Q fever[61] and malaria.[60] Furthermore, epidemics of subacute thyroiditis have been described.

Autoimmunity does not appear to play a role in the pathogenesis of subacute thyroiditis.[76] Significant levels of thyroid autoantibodies are found in a variable number of patients with subacute thyroiditis in the early phase; even in those patients, the presence of antibodies is generally transitory, and the antibodies have usually disappeared several months after the onset of the condition.[14, 42, 76] The transient phase of hypothyroidism correlated with the presence of microsomal antibodies, although this was not true for the rare development of permanent hypothyroidism.[42]

Of interest has been the finding of an antibody to the thyroid-stimulating hormone (TSH) receptor by a few workers during the early phase of either the classic subacute thyroiditis or the silent form.[26, 71, 72] The method employed in those studies was the radioreceptor assay, in which the antibody (thyrotropin binding inhibitory immunoglobulin, TBII) prevents the binding of TSH to thyroid cell membranes.[47] This assay, however, does not measure the ability of the antibody (thyroid stimulating antibody, TSAb) to stimulate thyroid cells.[77] Because there was a good correlation between the presence of TBII and the

hyperthyroid phase of the disorder in one study, the investigators suggested that the antibody might be pathogenetically related to the hyperthyroidism that occurs in thyroiditis.[26] However, in most studies, no correlation is found between the presence or absence of this antibody and thyroid status.[71, 72] The test result was positive in some patients in the hyperthyroid phase, and negative in others; when positive, it ultimately became negative without regard to the status of thyroid function. In studies carried out in our own laboratory, TBII was found to be positive in one of five patients with subacute thyroiditis; when the same samples were measured in an assay system of thyroid stimulation, i.e., generation of cAMP, the results were negative, i.e., there was no stimulation of the thyroid cells.[72] From these studies, three following points may be made:

1. In some patients with subacute thyroiditis, or painless thyroiditis, TBII may appear in the early phase.
2. In at least one study in which thyroid stimulation was also measured, the same antibody did not cause stimulation.
3. In all instances in which it had been found, the antibody then disappeared within several weeks and did not relate to thyroid status.

It thus seems that this antibody is generally *not* a TSAb; it is merely an antibody to the TSH receptor that is not stimulatory. Furthermore, since its presence or absence does not closely correlate with the hyperthyroid phase of the condition, it is not pathogenic. The antibody probably represents a secondary and appropriate immune response to the liberation of antigen and is, thus, another thyroid autoantibody running a course similar to that just described.

Evidence of cell-mediated immunity has been studied in a few laboratories.[14, 76] The evidence indicates transient sensitization of T lymphocytes against thyroid antigen. Antigen-reactive T lymphocytes have been shown to be present within the thyroid gland in large numbers in this disorder.[75] Although the percentage of T lymphocytes in the peripheral blood has been shown to be low in subacute thyroiditis,[83] the number of Fc receptor–bearing blood mononuclear cells is elevated.[6] These findings may represent nonspecific or specific responses to the inflammatory process or to the thyroidal antigenic release. This T lymphocyte sensitization, similar to the humoral response previously discussed, is transitory and almost certainly also represents another element of the appropriate secondary immune response to the inflammatory release of antigen.

An association between the painful type of subacute thyroiditis and HLA-Bw35 has been found in whites[2, 4, 43, 53, 54] and in the Chinese.[89] This genetic relationship may indicate a particular susceptibility to viral infections of the thyroid in these populations.[73] However, the precise significance of this association is not yet clear. It is of interest that in the Japanese, Graves' disease is associated with an increased incidence of HLA-Bw35.[77] However, in whites with Graves' disease, the increase in the HLA B locus is HLA-B8 not HLA-Bw35, and thus the finding of an increased incidence of HLA-Bw35 of about 72% in whites with subacute thyroiditis does not relate in any way to the increased incidence of the same marker in the Japanese with Graves' disease.[77]

In any event, there is no association with other autoimmune diseases and it is very rare for this condition to progress to either Hashimoto's or Graves' disease, both now considered related autoimmune disorders.[77] There have been a few cases in which such evolution has taken place, but the incidence is extremely small. Werner[85] has reported a case of painful thyroiditis that progressed to Graves' disease and has reviewed the literature in 1979. He argues that such inflammatory lesions of the thyroid with antigenic alteration may initiate Graves' disease. Because this development occurs so rarely and Graves' disease usually occurs *de novo*, this possibility seems very remote; subacute thyroiditis may therefore occasionally act only as a nonspecific stress in precipitating hyperthyroidism.[77]

Thus, autoimmune phenomena in subacute thyroiditis appear to be secondary to the release of antigenic material from the thyroid, and they are almost certainly not related to the pathogenesis of this disease. Furthermore, from these observations, it would appear unlikely that viral inflammation with thyroidal antigenic change is a causative factor in either Hashimoto's thyroiditis or Graves' disease.[77]

Pathology

The thyroid gland is often found to be slightly adherent to adjacent structures, but aside from occasional minimal adhesions it may be freed from these without difficulty.[1, 14, 27, 76] This point is useful in distinguishing subacute thyroiditis

from Riedel's struma. Microscopically, the process often commences with extensive cellular destruction and desquamation (Fig. 10–1). The follicular cells sometimes virtually disappear, leaving a fragile and fine follicular lining. Neutrophils appear to enter the follicles initially, followed by large mononuclear cells and lymphocytes. The follicles then appear much larger than normal, with disruption of the epithelial lining; some of the surviving follicular cells appear hyperplastic. Histiocytes tend to congregate around masses of colloid, both within the follicles and in the interstitial tissue, producing "giant cells." These, of course, are not *true* giant cells (pseudogiant cells), but on superficial inspection do resemble giant cells. An unpublished study from our laboratory has shown that these "cells" invariably consist of masses of colloid surrounded by large numbers of individual histiocytes. Since some of the colloid extravasates into the interstices, these pseudogiant cells are also seen in the interstitial tissue. There is considerable edema with histiocytes and lymphocytes in the interstitial tissues. This process is often irregularly distributed within either lobe of or throughout the thyroid gland. With recovery, the inflammatory reaction begins to recede, and a variable amount of fibrosis may then occur. Areas of follicular regeneration may appear, but there is no caseation, hemorrhage, or calcification. Aside from some residual fibrosis, the degree of recovery is almost always complete. Only in rare instances is there complete destruction of the thyroid parenchyma, leading to permanent hypothyroidism. Viral inclusion bodies have not been demonstrated in the few electron microscopic studies that have been reported.[1] There is, however, marked thickening of the basement membrane and some evidence to suggest increased cellular activity, although there are no apical pseudopods with colloid droplets that are seen after TSH stimulation.[1] The pathology of the "painless" form of thyroiditis will be considered separately (see Subacute Lymphocytic Thyroiditis).

Clinical Manifestations[1, 14, 23, 70, 76]

This disorder may be preceded by a prodromal phase with malaise and feverishness or by an upper respiratory tract infection. There is considerable variation in the mode of onset and the severity of the disease. Pain in the region of the thyroid gland may be lacking altogether or may be moderate or severe. Likewise, there may be little or no tenderness or, conversely, the tenderness might be exquisite. The thyroid gland may be of normal size but usually is moderately enlarged, quite firm, and tender. Rarely is the thyroid more than twice the normal size. One or other of the lobes may be involved or the symptoms may commence in one lobe and "creep" to the opposite lobe ("creeping thyroiditis"). Fever may reach as high as 40°C, and the systemic reaction may be minimal or marked. In former years many of the patients who exhibited few or no local symptoms aside from the presence of a goiter underwent surgical thyroidectomy, and the di-

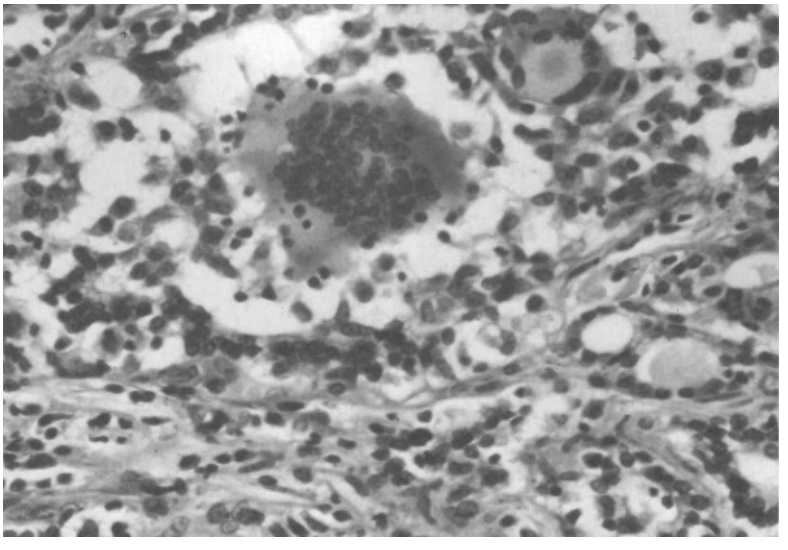

Figure 10–1. Pathology of subacute thyroiditis. Note the severe destruction of the thyroid follicle, with the remaining colloid being surrounded by large numbers of histiocytes giving a picture of a giant cell (pseudogiant cell). There is also marked interstitial edema with a cellular infiltration and considerable destruction of the thyroid parenchyma.

agnosis was made only by examination of the pathologic specimens.[25, 78]

When patients complain of pain in the region of the thyroid gland, they can usually localize it well to the region of the thyroid, over one or both lobes. They may inadvertently describe their symptoms as a "sore throat," but under appropriate questioning one will recognize that the pain is not within the pharynx. The pain typically radiates from the thyroid region up to the angle of the jaw to the ear on the affected side or sides. The pain may also radiate to the anterior part of the chest or may be centered only over the thyroid itself. Coughing, swallowing, or turning the head may aggravate the pain; many patients are aware of tenderness and thyroid swelling in the same region.

Systemic symptoms are common, although occasionally patients have local but no systemic symptoms. Most commonly, patients complain of malaise, fatigue, myalgia, and mild feverishness. In addition, many complain of mild or moderate nervousness, tremulousness, some weight loss, intolerance to heat, and rapid heart beat.

On physical examination, most patients appear flushed and uncomfortable, although usually not seriously ill. Fever may be absent or may reach as high as 40°C. The thyroid gland is characteristically only slightly to moderately enlarged with one lobe being larger than the other. If the thyroid gland is much larger than this, the diagnosis of subacute pseudogranulomatous thyroiditis should be considered unlikely. The consistency of the involved lobe, or sometimes both lobes, is usually quite firm to hard, and tenderness may be very marked or even exquisite in the involved area. Even after the tenderness subsides, the goiter may maintain its size and consistency for several weeks. Occasionally, however, tenderness may be minimal or absent. Cervical lymphadenopathy is rare. Physical signs of mild to moderate hyperthyroidism are often present. A history of pre-existing goiter is found in about 8 to 16% of patients with subacute thyroiditis.[23, 76, 78]

SUBACUTE LYMPHOCYTIC THYROIDITIS (SILENT OR PAINLESS THYROIDITIS)

The condition of subacute lymphocytic thyroiditis (silent or painless thyroiditis) is not fully understood, and it indeed may be clinically homogeneous but etiologically heterogeneous. Even in reports of over two decades ago, it was noted that some patients with subacute thyroiditis suffered no pain or tenderness.[79] Indeed, in some glands removed surgically for painless goiter, histologic examination revealed the "classic" appearance of DeQuervain's thyroiditis.[25, 78] In recent years, however, the number of patients with painless thyroiditis seems to have markedly increased. Moreover, certain features of the disorder are clearly different from the painful form of thyroiditis. Primarily, while the pathologic picture in a few of these patients does not differ from the classic form,[25, 78] in most biopsy specimens obtained during the course of this painless disorder, lymphocytic infiltration predominates.[12, 50, 51] The question of the relationship of this entity to autoimmune thyroiditis is discussed further.

Painless thyroiditis is now generally recognized clinically by the patient's presentation of symptoms and signs simulating hyperthyroidism.[12, 50–52] In fact, this disorder may represent about 15% of all hyperthyroid patients in North America, and it must be kept in mind as an important cause of hyperthyroidism.[50, 51] There are certain clinical features that may suggest such a diagnosis. The history is usually of short duration, eye signs are absent, the clinical signs of hyperthyroidism may be mild, and there may be only moderate or no enlargement of the thyroid gland.

The laboratory test findings are, for the most part, identical to those found in painful thyroiditis (see subsequent discussion). As anticipated by the clinical manifestations, the serum T_4, T_3, T_3 resin uptake, and free T_4 are elevated.[50–52] Unlike Graves' disease, the T_3/T_4 ratio is not increased.[84] Moreover, the ^{131}I, ^{123}I, or technetium thyroidal uptake is very low.[50–52] Thus, it is essential to perform uptake procedures in cases of clinical hyperthyroidism in areas where this condition is common, so as to avoid making an erroneous diagnosis of Graves' disease with consequent mismanagement. Plasma thyroglobulin level is also elevated, thus distinguishing the syndrome from surreptitious thyroxine ingestion.

There are, however, some differences in the results of laboratory procedures between painful and painless thyroiditis. In the painful variety, the sedimentation rate is almost invariably, markedly elevated,[1, 14, 23, 42, 70, 76, 79] whereas in the painless variety, the rate may be normal or only slightly above normal.[50–52, 86] The plasma thyroglobulin level is much higher in

the painful group.[34] Thyroid autoantibodies appear in about half of the patients with painful thyroiditis, although rarely do they reach high titers[14, 42, 76, 81]; in the painless variety, thyroid autoantibodies are often not detectable by ordinary serologic techniques, at least in most of the patients in whom the disease is *not* related to pregnancy, although such antibodies have been found by sensitive radioassays.[87] In comparison, post-partum thyroiditis is associated with significant thyroid enlargement and the presence of thyroid autoantibodies, and it may represent a different etiology from the form of the condition seen *without* the relationship to pregnancy (see subsequent discussion).

The course of the painless form of thyroiditis is very similar to that in the painful form and is discussed further. However, in the painless form, the course seems to be shorter, lasting several weeks rather than several months. The type and results of management of the two forms of thyroiditis differ and are discussed subsequently.

It may be emphasized that whereas this "atypical," painless form of thyroiditis once seemed uncommon, it now constitutes about 40% of all cases of "subacute" thyroiditis. In fact, it is likely that not all patients with this condition have a hyperthyroid phase, and thus the disorder may be far more common than is clinically evident.

RELATIONSHIP OF PAINLESS (SILENT) SUBACUTE LYMPHOCYTIC THYROIDITIS TO CHRONIC AUTOIMMUNE THYROIDITIS

It has been suggested that painless subacute thyroiditis is a new form of transient hyperthyroidism; in fact, this condition has been observed for many decades.[79] It is nevertheless true, as noted, that the number of such cases seems to have markedly increased; moreover, certain features of this variant are clearly different from the painful form of subacute thyroiditis.

In most biopsy specimens obtained during the course of this painless disorder, lymphocytic infiltration predominates. This finding has led some workers to the conclusion that this malady represents an unusual form of chronic lymphocytic thyroiditis.[86] However, only about a third of the biopsy specimens show evidence of Hürthle cells, which when present are generally infrequent as well.[50, 51] Fibrosis is usually absent or minimal. These last two features are certainly unlike chronic lymphocytic thyroiditis, in which Hürthle cells and fibrosis are common features. Moreover, the condition generally goes on to a full histologic recovery, leaving virtually no trace after several months in those cases unrelated to pregnancy.[31]

Moreover, thyroid autoantibodies are often undetectable in this condition when unrelated to pregnancy by ordinary serologic techniques, although such antibodies have been detected by radioassay.[12] There is, however, a discrepancy between the serologic procedures for thyroid antibodies and the findings by radioassay, which may be a result of interference by excessive amounts of thyroglobulin liberated during the course of the thyroiditis, giving artefactual levels of antithyroglobulin by radioassay.[77] Thus, there is no certainty that there are significant thyroid autoantibodies appearing often in this entity.

The HLA typing in this condition differs from that of classic painful thyroiditis and differs from that of Hashimoto's thyroiditis. In one study of patients with silent thyroiditis *unrelated* to pregnancy, there was no particular HLA type,[16] whereas in the painful form of subacute thyroiditis, HLA-Bw35 predominated.[4, 43, 53, 54] Conversely, in one study of post-partum thyroiditis, there appeared to be an increased incidence of HLA-DR5, similar to that seen in Hashimoto's thyroiditis.[16] This finding has been disputed by Jansson and colleagues[35] who have found an increased incidence of HLA-DR4 in post-partum thyroiditis.

It would thus appear that when silent thyroiditis occurs in *no* relationship to pregnancy, it does *not* usually represent chronic autoimmune thyroiditis, since thyroid enlargement is minimal or lacking, thyroid autoantibodies are often not present or present in very low titer, and recovery is quick and complete.[31] While the etiology of this condition is not clear, it still may be a variant of subacute (De-Quervain's) thyroiditis in persons who are genetically different and thus respond variously to the viral or other etiologic agent. It must be conceded, however, that the etiology is simply unknown at the present time.

It is, however, probable that the form of silent thyroiditis seen commonly in the post-partum period is indeed autoimmune in nature, despite the fact that it resembles the aforementioned form of painless thyroiditis, both clinically and pathologically. The possible

autoimmune nature of post-partum thyroiditis is discussed in Chapter 11.

At the present time, therefore, I believe that painless silent thyroiditis may be etiologically heterogeneous, albeit clinically homogeneous. At least in those patients in whom there is no relationship to pregnancy, there does not appear in most cases, at least, to be any demonstrable relationship to autoimmune processes. My current conception, therefore, is that while the histologic appearance of these cases does seem similar to chronic autoimmune thyroiditis, at least in those instances where there is no relationship to pregnancy, there is probably some other etiologic explanation. Many observers have not seen fit to categorize cases in this manner, and this lack of definition has undoubtedly led to the confusion that has marked this subject.

Course of the Disease

As noted previously, the course of subacute thyroiditis is quite variable.[1, 14, 23, 70, 76] The average duration for the painful variety is approximately 2 to 5 months, although the duration is shorter for the atypical painless disorder.[50–52, 86] Moreover, in about one fifth of patients, recurrences tend to prolong the course.

A clinical state simulating hyperthyroidism is initially present in approximately half of the patients with pain.[1, 14, 23, 70, 76] There is no way of knowing what the proportion of hyperthyroidism is in the painless variety, since those patients who are not hyperthyroid could not be diagnosed. With progression of the disease in either the painful or painless form, complete depletion of the colloid stored within the thyroid gland may be caused by continuous leakage of the colloid into the interstitial tissue. The result is the virtual failure of formation of new thyroid hormone, because of the disruption of the thyroid parenchyma.[79] If the disease is severe or prolonged enough, a phase of transient hypothyroidism will appear and may persist for several weeks or months.[1, 14, 23, 70, 76, 79, 80] With recovery, the thyroid gland is finally repleted with colloid and thyroid function is restored. Such transient hypothyroidism is seen in about up to 56% of the patients, particularly if a thyrotropin-releasing hormone (TRH) test is utilized to detect some of the previously undetected subclinical cases (Fig. 10–2).[42]

It is a rare event for a patient with subacute

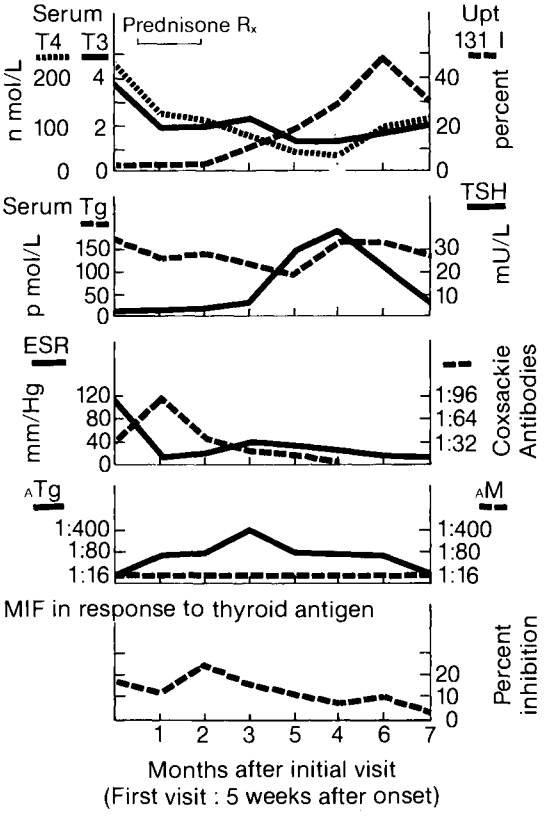

Figure 10–2. Laboratory values from a typical case of subacute (DeQuervain's) thyroiditis (T_4 = thyroxine; T_3 = triiodothyronine; Upt ^{131}I = 24-hour radioactive iodine uptake; ESR = erythrocyte sedimentation rate; Tg = thyroglobulin; $_A$Tg = antithyroglobulin; $_A$M = antimicrosomal antibody; and MIF = migration inhibition factor). Note the proportionate elevation of serum T_4 and T_3 at the outset followed by a fall to subnormal levels, the hypothyroid phase lasting approximately 2 months. The 24-hour radioactive iodine uptake was initially very low, rose only slowly, but eventually became supranormal 6 months after the initial visit. It returned to normal some time after the serum T_4 and T_3 had become normal. Serum Tg however remained elevated throughout this period although after 1 year it had returned also to normal. There were transient elevations in $_A$Tg and MIF in response to thyroid antigen, falling ultimately back to normal. While prednisone therapy rapidly ameliorated the clinical signs and symptoms, it did not prevent the evolution of these changes.

thyroiditis to progress to permanent myxedema.[1, 14, 33, 42, 70, 76, 79] The progression may be due to complete destruction of the thyroid gland with consequent complete fibrosis; in even more rare instances, the disorder may culminate in autoimmune thyroiditis associated with hypothyroidism. A few patients have developed hyperthyroidism after recovery from subacute thyroiditis.[14, 76, 85] The nature of this sequence is unknown, but it is possible that

the thyroid inflammation may have acted as a nonspecific stress in inducing the subsequent autoimmune hyperthyroid state.[77]

Thyroid function undergoes dynamic changes consequent to the pathologic process. Initially, the inflammatory destruction of the thyroid gland results in marked leakage of colloid from the damaged follicles into the interstices and then into the circulation and includes a variety of iodinated materials—proteins, proteases, peptides, and amino acids.[80] This process results in an increased protein bound iodine (PBI) concentration.[1, 14, 76]

Although there is often an increase in serum T_4 and T_3, resulting from cleavage of the thyroid hormone from the discharged colloid, the remainder of the PBI not accounted for by the discharge of the hormones is a result of the discharge of iodoproteins, such as thyroglobulin[34] and iodoalbumin. In any event, the increase in serum T_4 and T_3 consequent to the thyroid inflammation accounts for the manifestations of hyperthyroidism.[1, 7, 14, 21, 38, 76] It is curious, however, that the levels of T_3 are less elevated in this form of hyperthyroidism than in Graves' disease.[38, 84] This situation may account for the mildness of the clinical manifestations of hyperthyroidism in both painful and painless thyroiditis. In Graves' disease, in addition to the T_3 produced by the peripheral monodeiodination of T_4, the stimulated thyroid cells produce disproportionate amounts of T_3, which then are secreted into the circulation.[78] In comparison, in subacute thyroiditis, only preformed thyroid hormones, in normal proportions, are discharged.

The fall in plasma T_4 is exponential during the first week.[21] This phase of hyperthyroidism can continue only until the gland reaches a stage of depletion of preformed colloid—a stage that can last only several weeks at best. The plasma thyroglobulin, however, may remain elevated long after all other evidence of the inflammation has subsided.[34]

At the same time, the damaged thyroid follicular cells are incapable of functioning effectively and, thus, cannot trap iodine; therefore, the 24-hour radioactive iodine uptake is characteristically suppressed to values in the order of 0 to 1%.[1, 7, 14, 21, 38, 76] Even if only part of the gland is involved, the uptake may be similarly depressed as a result of suppression of pituitary TSH due to the elevated levels of thyroid hormone.[40] This condition explains the apparent paradox of a high serum PBI, T_4, or T_3, with a very low radioactive iodine uptake, a characteristic set of findings in the early phase of subacute thyroiditis.[1, 7, 14, 21, 38, 76] Under these circumstances, very minimal hormone biosynthesis persists and what is produced leaks out.[32]

In the early stages of the disease, the scans of the thyroid gland reveal a patchy and irregular pattern of distribution of the tracer or no uptake whatever.[24, 40] Measurements of serum TSH are usually undetectable at this stage, because of the high concentrations of thyroid hormones.[21, 36, 38] In addition, there is diminished TSH response to TRH in this phase.[3, 9, 20, 37, 38, 69, 84] Generally, perchlorate or thiocyanate administration does not release excessive amounts of iodine from the gland[13, 32, 39]; large doses of TSH may cause a rise in the radioactive iodine uptake, but only when there are some uninvolved parts of the gland.[40]

Most commonly, there is no rise in the radioactive iodine uptake after exogenous TSH injection during the first weeks of the disease, indicating that the impairment of the thyroid cell remains a crucial factor in the disturbance of the iodide concentrating mechanism.[13] Radioactive iodine uptake may remain suppressed for 6 weeks or more after the onset of symptoms.[80] The suppression indicates the complete blocking of the various stages of intrathyroidal metabolism during this interval. If recovery of cellular function is prolonged, a phase of transient hypothyroidism may appear, which may last for months.[14, 42, 74, 76] This phase, associated with an elevated TSH level, is seen in about 25% of patients. Ultimately, recovery is the rule, and conversely permanent myxedema is uncommon (about 5%).[42] There may be a lag between the reestablishment of the iodide-concentrating process within the thyroid and the resumption of hormonal synthesis, secretion, and repletion within the gland.[36]

The erythrocyte sedimentation rate is characteristically markedly elevated in the painful form of thyroiditis, often to about 100 mm/hour[1, 14, 23, 70, 76]; if the sedimentation rate is normal in the painful form, the diagnosis should be suspected. In the painless form, however, the sedimentation rate may be normal or minimally elevated.[86] The leukocyte count is usually normal but has been reported as high as 18×10^9/L in the painful variety.[1, 76] An increase in alpha-2-globulin is a nonspecific inflammatory response.[64] The alkaline phosphatase and other hepatic enzyme levels may be elevated in the early phase.[8] Thyroid autoantibodies appear in a minority of patients

some weeks after onset, usually reach only modest titers, and decline to zero after several months.[14, 42, 76] In the painless variety unrelated to pregnancy, serologic findings for thyroid antibodies are usually negative, although radioassay findings for antithyroglobulin have been reported to be positive.[87] The significance of these findings has been discussed. The appearance of antibodies directed against the TSH receptor was discussed previously.

Diagnosis

In a patient with fairly typical manifestations, the diagnosis of subacute thyroiditis should present no difficulties. Nevertheless, pharyngitis is a common misdiagnosis in the early stages, since "sore throat" is a characteristic complaint.[79] However, when the pain is precisely localized in the anterior neck area, associated with swelling and tenderness of the thyroid gland itself, together with prolongation of the complaint to months, the physician should be led to the correct diagnosis.

A small percentage of patients with Hashimoto's thyroiditis may have painful tender thyroid enlargement, which may be indistinguishable initially from subacute thyroiditis.[76] However, the uptake of ^{131}I is rarely as completely suppressed as in the early stages of subacute thyroiditis and may be elevated; also, the titers of thyroid antibodies are usually high enough to suggest the presence of chronic thyroiditis.

Initially, acute suppurative thyroiditis may be difficult to distinguish from subacute thyroiditis.[76] However, definite clinical signs of suppuration soon are evident. Another disorder often mistaken for subacute thyroiditis is *globus hystericus*. The patient complains of a sense of pressure or a feeling of a "ball" or "lump" in the throat. The patient may have mild tenderness, but this proves to be diffuse and is not associated with any tender thyroid enlargement.

Rapidly growing anaplastic carcinomas of the thyroid may be associated with severe pain and tenderness within the thyroid,[58] but the large size of the malignant goiter, the usual adherence to adjacent structures, the lymphadenopathy, and the characteristic course soon lead to the correct diagnosis.

Occasionally, patients with painful subacute thyroiditis are diagnosed as suffering from hyperthyroidism in the early phases, and indeed some patients with true hyperthyroidism may have a mildly tender thyroid gland.[1] The presentation is that of clinical hyperthyroidism in the painless variety. It should be emphasized that this possibility must be kept in mind when patients are suffering from hyperthyroidism in areas of high prevalence of subacute thyroiditis, and appropriate tests must be carried out to establish the correct diagnosis. Factitious hyperthyroidism is an important differential diagnosis, but is associated with a low serum thyroglobulin level. While painless thyroiditis associated with goiter has also been diagnosed in euthyroid persons, most usually by retrospective histologic examination of an excised goiter, it is almost certain that many patients with painless thyroiditis with small glands must escape detection.

Later, during the hypothyroid phase, through which some patients pass, a diagnosis of permanent hypothyroidism may be made.[79] When local symptoms have subsided, palpation of the firm goiter may suggest diagnoses such as colloid goiter, nodular goiter, thyroid adenoma, carcinoma of the thyroid, and even Riedel's struma and may lead erroneously to surgery.[79]

Therapy

There is no definitive therapy for painful subacute thyroiditis, but the use of corticosteroids has proved to be valuable in the treatment of the vast majority of patients.[1, 14, 23, 70, 76] The clinical response is rapid, often with relief of symptoms within 24 hours. Corticosteroids are highly effective in relieving symptoms and do not appear to alter the basic disease process. Presumably, corticosteroids act by suppressing the inflammatory response and by allowing the basic disease process to run its now subclinical course.

Generally, prednisone or a similar analogue is prescribed. It is necessary to start with pharmacologic dosages, e.g., 10 mg four times daily; the dosage is then slowly reduced over approximately 1 month and can be discontinued in most instances thereafter. However, as the dosage is decreased, there are recurrences in about 20% of patients that necessitate the restoration of a higher dose. It is sometimes necessary to give repeated courses of treatment before ultimate recovery. The development of viral and thyroid antibodies does not appear to be affected by this form of therapy. In most instances, however, exacerbations do not occur, and patients go on to full recovery.

Triiodothyronine has been reported to bring about rapid relief of symptoms in the acute phase,[28, 84] but in my experience this does not appear to be of benefit if the patients are chemically hyperthyroid at the time of therapy. However, in the event of repeated exacerbations, the addition of thyroxine or triiodothyronine to the therapeutic regimen may often result in considerable benefit and appears to prevent further recurrences.[1, 14, 23, 70, 76] It may be that, in these instances, endogenous TSH is playing some role in maintaining the disorder, and indeed there are a few reports in which exogenous TSH has either aggravated or precipitated thyroiditis.[23]

Irradiation was a popular form of therapy for subacute thyroiditis in past decades.[14, 76, 79] Doses varied between 200 and 2000 rads, although it was reported that doses in the order of 200 to 400 rads were quite effective. Nevertheless, there was a failure rate of about 25%, and the response was considerably slower than with corticosteroids and certainly less predictable. In the past two decades or more, this therapy has not been employed to any degree. Moreover, in view of the major concern about the effects of low-dose radiation in inducing late thyroid carcinoma, such therapy is contraindicated.[82]

Salicylates, phenylbutazone, and propoxyphene have frequently been administered with good success.[1, 13, 14, 76] In my view, however, such agents are effective only in the milder cases of this disease and are much less potent and predictable than corticosteroids.

Antibiotics are generally useless. Thiouracil and thyrotropin have been reported to exert beneficial effects by some workers, but such agents have not found general favor.[23] The high incidence of postoperative myxedema has discouraged thyroidectomy as treatment for subacute thyroiditis, and since recovery is the general rule thyroidectomy almost never needs to be recommended.[23] However, the rare complication of a very prolonged course with malaise and local distress continuing almost indefinitely may justify thyroidectomy, which will often then result in relief.[14]

Such measures as are outlined above need not be employed in the treatment of painless thyroiditis. In this condition, it is necessary only to treat symptomatically those manifestations related to the excess release of thyroid hormones. Thus, propranolol in modest dosages is quite useful.[50–52, 86] Since the course is generally more rapid than in the more classic form, the medication can usually be withdrawn after a few weeks. It is rarely necessary to employ any further form of therapy.[52]

After complete recovery, late recurrences are rare in the painful form of the disease. However, it is not uncommon to have the painless variety recur months or even years later. It has been noted that this condition may appear following pregnancy. The painless form varies in its outcome. While most patients recover completely and easily, those patients whose condition is due to autoimmune processes may suffer recurrences or may go on to permanent goiter and hypothyroidism, thus requiring thyroxine therapy.

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11
Autoimmune Thyroiditis

ROBERT VOLPÉ

The prototype of autoimmune thyroiditis, i.e., struma lymphomatosa (Hashimoto's thyroiditis), was first described by Hashimoto in 1912.[34] He reported four patients with goiter in whom the histology of the thyroid was characterized by diffuse lymphocytic infiltration, atrophy of the parenchymal cells, fibrosis, and eosinophilic change in some of the parenchymal cells. While this condition as described remains common, there are several variants that differ slightly from the prototype (Table 11–1).[37] In the "chronic fibrous" thyroiditis variant, fibrosis predominates and lymphocytic infiltration is less marked. In lymphocytic thyroiditis of childhood and adolescence, fibrosis, Askanazy cells, and germinal centers are less obvious than in adulthood. The titers of thyroid autoantibodies are generally lower in this category when compared with those in adults and indeed are often negative.[50] Postpartum thyroiditis is often a transient form of autoimmune thyroiditis, occurring during the course of a more occult disorder; it may indeed culminate in a chronic form.[1,2] In "idiopathic myxedema" the gland is characterized by atrophy rather than hypertrophy. The atrophic asymptomatic form is clinically occult and often discovered at autopsy.[4] There appear to be several genetic and pathogenetic differences between these variants. However, the variants appear to share at least a similar pathogenesis; the term "autoimmune thyroiditis" should thus be used as a generic term for this group.

In past generations, Hashimoto's thyroiditis was considered uncommon, and the diagnosis was often made only at thyroidectomy. Increased awareness, associated with improved diagnostic procedures, has resulted in improved recognition. The disease may also have actually increased in frequency, perhaps owing to the sharp increase in iodine intake that has occurred in the Western world in the past two

Table 11–1. Autoimmune Thyroid Disease

Autoimmune thyroiditis
Lymphocytic thyroiditis (struma lymphomatosa, Hashimoto's thyroiditis)
Chronic fibrous thyroiditis
Lymphocytic thyroiditis of childhood and adolescence
Post-partum thyroiditis
Idiopathic myxedema
Atrophic asymptomatic thyroiditis
Autoimmune hyperthyroidism (Graves' disease, Parry's disease, Basedow's disease, diffuse toxic goiter, exophthalmic goiter)

generations.[80] It is now considered that approximately 3 to 4% of the population has significant autoimmune thyroid disease.[27] About 4.5% of the population has some functional deficiency of the thyroid, secondary to autoimmune thyroid disease.[35, 36] In elderly women, about 16% are found to have thyroid autoantibodies; these correlate with at least some degree of lymphocytic infiltration of the thyroid gland, although this cannot be recognized clinically.[91]

A very close relationship exists between Graves' disease and autoimmune thyroiditis; the term autoimmune thyroid disease would certainly encompass Graves' disease as well as the other disorders listed in Table 11–1. Although Graves' disease and autoimmune thyroiditis appear to be caused by closely related genetic and immunologic disorders, there are at least a few aspects of these conditions that continue to separate the two maladies, so that they cannot be considered merely as different expressions of a spectrum of a single entity (see subsequent discussion).[80]

ETIOLOGY AND PATHOGENESIS

The first indications of an immunologic abnormality in lymphocytic thyroiditis came from reports of an elevation of plasma gamma globulin level and of an abnormal serum flocculation test result.[80] These observations formed the background of the classic discoveries of antithyroid antibodies in the serum of patients with Hashimoto's thyroiditis in 1956[63] and the production of experimental autoimmune thyroiditis in the same year.[66] These pioneer observations led to innumerable investigations into the nature of the immunologic disturbance in autoimmune thyroid disease; indeed, these have served largely as prototypes for autoimmune disease generally.

While the pathogenesis of autoimmune thyroid disease is not fully clarified, a working hypothesis may now be outlined (Fig. 11–1).[80] The evidence suggests that both Graves' and Hashimoto's diseases are due to specific genetic defects in immunoregulation (see Chapter 13, Graves' disease, for a more detailed treatment of autoimmune factors). Evidence is accruing that the actual immunoregulatory defect in each organ-specific autoimmune endocrinopathy is a qualitative or quantitative abnormality of an organ-specific clone of suppressor thymic-dependent (T) lymphocytes.[81a] In each condition, given such a defect, a normally, randomly mutating thyroid-directed, self-reactive clone of helper T lymphocytes is permitted to survive. All normal persons have the capacity to produce clones of self-reactive lymphocytes, arising by either a process of random mutation or perhaps viral induction, directed against normal body constituents. In normal persons, specific clones of suppressor T lymphocytes suppress specific autoreactive clones of helper T lymphocytes, thus preventing them from interacting with their complementary autoantigen (resulting in normal tolerance). The mechanism of immunoregulation thus may largely be related to specific as well as nonspecific suppressor T lymphocyte function, although other mechanisms for tolerance clearly exist. Anti-idiotypic antibody has been proposed as another regulatory mechanism.[25]

If in organ-specific autoimmune thyroid disease there is a single genetic (or occasionally sporadic?) defect in immunoregulation, then the random appearance of the appropriate thyroid-directed autoreactive clone of helper T lymphocytes, which has escaped normal control as a result of the defect, would initiate the disease.[80] These T lymphocytes would then interact with their complementary antigen on the thyroid cell membrane and set up a localized cell-mediated immune (CMI) response. This would not require any antigenic alteration, only antigenic availability, and requires the expression of HLA-DR antigen, either on the thyroid cell membrane itself[6] or via macrophage presentation of the antigen to the lymphocytes.[29] The expression of HLA-DR antigen on thyroid cells has now, in any event, been demonstrated to be a secondary event.[81b] There is no convincing evidence that there is any antigenic alteration in these disorders[43]; my view is that the thyroid gland is merely a passive captive to specific events within the immune system.[80] When these thyroid-directed autoreactive helper T lymphocytes have escaped suppression, as outlined, and have interacted with their complementary thyroid cell membrane antigen, they would then direct groups of existing bursa-equivalent (B) lymphocytes, which in consequence would produce the appropriate thyroid autoantibodies.

Antibodies to various antigens have been identified in autoimmune thyroid disease (Table 11–2 and Humoral Mechanisms), which exert deleterious or occasionally stimulatory effects on the thyroid parenchymal cells, alone as immune complexes or in association with lymphocytes, e.g., antibody-dependent cellu-

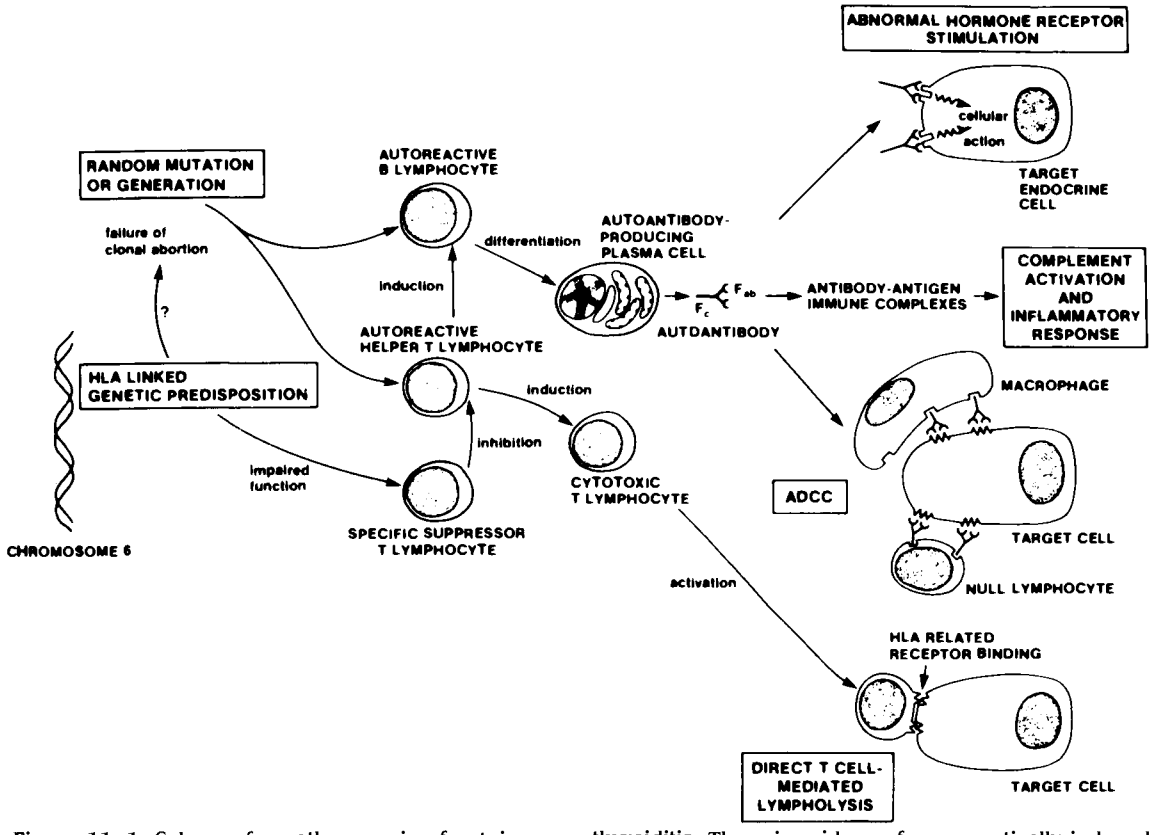

Figure 11–1. Schema for pathogenesis of autoimmune thyroiditis. There is evidence for a genetically induced human leukocyte antigen (HLA)-linked, organ-specific defect in suppressor T lymphocytes, which may be the fundamental abnormality in autoimmune thyroid disease. This may be augmented by environmental influences on generalized suppressor T lymphocyte function. With the availability of the thyroid cell membrane antigen, via antigen-presenting cells initially and directly via the thyroid cells (through gamma interferon–induced thyrocyte HLA-DR expression) subsequently, the no longer suppressed thyroid-directed T lymphocytes become activated and, in turn, stimulate thyroid-directed B lymphocytes to produce thyroid autoantibodies. Cytotoxic factors are noted on this figure and are amplified in Figure 11–2. Topliss, D. J., Lewis, M., Hoos, J., Row, V. V., and Volpé, R. (1981). Autoimmunity in endocrine disease. Med. Clin. North Am. 10:1053–1064.

lar cytotoxicity (ADCC), macrophages, or "killer" cells (Fig. 11–2).[70] It may also be emphasized that various other organ-specific autoimmune disorders occur in the same patients and in their families much more often than chance alone would dictate (Table 11–3).[80] The presence of two or more such conditions, which often do not commence at the same time, could be explained by two variables as follows: (1) the magnitude of the genetic defect in immunoregulation in respect to two or three or more autoreactive clones of lymphocytes and (2) the random appearance by mutation of the appropriate autoreactive clone of helper T lymphocytes, which consequently escape regulation (see Chapter 13, Graves' disease, for discussion of the pathogenesis of autoimmune thyroid disease).[80]

PATHOLOGY

The histology is characterized by an inflammatory infiltrate with areas of follicular hyperplasia and tall columnar follicular cells, although often the follicles are small and contain little colloid.[4, 37] Follicular cells may be arranged in the form of masses, with vacuolized and very eosinophilic cytoplasm (Hürthle or Askanazy cells). These cells are enlarged, and the cytoplasm contains eosinophilic granules that prove to correspond to increased numbers and size of mitochondria on ultrastructural studies. There is, of course, widespread lymphocytic infiltration, which may be arranged in germinal centers. Large numbers of plasma cells are also seen (Fig. 11–3). There is variable fibrosis, which in some instances may be extreme (i.e., chronic fibrous variant). The gland

Table 11–2. Antigen-Antibody Systems Involved in Humoral Responses of Thyroid Autoimmune Disease

Antigen	Antibody (Function)	Antibody Detection
Thyroglobulin	Thyroglobulin antibody (No clear function)	Precipitin technique; tanned red blood cell hemagglutination; immunofluorescence on fixed thyroid sections; competitive binding radioimmunoassay; coprecipitation with ^{125}I thyroglobulin; microenzyme-linked immunoassay (ELISA); plaque-forming assay
Microsomal antigen	Microsomal antibody (Cytotoxic in conjunction with lymphocytes)	Complement fixation; immunofluorescence on unfixed thyroid sections; cytotoxicity test on cultured thyroid cells; competitive binding radioimmunoassay; tanned red blood cell hemagglutination; microELISA
Second colloid component	CA_2 antibody (No clear function)	Immunofluorescence on fixed thyroid sections
Cell surface antigens	Membrane antibodies (Cytotoxic with lymphocytes)	Immunofluorescence on viable thyroid cells; mixed hemadsorption binding assays
Thyroxine and triiodothyronine	Thyroid hormone antibodies (Bind and prevent hormone action)	Antigen-binding capacity
Antigen not defined	Growth-stimulating and growth-inhibiting antibodies (May induce or inhibit thyroid growth)	Effects on DNA content per thyroid cell nucleus or glucose-6-phosphate dehydrogenase activity per cell
TSH receptor–related antigen	TSH receptor antibodies (May stimulate thyroid cells, inhibit TSH effect, or exert both or neither)	Stimulatory assays: current terms employed for stimulatory assays include human thyroid stimulator; human thyroid stimulating immunoglobulin (TSI); thyroid stimulating antibody (TSAb). Long-acting thyroid stimulator (LATS) bioassay; colloid droplet formation in human thyroid slices; stimulation of human thyroid adenylate cyclase *in vitro*; cytochemical assay Binding asays: LATS protector assay; inhibition of ^{125}I-thyrotropin binding to human thyroid membranes (thyrotropin displacement activity, TDA); TSH-binding inhibitor immunoglobulin, TBII; fat cell membrane radioligand assays; fat cell ELISA

itself may be either hypertrophied or atrophied, and in the atrophied cases fibrosis tends to be more dominant. Ultrastructural studies additionally show dilatation of capillaries, exudation of lymphocytes, and damage to the basement membranes of the thyroid follicles.[4]

The various subsets of lymphocytes within the thyroid infiltrate in Hashimoto's thyroiditis have also been studied.[80] These include both B and T lymphocytes in proportions similar to those observed in the peripheral blood, although there is a suggestion of a higher ratio of B lymphocytes within the thyroid. The ratio of helper to suppressor cells has been the subject of some controversy with some investigators describing a relative decrease in intrathyroidal suppressor T lymphocyte numbers and others demonstrating proportions equal to those in peripheral blood. However, there does seem to be a functional reduction in the

Figure 11–2. Mechanisms of effector interactions in Hashimoto's thyroiditis. (A) Sensitized cytolytic T lymphocytes might interact with specific antigens on the thyroid follicular cells and induce direct T lymphocyte–induced cytolysis. (B) Complement fixing antibody may produce thyroid cell cytolysis by activating complement. (C) Antibodies against cell membrane constituents can attract "killer" cells (antidody-dependent cellular cytotoxicity; ADCC), thus producing cytolysis. (D) Thyrotropin receptor antibodies may block the action of thyrotropin (TSH) and thus act as a cause of hypothyroidism. (E) There is some evidence for a thyroid growth promoting antibody and a thyroid growth inhibiting antibody, but it is not clear whether these are separate from thyrotropin receptor antibodies.

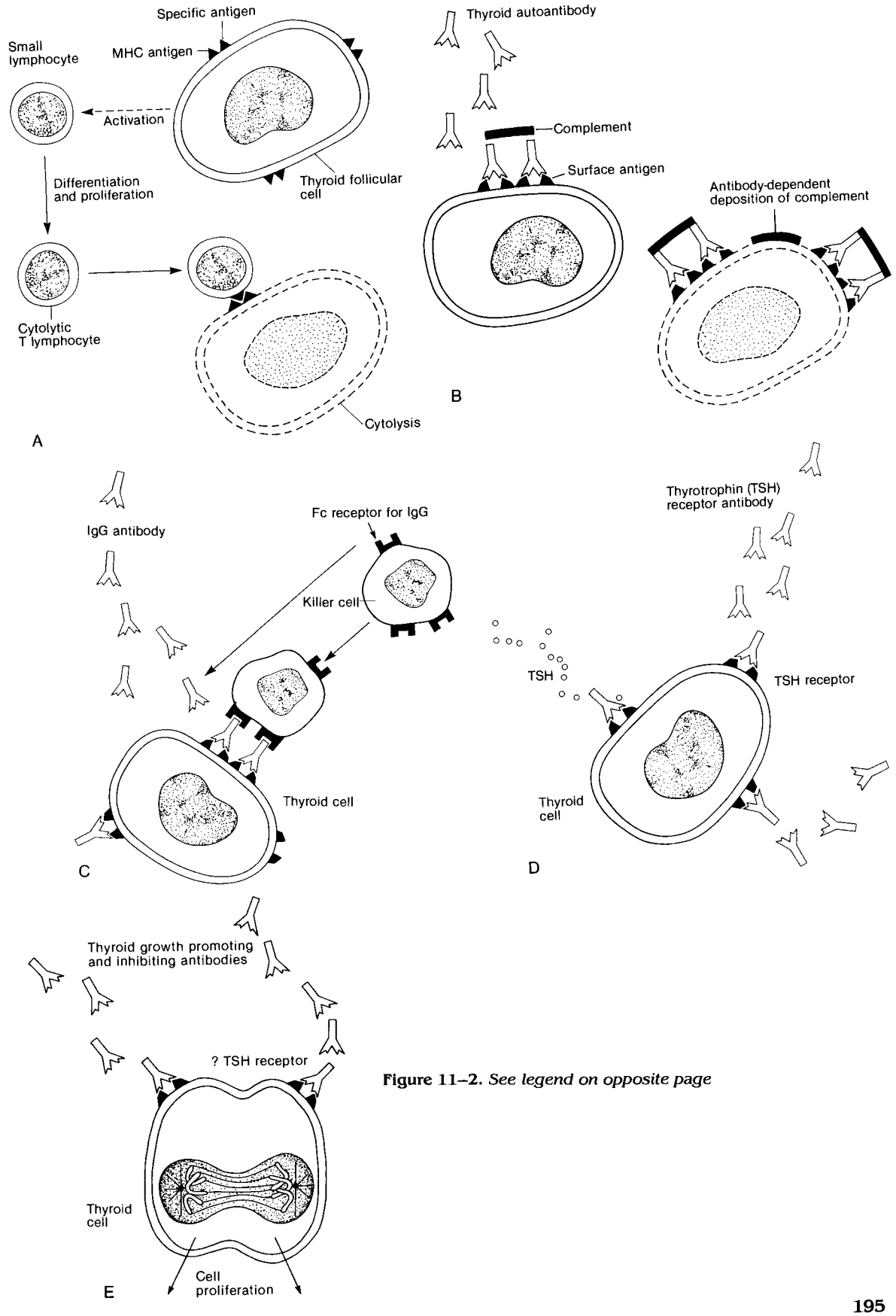

Figure 11–2. *See legend on opposite page*

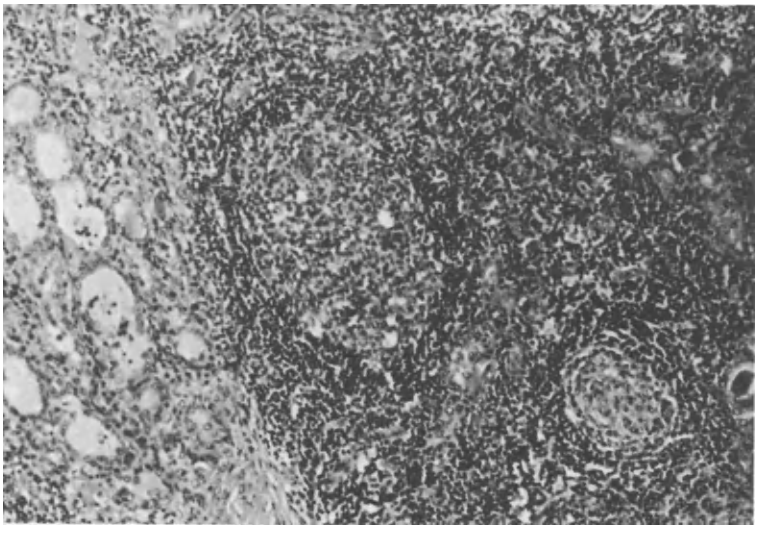

Figure 11–3. Pathology of Hashimoto's thyroiditis.

suppressor/helper T cell ratio within the intrathyroidal lymphocytes in Hashimoto's thyroiditis.[80]

PREVALENCE

One recent survey in adolescent subjects suggests a prevalence of 1 to 2% of autoimmune thyroiditis during the second decade of life.[62] Since age-specific incidence rates increase fourfold over 20 years and the peak incidence for this disorder is at 50 to 60 years,[82] the prevalence of thyroiditis in the older age group may be as high as 3 to 4%.[20] Since focal lymphocytic infiltration correlates with the presence of thyroid autoantibodies, the incidence in elderly females appears to be as high as 16 to 23%; in these patients, the disorder is generally occult.[91] The disease is four times more common in women than in men and four times more common in whites than in blacks.[20] The perceived marked increase in prevalence over the past 50 years may be related to the greater intake of iodine that has occurred during this interval.[80] In countries where iodine deficiency exists, Hashimoto's thyroiditis is rare. Alternatively, excessive iodine intake can unmask thyroiditis both in animals and humans.

GENETICS

These disorders tend to aggregate in specific families and thus appear to be genetically induced.[80] Indeed, Graves' and Hashimoto's diseases tend to occur in the same families and may even coexist within the same patient's thyroid gland. Moreover, homozygous twins have been reported in whom one twin has Graves' disease and the other has Hashimoto's thyroiditis. The increased incidence of other organ-specific autoimmune diseases in patients with both of these disorders, as well as in their families, has been noted (see Table 11–3). Finally, Graves' disease may culminate in Hashimoto's thyroiditis; conversely, patients with hypothyroidism and Hashimoto's thyroiditis may recover and then ultimately develop hyperthyroidism due to Graves' disease.[80]

Studies of the age-specific incidence rates in both Graves' and Hashimoto's diseases have indicated that these disorders occur at random in genetically predisposed populations.[82] There is an interesting correlation with HLA genes in autoimmune thyroid disease; these are the histocompatibility genes, which are also related to immune reactivity.[24] In whites with Graves' disease, there is an increased incidence of HLA-B8 and HLA-DR3. Patients with atrophic thyroiditis also have an increased incidence of HLA-DR3, whereas those with goitrous Hashimoto's thyroiditis have been reported to have an increased incidence of HLA-DR5. Thus, there appear to be significant genetic differences between goitrous Hashimoto's thyroiditis and atrophic thyroiditis. Further, it has been shown that patients with Hashimoto's thyroiditis from the same family as those with Graves' disease share the same haplotype (i.e., they seem to have the same genetic background), and often under these

Table 11–3. Immune Stigmata Associated with Graves' Disease and Hashimoto's Thyroiditis

Stigma	Graves' Disease	Hashimoto's Disease
Lymphocytic infiltration in thyroid	Frequently present	Almost invariable
Immunoglobulins in thyroid stroma	Yes	Yes
Type of infiltrating lymphocytes in thyroid	B and T lymphocytes, some unidentified lymphocytes	B and T lymphocytes, some unidentified lymphocytes
Immune complexes in circulation	Common	Common
Thymic enlargement	Common	Common
Lymphadenopathy and splenomegaly	Infrequent	—
Relative lymphocytosis	Common	—
Hypergammaglobulinemia	—	Common
Benefit from corticosteroid therapy	Yes	Yes
Thyroid-stimulating immunoglobulin	Almost all	Infrequent
Exophthalmos	Common	Occasional
Evidence of cell-mediated immunity	Yes	Yes
Evidence for a defect in suppressor T lymphocytes	Yes	Yes
Other autoimmune diseases in patients	Pernicious anemia, diabetes mellitus, myasthenia gravis, Addison's disease, idiopathic thrombocytopenic purpura, and so forth	Pernicious anemia, diabetes mellitus, myasthenia gravis, Addison's disease, chronic active hepatitis, and so forth
Thyroid antibodies in relatives	50%	50%
Thyroid and other autoimmune diseases in relatives	Common	Common
HLA genes (whites)	HLA-B8-Dw3	Atrophic form: HLA-B8 and HLA-DRw3 Goitrous form: HLA-DR5
Animal models	—	Yes

circumstances they share HLA-DR3.[73] The last finding in these families suggests that the immunogenetic background for both Graves' and Hashimoto's diseases, while not identical, is certainly very similar.

Not all patients with these disorders have these particular HLA genes; conversely, if a patient does have the "appropriate" gene the relative risk of developing these disorders is only increased about fivefold.[23] It is therefore clear that these are *not* specific "disease susceptibility" genes, which may, however, lie in close "linkage disequilibrium" with the above genes. Since it is known that the HLA genes are closely related to immunoregulation, it would seem likely that the putative disease susceptibility gene is the gene that results in the defect in immunoregulation.[71] It is known that expression of DR antigen on the surface of target cells, macrophages, or both is important for the presentation of antigen to the lymphocytes[6, 29]; it is not quite clear whether or how this would relate to the immunoregulatory defect or whether the effect of the gene is translated by other mechanisms to the immune system. While it seems probable that there is indeed an organ-specific suppressor T lymphocyte defect as a basic for autoimmune thyroid disease, what the genetic basis in molecular terms for such a defect may prove to be has not yet been clarified.[80]

The association of autoimmune thyroiditis with other organ-specific autoimmune diseases probably relates to the likelihood that the genes all lie close together on chromosome 6 in linkage disequilibrium. Most of the disorders that are associated with autoimmune thyroiditis have an increased incidence of HLA-DR3. It is of further interest, however, that autoimmune thyroiditis is much more common than expected with Down's syndrome and Turner's syndrome (gonadal dysgenesis), each caused by quite different chromosomal abnormalities; how these influence the expression of autoimmune thyroid disease has yet to be determined.[80] Even the remarkable sex difference whereby females outnumber males in these disorders by approximately 4:1 may prove to have a genetic basis, i.e., the presence of the X or the lack of the Y chromosome may modify the expression of the putative "disease susceptibility" gene on chromosome 6. Thus,

the sex incidence and the incidence of these diseases in chromosomal abnormalities may be manifestations of one gene modifying the expression of another gene. There is some evidence, also, that estrogen may influence the immune system and thus the expression of these diseases.[80]

HUMORAL MECHANISMS

Thyroglobulin and Antithyroglobulin Antibodies

It was once believed that thyroglobulin represented a sequestered antigen within the thyroid follicle, which was not recognizable to the organism as "self," and destruction of the follicles with escape of thyroglobulin might lead to autoimmune thyroiditis.[59] However, it is now known that thyroglobulin begins to leak into the circulation *in utero* in all humans and in fact is a normal circulating constituent even before birth.[64] Thus, the "sequestered" antigen theory can be dismissed.[79] Moreover, thyroglobulin binding B lymphocytes are present even before birth.[79] By the common technique of tanned red blood cell agglutination, significant titers of antithyroglobulin are found in about 70% of patients with Hashimoto's thyroiditis, in about 60% of those with newly diagnosed idiopathic myxedema, in about 30% of those with Graves' disease, and in a small percentage of those with thyroid carcinoma and other thyroid disorders.[61] However, by radioassay, a larger number of sera that gave negative results by the hemagglutination test were found to give positive results by this same group.[61] However, the reason for this discrepancy proved to be an increased level of serum thyroglobulin, which may produce false positive results in the measurement of antithyroglobulin by radioassay. Interpretation of radioassay results for antithyroglobulin must be tempered by these considerations. Another technique, the enzyme linked immunoabsorbent assay (ELISA), appears to be more sensitive than any other so far utilized.[54]

Antimicrosomal Antibodies

The thyroid microsomal antigen has been localized by immunofluorescence in the apical cytoplasm of follicular cells.[79] It now seems evident that an important component is that of thyroid peroxidase[61a] as well as other cell membrane antigens. However, the precise antigen has yet to be totally characterized. Antibodies to the microsomal antigen have been shown to be complement fixing and have the ability to induce cytotoxic changes in monolayers of cultured thyroid cells. Correlation exists between the titers of this antibody and the histologic lesion of Hashimoto's thyroiditis.[35, 36] The correlation between this antibody and laboratory or clinical evidence of thyroid dysfunction is likewise good. The titers of thyroglobulin antibodies do not correlate nearly as well. Antimicrosomal antibodies may be detected by immunofluorescence, complement fixation, hemagglutination, radioassay, or ELISA techniques.[79] The hemagglutination test is currently the favorite procedure, but it may be supplanted by a microELISA procedure.[67] Microsomal antibodies may be detected in virtually all patients with Hashimoto's thyroiditis, in most of those with idiopathic myxedema or Graves' disease, but much less frequently in other thyroid disorders.[79] There are marked discrepancies between antimicrosomal and antithyroglobulin antibodies. Moreover, high levels of antithyroglobulin antibody may produce false positive results in measurements of antimicrosomal antibodies by hemagglutination, but this interference may be overcome by adding an excess of thyroglobulin to the system.[61] This problem is of minimal importance since antimicrosomal antibodies are present much more commonly than antithyroglobulin antibody, whereas conversely antithyroglobulin antibodies are rarely present in the absence of antimicrosomal antibodies. A preponderance of antimicrosomal antibodies is found to be even more pronounced in patients with Graves' disease.

There is a close association between the presence of elevated antimicrosomal antibody titers and elevated antibodies to the cell surface antigens, and there appear to be common antigens involved in both systems.[61] However, this relationship is not absolute. Antimicrosomal antibodies tend to fall in many but not in all patients who become severely myxedematous spontaneously[4] or in hypothyroid Hashimoto's patients receiving thyroxine therapy.[42, 65]

Other Antibodies

Antibodies to a colloid component other than thyroglobulin have been identified but their significance is unclear.[79, 80] Antibodies to the thyroid hormones, thyroxine and triiodothy-

ronine, are uncommonly found in autoimmune thyroid disease and are generally associated with very high titers of antithyroglobulin.[80] These may become important when measuring circulating levels of thyroid hormones, as they may result in spuriously high or low T_4 or T_3 concentrations, depending on the particular technique employed. These antibodies will not affect thyroid function as long as the thyroid is capable of responding to thyroid-stimulating hormone (TSH) normally.

Antibodies have been detected in goitrous Hashimoto's thyroiditis that are capable of stimulating *growth* of the thyroid cells without stimulating thyroid activity.[18] Moreover, antibodies that *inhibit* the trophic effects of TSH have been detected in some cases of atrophic primary myxedema.[19]

Antibodies to the TSH receptor are demonstrable in a small proportion of patients with Hashimoto's thyroiditis.[80] Some of these antibodies prove to be stimulatory and, thus, can be categorized as thyroid stimulating. When such antibodies are found in patients who are either euthyroid or hypothyroid, associated with the clinical picture of Hashimoto's thyroiditis, it may be presumed that they would be hyperthyroid if there was sufficient thyroid parenchyma to respond to this antibody, which is considered to be the direct cause of Graves' disease. Some of the antibodies to the TSH receptor found in these patients, however, are not stimulatory in nature, and some even inhibit the effects of TSH and thus contribute to hypothyroidism.[20, 44] Antibodies to other cell membrane components have likewise been identified in Hashimoto's thyroiditis.

In addition to the aforementioned thyroid autoantibodies, immune complexes are found in about a third of patients with this disorder.[79] Lymphocytotoxic antibodies are likewise found in a significant minority of patients with autoimmune thyroid disease.[80] Autoantibodies to certain other organs are found more often than would be expected by chance association alone, e.g., antibodies to gastric constituents, islets of Langerhans, and adrenal cortex.[80]

NATURE OF THE THYROID CELLULAR DAMAGE

Destruction of thyroid cells (see Fig. 11–1), a common occurrence in Hashimoto's thyroiditis, usually involves one of three mechanisms.[80] Firstly, antibodies directed against the cell membrane constituents or "microsomal" antibodies can attract nonspecific killer lymphocytes to the site, thus inducing antibody-dependent cellular cytotoxicity. Secondly, antigen-antibody complexes on the cell surface membrane may activate complement, and cellular damage may occur as a result of this complement cascade. Thirdly, the specific sensitized cytotoxic T lymphocytes interacting with their complementary antigen on the thyroid cell membrane may directly cause cytolysis. Finally, as noted previously, a less common cause of hypothyroidism may be due to the blocking of TSH from access to its receptors by means of a thyrotropin receptor antibody, and this form of hypothyroidism occurs without cellular damage.[20, 44] Graves' disease, conversely, is caused by another thyrotropin receptor antibody, namely thyroid stimulating antibody, which mimics the action of TSH but is not subject to biologic feedback that governs that hormone.[70, 80] Thyroid growth–promoting antibody may promote the growth of the gland, without inducing hyperactivity,[18] and conversely a thyroid growth–inhibiting antibody may inhibit the growth of thyroid cells and thus be a factor in inducing atrophic thyroiditis.[19]

ABNORMALITIES OF THYROID FUNCTION

Abnormalities of thyroid function may be seen in patients with autoimmune thyroiditis, varying in severity and frequency.[4, 27] In most patients, the 24-hour thyroidal ^{131}I uptake is within normal limits.[4, 27] However, in about 15 to 20% these values are low, suggesting hypothyroidism,[30, 84] whereas in other patients the values are *above* normal.[4, 27, 49, 68] In most instances, the ^{131}I uptake will be suppressible with thyroid hormone administration, but in about 10% of patients it will not be suppressible, owing to the concomitant presence of thyroid stimulating antibodies.[27] Not uncommonly, early ^{131}I uptake results at the 2- to 6-hour stage are increased and may exceed the 24-hour value, owing to defective organification of the iodide.[9, 57] Indeed, using a potassium perchlorate "flushing" test, about 25 to 40% of patients with Hashimoto's thyroiditis manifest a positive discharge response following the administration of potassium perchlorate; this response indicates a defective iodide–iodine organification process.[9, 57, 69, 72] In addition to this defect in organification of iodide, patients with this condition may have an increased ra-

tio of monoiodotyrosine/diodotyrosine (MIT/DIT) within the thyroid gland as well as an increased thyroidal T_3/T_4 ratio. A reduction in the intrathyroidal iodide pool has been demonstrated.[4] Decreased responsiveness to exogenous TSH indicates decreased thyroidal reserve.[28, 30] There is often an increased secretion of nonmetabolically active iodoproteins, and this may be partially caused by the secretion of thyroglobulin and iodoalbumin.[13, 76, 77] The greater sensitivity to iodide has been mentioned,[7, 9, 48, 57] and there is also increased sensitivity to lithium.[47] Autoimmune thyroiditis is frequently associated with reduced biosynthesis of thyroid hormones, resulting in hypothyroidism,[4, 27, 84] and with incipient thyroid failure. The first and most sensitive indicator is an elevation of TSH *before* there is any decline in the thyroid hormone concentrations in the blood (i.e., compensated hypothyroidism).[8] If the lesion progresses the serum thyroxine level falls first, and serum T_3 may remain normal until thyroid failure becomes severe.[46]

CLINICAL ASPECTS

There is a wide variation in clinical presentation encountered in this disorder.[4, 27, 30, 48] Some patients may have no clinical signs or symptoms whatever, and a diagnosis may be made only by inadvertently testing for thyroid function or thyroid autoantibodies, by screening in geriatric institutions, or by finding lymphocytic infiltration in the gland unexpectedly at operation or autopsy. Other patients may be found to have enlarged thyroid glands as their only clinical manifestation.[4, 27] At the opposite extreme patients may present with overt evidence of thyroid dysfunction, usually hypothyroidism.[4, 27, 49, 78] As mentioned, females predominate in this disorder (4:1).[4, 27, 49] Either iodide[7, 9, 69] or lithium[47] ingestion can unmask occult thyroiditis rendering it overt with goiter, thyroid antibodies, or both with or without thyroid dysfunction.

Pressure symptoms within the neck are occasionally encountered; there may be some mild discomfort, hoarseness, dysphagia, or cough, although most commonly there are no local symptoms in the neck. Moderate pain occurs infrequently, but severe pain reminiscent of subacute thyroiditis is seen in about 5% of patients, and in these the gland may be correspondingly tender.[16, 21, 78]

Hyperthyroidism may occur in autoimmune thyroiditis and may be of two types.[4, 16, 80] *The first* of these is due to Graves' disease, occurring concomitantly with Hashimoto's thyroiditis.[15, 16, 26, 80] This condition clearly is a combined disorder and is indistinguishable from Graves' disease with the exception of the firmness of the goiter and the high titers of the thyroid antibodies. Some patients with euthyroid Hashimoto's thyroiditis have exophthalmos,[3, 11, 51, 52, 80] with or without thyroid stimulating antibodies. It may be noted that when thyroid stimulating antibodies are present in a patient with Hashimoto's thyroiditis, the thyroid status will depend on the state of the parenchymal integrity (i.e., the number of remaining intact thyroid follicular cells); this factor will determine whether the patient is hyperthyroid, euthyroid, or hypothyroid.[80]

The second form of hyperthyroidism may occur transiently in the initial phase of Hashimoto's thyroiditis, once again simulating subacute thyroiditis.[16, 21] This form may or may not be associated with a painful thyroid gland. In this condition, the hyperthyroid phase would appear to be due to discharge of preformed thyroid hormone, resulting from the inflammatory process; this uncommon condition must occur acutely and severely enough to permit rapid elaboration of the excessive amounts of thyroid hormone so as to manifest a hyperthyroid phase. The form of "painless" thyroiditis seen in the postpartum period (postpartum thyroiditis)[1] is, at least in most instances, a form of autoimmune thyroiditis.[1, 2]

Many, perhaps 25%, of patients will present with clinical hypothyroidism (Fig. 11–4), but the percentage of all cases is difficult to determine, owing to the very large number of asymptomatic patients.[32, 45] Of course, an atrophied thyroid gland as an expression of autoimmune thyroiditis is commonly associated with hypothyroidism; less commonly, goitrous Hashimoto's thyroiditis is also associated with hypothyroidism, usually less severe than in the atrophic form.[15, 16] The most common cause of spontaneous compensated hypothyroidism, characterized with normal plasma concentrations of thyroid hormones but elevated TSH values, is indeed autoimmune thyroiditis; patients are *clinically* euthyroid with this condition.[45, 74]

The thyroid functional state may vary considerably with time in this disorder. Most characteristically, patients may remain euthyroid for years, although about 10% of those presenting with goiter and high titers of thyroid

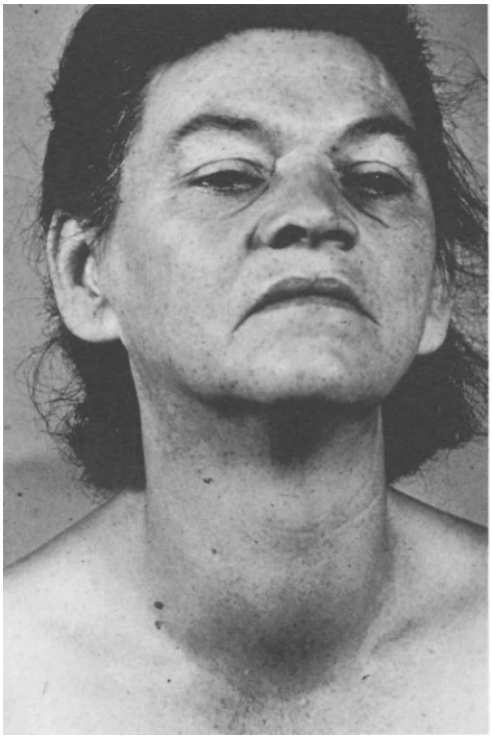

Figure 11–4. Patient with Hashimoto's thyroiditis and hypothyroidism.

autoantibody ultimately become hypothyroid.[45] Some patients may even remit completely, and those patients either may remain clinically normal throughout their life or may suffer a recurrence of clinical abnormalities.[14, 45, 80]

Patients who have Hashimoto's thyroiditis and even severe hypothyroidism may ultimately recover and may even develop hyperthyroidism due to Graves' disease.[80] Conversely, hyperthyroidism due to Graves' disease may ultimately culminate spontaneously as hypothyroidism secondary to autoimmune thyroiditis.[80] Thus, a patient with a given thyroid status may not remain stable when autoimmune thyroiditis is encountered.[14]

In most patients with goitrous Hashimoto's thyroiditis, the goiter is usually diffusely, but not necessarily symmetrically, enlarged and may vary in size and degree of enlargement. It is characteristically associated with increased firmness and bosselation without clearly distinguishable thyroid nodules.[4, 16] The gland is only occasionally tender, and bruits are not heard. The relationship of autoimmune thyroiditis to thyroid malignancy is discussed subsequently.

An enlarged thymus gland is frequently found in autoimmune thyroiditis and may be important in relation to the pathogenesis of the disorder.[4, 55, 80] It may also be noted that autoimmune thyroiditis may be associated with other organ-specific autoimmune diseases, such as insulin-dependent diabetes mellitus, pernicious anemia, Addison's disease, vitiligo, and others, in the patient or the relatives.[80] Indeed, there is often a family history of autoimmune thyroid disease, so that relatives should always be tested for this disorder.

As mentioned, there is an increased incidence of autoimmune thyroiditis in the older population.[32, 85, 91] Because of the frequent subtleness of expression of this disorder, there has been some suggestion that screening procedures in geriatric institutions might be useful to detect mild or occult forms of autoimmune thyroiditis.[85]

In children or adolescents, juvenile lymphocytic thyroiditis is the cause of diffuse euthyroid goiter in about two thirds of instances.[31, 48, 58] Thyroid autoantibodies are not increased as much as in adults, and often the diagnosis can be verified only by biopsy.[50] Thyroid autoantibodies cross the placenta freely; it has been suggested that the passive transfer of thyroid autoantibodies could produce congenital transient hypothyroidism.[56, 60] However, most infants with congenital hypothyroidism do not have these antithyroid antibodies, and most infants born to mothers with high titers of thyroid autoantibodies are normal; it would seem that there is no lasting effect from the transplacental transfer of the "conventional" thyroid autoantibodies.[80] Transient hypothyroidism resulting from the placental transfer of a TSH receptor antibody, which blocked the effect of TSH, has been reported in newborns.[53] Further, in some cases of congenital athyreotic hypothyroidism, thyroid growth–inhibiting antibodies have been found.[75]

RELATIONSHIP TO SILENT (PAINLESS) THYROIDITIS AND POST-PARTUM THYROIDITIS

The relationship of autoimmune thyroiditis to silent (painless) thyroiditis is dealt with in subacute thyroiditis in Chapter 10. Suffice it to say that I consider silent thyroiditis to be a disorder of heterogeneous causation, and in many instances the disorder does not appear to be of autoimmune origin.[80] Most of the cases that occur *without* relationship to the post-partum period, do not have serologic evidence of thyroid autoantibodies despite having lymphocytic infiltration within the thyroid

gland reminiscent of Hashimoto's thyroiditis.[17] Moreover, the histologic appearance of the thyroid returns to normal after several months.[39] However, the circumstances are very different in post-partum thyroiditis.

Pregnancy and delivery are now known to influence the clinical course of autoimmune thyroid disease.[1, 2] Changes in serum thyroid antibodies and thyroid stimulating antibody are common during pregnancy and delivery in Graves' disease and in autoimmune thyroiditis; both of these types of antibodies decrease as pregnancy progresses and sometimes test results for those antibodies are negative in the third trimester. Of course, in some patients, these antibodies persist throughout the pregnancy. Following delivery, however, the antibodies increase again, reaching peaks 3 to 4 months post-partum in more than half of the patients. In some, antibodies develop *de novo* following delivery. Similar transient increases in antibodies are observed after spontaneous and therapeutic abortions. These changes appear to be induced by physiologic and immunologic changes occurring during pregnancy and delivery.

There have now been several studies of painless thyroiditis that appears in the post-partum interval (post-partum thyroiditis), both in Japan and in North America.[1, 2] As many as 5 to 8% of all women who deliver have been estimated to suffer from this condition. It is frequently associated with a hyperthyroid phase, often subsequently progressing to a hypothyroid phase then on to complete or incomplete recovery. The hyperthyroid phase is characterized by proportionate rises in both serum T_4 and T_3 which is unlike the usually disproportionate rise in serum T_3 in Graves' disease, and by a very low 24-hour ^{131}I thyroidal uptake of 0 to 2%. Goiters often decline in size but may persist permanently; most patients manifest thyroid autoantibodies sometimes in high titers. These may also decline or disappear, or test results may be negative at all times throughout the course. It would appear that post-partum thyroiditis may be due to the transient rebound of autoimmune processes following cessation of pregnancy.[1, 2] Many of these patients who go into remission spontaneously have recurrences following later pregnancies, which may ultimately culminate in chronic thyroiditis.

ASSOCIATION WITH THYROID MALIGNANCY

While in the past surgery has been proposed for Hashimoto's thyroiditis on the basis that the incidence of histologic thyroid carcinoma is significant in such cases, this has never been conclusively verified.[4, 20, 27, 49, 80] Indeed, it is quite possible that the focal thyroiditis seen in many patients with thyroid malignancy is actually a secondary immune response to the malignancy itself. Conversely, clinical thyroid carcinoma is not observed in increased frequency in patients with diffuse goitrous Hashimoto's thyroiditis. There is little evidence at present to suggest that Hashimoto's thyroiditis is a precarcinomatous lesion.

Lymphoma of the thyroid, a rare condition, is increased in incidence in coexistent Hashimoto's thyroiditis.[20, 33, 40, 49, 87] The precise incidence of lymphoma of the thyroid in relation to Hashimoto's thyroiditis is unclear, as the proportion has not been systematically calculated and is obviously uncommon, even in this group. However, the finding of Hashimoto's thyroiditis in association with malignant lymphoma of the thyroid is very common. The conclusion is almost inescapable that the autoimmune process of Hashimoto's thyroiditis can occasionally evolve in some manner into an immunologic neoplasm.

DIAGNOSIS

The diagnosis of autoimmune thyroiditis is frequently not made unless the patient presents with overt manifestations. Many patients are asymptomatic and are unaware of the presence of the goiter. In the atrophic form of the disease, there patently is no goiter and if these patients are clinically euthyroid, diagnosis is exceedingly difficult to make, and often the disease is only discovered inadvertently or at necropsy. Alternatively, if an asymptomatic, diffuse, firm, bosselated goiter or lobulated goiter is discovered, the suspicion of thyroiditis is certainly appropriate. Even soft goiters may prove on biopsy to be caused by lymphocytic thyroiditis; conversely, even frankly nodular goiters may also be caused by this disorder. Thus, an ultimate diagnosis is based on biopsy of the thyroid gland; a Tru-cut or Silverman needle biopsy will provide the most information of the histologic architecture and is readily done in a patient with a large, firm goiter. In comparison, such procedures are scarcely necessary from the viewpoint of clinical management and cannot be advised in the vast majority of cases. Fine needle aspiration biopsies, while much simpler, provide only cytologic material; this sometimes can be confusing in

cases of Hashimoto's thyroiditis, since atypia is occasionally encountered.[68] However, characteristically, large numbers of lymphocytes, Hürthle cells and plasma cells are highly suggestive of autoimmune thyroiditis in such preparations.[86] Even this procedure is not necessary in the assessment of most patients in whom a presumptive diagnosis rather than an "ultimate" diagnosis would suffice. Thyroid autoantibody values have been utilized as diagnostic aids. When titers of antimicrosomal, antithyroglobulin, or both antibodies are found in dilutions of 1:2000 or above, then clearly one is dealing with autoimmune thyroid disease. However, even low titers of these thyroid autoantibodies may be associated with at least focal infiltrations of lymphocytes within the thyroid gland.[10, 82] Conversely, even diffuse Hashimoto's thyroiditis may be associated with lower titers of thyroid autoantibodies than anticipated, and a small proportion of patients with classic goitrous Hashimoto's thyroiditis will have no detectable thyroid autoantibodies.[16, 50] Moreover, 30% of patients with atrophic myxedema will have no detectable thyroid autoantibodies[15, 16]; as mentioned previously, children and adolescents with lymphocytic thyroiditis often have low or no detectable thyroid autoantibodies.

Conversely, thyroid autoantibodies in low titers may be found in many thyroid disorders other than autoimmune thyroiditis, so that the finding of such antibodies is not diagnostic of primary forms of thyroiditis.[15, 16] It may also be mentioned that the finding of antimicrosomal antibody is much more frequent than that of antithyroglobulin antibody; the titers are higher in the former, and there is a closer relationship to thyroid dysfunction.[80]

The demonstration of clinical, biochemical, or both types of hypothyroidism will itself at least suggest the likely possibility of autoimmune thyroiditis, since it is the commonest cause of acquired spontaneous hypothyroidism in most Western countries. When "compensated hypothyroidism" (normal T_4 and T_3 with high TSH levels) is detected with or without thyroid autoantibodies in middle-aged or older people, the diagnosis may be presumed to be autoimmune thyroiditis unless other causes for this syndrome are apparent.[45]

Other abnormalities in thyroid function are nonspecific but may be of some use in a diagnostic process. The detection of defective organification of iodide as manifested by a higher early uptake value compared with a lower 24-hour uptake, or alternatively a positive result from the potassium perchlorate "flushing" test would certainly be compatible with autoimmune thyroiditis, although these procedures are not commonly performed and are not specific.[4, 9, 27, 32, 57, 69, 72] The factors are also true for the finding of an abnormal butanol-insoluble iodoprotein, which is likewise unnecessary, rarely sought, and nonspecific.[13]

Practically, the observation of a patient with a diffuse goiter associated with moderate or high titers of thyroid autoantibodies, with or without any degree of hypothyroidism, would permit a presumptive diagnosis of autoimmune thyroiditis; no further definitive diagnostic procedures, such as biopsy, need to be performed. If a diffuse goiter is encountered without hypothyroidism and without antibodies, then one can suspect autoimmune thyroiditis, but without a biopsy a diagnosis cannot and need not be established, since it does not influence the management of the patient in any significant fashion. Biopsy procedures cannot be justified in patients with clearly benign lesions in whom decisions about treatment can be readily made without a definitive diagnosis. Many euthyroid goiters will fall into this category and should occasion no concern. Only when there are distinct nodules or rapid growth or irregularity of the goiter, unusual hardness or fixation to adjacent structures, or local symptoms within the neck, will it be quite important to establish a firm histologic diagnosis in a patient in whom the thyroid functions tests and thyroid autoantibodies do not direct one towards a diagnosis. Indeed, it must be emphasized that solitary benign or malignant nodules may occasionally coincide and coexist with autoimmune thyroiditis, so that the presence of such nodules, despite high thyroid autoantibody titers, must lead to some further suspicion and investigation.[4, 20, 27, 49]

TREATMENT

Since the evidence is overwhelming that autoimmune thyroiditis is due to an immune process, one might expect that rational therapy would include immunosuppressive treatment. Indeed, corticosteroid therapy will cause rapid regression of goiters in this disorder but this is of theoretic interest only.[5, 41] In view of the serious nature of the side effects of such agents and, conversely, the ease by which autoimmune thyroiditis can be treated with thyroid hormone suppression and replacement, corti-

costeroid or immunosuppressive therapy is unwarranted.

Thyroxine therapy is certainly necessary in all patients with the hypothyroidism that accompanies autoimmune thyroiditis.[4, 15, 16, 20, 32, 78] The treatment of the euthyroid, asymptomatic patient is not quite so clear-cut. In the presence of a goiter, it is often possible to cause regression in the Hashimoto's gland by the administration of thyroxine.[15, 16] On the one hand, such regression is variable in degree but may be quite marked; on the other hand, some goiters will not regress and some will continue to expand despite thyroxine therapy.[15, 16] Such further enlargement has been ascribed to the presence of thyroid growth–promoting antibodies.[18] When such thyroid enlargement occurs in Hashimoto's goiter, despite thyroxine therapy, it is also necessary to consider the rare possibility of a concomitant thyroid lymphoma, which has arisen in the context of Hashimoto's thyroiditis.[20, 33, 47, 49]

It appears that only about 10 to 15% of patients with euthyroid Hashimoto's thyroiditis go on ultimately to hypothyroidism.[45] In contrast, some cases of Hashimoto's thyroiditis may spontaneously remit.[14, 62, 80] Thus, there is not absolute direction as to whether one should utilize thyroxine indefinitely in euthyroid patients with Hashimoto's thyroiditis. A practical suggestion would be that if patients are not being seen frequently by physicians aware of all the vicissitudes of this disorder, it would seem that the most reasonable approach is to maintain such patients on thyroxine therapy indefinitely. Hypothyroidism in the elderly may be a subtle, surreptitious, easily missed and dangerous disease, and thus this approach seems to offer the most benefit to the most patients. Adverse effects from thyroxine in physiologic replacement dosages will occur primarily in those patients who suffer concomitant coronary artery disease and angina pectoris.[10] In patients with such disorders, the dose should be reduced to suboptimal levels.[81]

In young persons with autoimmune thyroiditis, thyroxine can be prescribed initially at full maintenance replacement or suppressive dosages (0.10 to 0.15 mg/day) without adverse effects.[8] In middle-aged or older persons, however, the initial dosage should be smaller with gradual increments until full replacement is reached. In the elderly, one should commence thyroxine in dosages in the order of 0.025 mg/day and increase cautiously at monthly intervals. One should not attempt to reach dosage levels beyond 0.1 mg/day in such elderly persons even if there is no evident cardiovascular disease.[10, 81]

Testing patients on thyroxine therapy is not completely straightforward. The estimation of serum thyroxine level is *not* the most appropriate test with which to monitor exogenous thyroxine therapy, and the level is often moderately elevated under these circumstances. One can utilize the TSH determination to indicate when the patient is taking enough thyroxine, but the total serum triiodothyronine by radioimmunoassay (T_3 RIA) procedure is the best test to determine whether the patient is receiving too much thyroxine.[89] In the vast majority of instances, dosage levels between 0.10 and 0.15 mg of thyroxine daily will yield values for serum triiodothyronine well within the normal range.[89] The TSH values should be kept in the lower part of the normal range by a sensitive TSH assay, for adequate goiter suppression.

Thyroid hormones may have some effect on the immune process although the histology of the gland seems to remain stable despite this therapy.[42, 65] However, a decline in thyroid autoantibodies often accompanies regression of the thyroid enlargement.[15, 16, 42, 65]

Surgery was recommended in some medical centers in a previous era because of the reportedly high incidence of coincidental thyroid malignancy.[12] It is generally accepted, however, that such risk has been vastly overrated.[4, 20, 81] Only when there appears to be a particular indication for surgery, such as further enlargement despite thyroxine therapy or the presence of a nodule which should be managed as any other thyroid nodule, should surgical treatment be considered.

If hyperthyroidism coexists, it should be treated appropriately. Thyroidectomy or radioactive iodine therapy appears to be associated with an increased risk of hypothyroidism in patients with the combined disorder when compared with patients with uncomplicated hyperthyroidism without associated thyroid autoantibodies.[40]

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12
Invasive Fibrous (Riedel's) Thyroiditis

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Invasive fibrous (Riedel's) thyroiditis may be defined as a disorder of unknown etiology in which aggressive fibrosis not only replaces part or all of the normal structure of the thyroid gland but also characteristically spreads across the thyroid capsule to invade adjacent structures, such as nerves, blood vessels, and muscles.[5, 28, 31, 36, 59, 63, 64] The initial description was by Riedel[48] in 1896; he described a chronic sclerosing thyroiditis that primarily affected women, frequently caused pressure symptoms in the neck, and tended to progress ultimately to complete destruction of the thyroid gland. In his reports, Riedel described a "specific inflammation of mysterious nature producing an iron-hard tumefaction of the thyroid."[48–50]

The term chronic invasive fibrous thyroiditis has a few synonyms, including Riedel's struma, struma fibrosa, ligneous (Eisenharte) struma, chronic fibrous thyroiditis, and chronic productive thyroiditis.[5, 24, 36, 59]

INCIDENCE

The condition is considered very rare. Its incidence has been reported as between 0.03 and 0.98% of thyroidectomies in numerous surgical series when the operations were performed for a variety of reasons.[13, 20] Experience at the Mayo Clinic between 1920 and 1984, when more than 56,700 thyroidectomies were performed, showed 37 cases of invasive fibrous thyroiditis identified among the 3.5 million registered patients.[26, 27, 63, 64] The operative incidence was 0.06%, and the overall incidence in outpatients at the Mayo Clinic was 1.06 per 100,000. The incidence among the thyroidectomies at the Mayo Clinic is almost certainly much higher than in the population at risk with goiters, since it may be expected that the manifestations of Riedel's struma compared with other types of goiters are much more likely to lead to surgical intervention.

ETIOLOGY

The etiology remains unknown. A generation ago, chronic lymphocytic thyroiditis was considered to be an earlier stage of invasive fibrous thyroiditis.[16, 18, 47, 62] However, patients with lymphocytic thyroiditis, when followed for many years, almost never show progression to Riedel's struma[19, 21, 22, 30, 52]; there have been two instances where this type of progression seems to have occurred.[6, 31, 32] However, a

patient who presents with Riedel's thyroiditis does not have a history of Hashimoto's thyroiditis.[59] It now seems evident that the two entities are separate.[5, 36, 55, 56]

Thyroid function is usually within normal limits in this disorder, unlike frequent hypothyroidism in lymphocytic thyroiditis. While thyroid autoantibodies have been considered to be absent or present in only low titer in Riedel's struma, which is in contradistinction to the findings in Hashimoto's thyroiditis,[58] a report from the Mayo Clinic indicates that appreciable titers of these antibodies may be found in 45% of the patients who have been tested for their presence.[26, 27] Morphologically, there are numerous dissimilarities (see following discussion).[25, 28, 63, 64]

Subacute nonsuppurative thyroiditis has also been suggested as a possible precursor of Riedel's struma.[60] However, these two disorders appear to be quite separate, aside from rare exceptions.[8] Preservation of the thyroid capsule in subacute thyroiditis is in marked contradistinction to the capsular invasion in chronic fibrous thyroiditis.[11, 12, 14] With the exception of the patient described by Chopra and coworkers,[8] no other instance of a transformation of subacute thyroiditis into Riedel's struma has been reported. Patients with Riedel's struma almost never have histories of the type of pain in the region of the thyroid that is observed in subacute thyroiditis.[5, 36] The histologic appearances of the two disorders are quite different (see subsequent discussion).[5, 28, 63, 64]

There is no adequate explanation for this type of fibroblastic proliferation. Riedel's struma, however, has been described in association with extracervical fibrosclerosis, as first reported by Bowlby[7] in 1885. Since 1962, the association has been extended to include salivary gland fibrosis,[29] fibrous mediastinitis,[11, 26, 27, 46] retroperitoneal fibrosis,[1, 4, 5, 9, 23, 31, 40, 42, 45, 48, 49, 63, 64] sclerosing cholangitis,[4, 9] pseudotumor of the orbits,[1, 2, 9, 36, 58, 59] and lacrimal gland fibrosis.[53] Of 37 patients with invasive fibrous thyroiditis at the Mayo Clinic who have been followed for a mean period of 10 years, 12 demonstrated extracervical fibrosclerosis located in the orbit, the mediastinum, or the abdomen.[26, 27] For these reasons, it has been proposed that these apparently disparate fibrotic lesions may be different manifestations of the same generalized fibrosing disease.[5, 36] In addition, the vascular process observed in Riedel's struma has been held to resemble the lesion described in Takayasu's arteritis.[43] Although there has been a suggestion that Riedel's thyroiditis could be the result of a generalized process such as a collagen disease,[36, 44] this is entirely speculative. Similarly, any suggestion that this disorder is a result of autoimmune processes is equally speculative, and there is no evidence for such a notion.[26, 27, 34, 59] Suggestions that the lesion may represent a response to an atypical fungal infection or to ergot-like drugs have no clear-cut basis to support them.[36] Finally, it has been noted that there are frequently benign follicular adenomas of the thyroid in the center of the Riedel's lesion.[5, 11, 28, 34, 36, 63, 64] The relationship of these adenomas to the Riedel's struma is unclear and occurs in about 25 to 50% of instances.

CLINICAL FEATURES

The reported age incidences are variable with a range from 23 to 78 years.[5, 11, 15, 17, 26, 63] Most cases are diagnosed in the fourth to sixth decades with an average age of about 50 years.[26, 63] A female preponderance has been noted with ratios of 2:1 to 4:1 (Table 12–1).[5, 11, 26, 36, 63, 64]

Whereas goiter may have been present for several months before the onset of symptoms in many instances, gradual or sudden enlargement of the thyroid precipitates the local symptoms within the neck.[5, 11, 26, 27, 35, 36, 59, 63] These usually consist of a marked sense of pressure or severe dyspnea, and symptoms may be out of proportion to the size of the goiter.[5] Patients often complain of feelings of suffocation, cough, and dysphagia.[10, 30, 50] Recurrent laryngeal nerve palsy with hoarseness has been reported.[28] Pain is rarely a major complaint, although the sense of pressure may be inappropriately described as pain by the patient.[26, 27]

The presence or degree of obstruction varies with the extent to which the surrounding structures have been invaded.[33] Some patients experience only mild and infrequent symptoms with minimal dysphagia and dyspnea. Others, with more severe compression of the trachea,[33, 52] may have stridor, severe dyspnea, or even attacks of suffocation.[10, 30, 32, 50]

There is usually no fever or systemic manifestations except in those few patients who have such widespread thyroid involvement that hypothyroidism results. Where there is extracervical fibrosclerosis, symptoms may ensue resulting from those areas of fibrous infiltration.

On physical examination the thyroid is of

Table 12–1. Clinical Features*

	Riedel's Disease	Subacute Thyroiditis	Hashimoto's Thyroiditis
Age incidence	30 to 70 years (most 50 years or over)	Any age (most 30 to 50 years)	Any age (most 20 to 50 years)
Sex incidence (F/M)	2 to 4/1	~ 4/1	4 to 10/1
Symptoms	Pressure, goiter	Pain, tenderness, goiter	± Goiter; hyperthyroid, euthyroid or hypothyroid
Thyroid involvement	Unilateral, 30%	Bilateral (one side may be more affected)	Focal or diffuse
Thyroid antibodies	None or very low	±	+
Follow-up	Hypothyroidism rare; may recur following treatment, stabilize, or regress	Thyroid function reverts to normal in almost all cases	Usually progresses to hypothyroidism

*From LiVolsi, V. A.: Riedel's struma. In: LiVolsi, V. A. and LoGerfo, P.: Thyroiditis. Boca Raton, CRC Press, 1981, with permission.

variable size and may even be small.[36, 59] The lesion may be limited to one lobe or may be present in both. The involved area is of a stony, hard consistency and densely adherent to adjacent cervical structures.[5, 24, 48–50, 63, 64] It has a harder consistency than carcinoma and is rarely tender. While adjacent lymph nodes are only occasionally enlarged,[15, 17] when they are present and associated with the hard thyroid mass, a diagnosis of carcinoma is often suspected.

As previously mentioned, manifestations of hypothyroidism are occasionally present. In two instances, hypoparathyroidism developed as well.[3, 8, 11, 36] It should be reemphasized that because there may be fibrosclerosis elsewhere in the body, the examination must include careful search for such associated disorders.

LABORATORY FINDINGS

Usually the patient is clinically euthyroid and the thyroid function test results provide correspondingly normal results.[5, 36, 38, 59] Only occasionally will thyroid function test findings indicate hypothyroidism.[8, 30, 64] Thyroid autoantibodies are usually absent or present in low titers,[5, 36] although, as mentioned, Hay and associates[26, 27] have reported that as many as 45% of patients have significant titers of thyroid autoantibodies. Thyroid scintiscans will show "cold" areas, corresponding to the extent of the lesion.[31, 38, 59]

The white blood cell count may be normal or elevated, and the sedimentation rate is usually elevated although not to the high rates in subacute thyroiditis.[5, 36]

PATHOLOGY

Characteristically, the fibrosing process involves part or all of the thyroid lobe, may be unilateral or bilateral, and has been described as woody or very hard.[5, 11, 21, 22, 24, 36, 39, 48–50, 63, 64] As mentioned previously, extension of the fibrosis beyond the capsule of the thyroid into adjacent structures is a characteristic feature. There are no tissue planes, making surgical extirpation very difficult. An adenoma may be found at the center of the fibrous mass.[5, 36] Isolated thyroid amyloidosis has been described in one case of Riedel's struma.[37]

Microscopic criteria for the diagnosis of Riedel's struma have been established by Woolner and associates.[63, 64] These include complete destruction of the involved thyroid tissue with absence of normal lobulation; lack of granulomatous reaction; and extension of the fibrosis beyond the thyroid into adjacent tissues, surrounding nerves, blood vessels, fat, and skeletal muscle. Lymphocytes and Hürthle cells are sparse, contrasting with Hashimoto's thyroiditis. An associated arteritis and phlebitis are observed with intimal proliferation, medial destruction, adventitial inflammation, and frequent thrombosis (Table 12–2).[5, 28, 51, 63, 64]

Similar features may be observed in the extracervical fibrosclerotic lesions, in the retroperitoneal or mediastinal regions, in the orbit or lacrimal glands, or in cholangitis.[5, 36, 63, 64]

DIAGNOSIS AND TREATMENT

Invasive fibrous thyroiditis may appear as a painless, fixed, hard goiter with either slow or rapid growth. Whether this is associated with local lymphadenopathy or not, it may be impossible to differentiate this disorder from carcinoma of the thyroid on the basis of clinical findings alone.[5, 36, 59] The disorder can be more readily distinguished from Hashimoto's thyroiditis or subacute thyroiditis as noted on

Table 12–2. Pathologic Features*

	Riedel's Disease	Fibrosing Hashimoto's Thyroiditis	Subacute Thyroiditis	Anaplastic Carcinoma	Fibrosarcoma
Gross					
Color	White	White-tan	Grey-white	White-tan	Tan
Consistency	Very hard, woody	Firm	Smooth	Fleshy, hard	Fleshy, firm
Lobulated	No	Yes	Yes	No	No
Extrathyroid	Yes	No	No	Yes	Yes
Extent necrosis	±	No	No	Common	Yes
Microscopic					
Adenoma	Yes (25 to 50%)	No	No	Yes (or low-grade carcinoma) common	N/A
Colloid	Normal in uninvolved areas	Depleted	Usually decreased	N/A†	N/A
Oxyphils	No	Yes	±	N/A	N/A
Lymphocytes	Sparse	Marked	Yes	N/A	N/A
Squamous metaplasia	No	Yes	Rare	N/A	N/A
Stroma	Fibrosis	Fibrosis	Fibrosis	N/A	N/A
Giant cells	No	No	Yes	Yes	±
Pleomorphism of cells	No	No	No	Marked	± to marked
Mitoses	No	No	No	Yes	± to Yes (depending on degree of differentiation)
Blood vessels	Vasculitis	Adventitial fibrosis may be seen	Adventitial fibrosis may be seen	N/A	N/A

*From LiVolsi, V. A.: Riedel's struma. In: LiVolsi, V. A. and LoGerfo, P.: Thyroiditis. Boca Raton, CRC Press, 1981, with permission.
†N/A = Not applicable.

Tables 12–1 and 12–2. Hashimoto's thyroiditis is not associated with any extension of the lesion beyond the capsule; the goiters are usually larger and lobulated; and the antibody titers are generally, markedly elevated.[59] Subacute thyroiditis is associated with severe pain and tenderness, frequent fever, and a rapidly evolving course. There is no extension of the lesion beyond the capsule.[14, 59] Closed biopsy findings in this condition are often difficult to interpret,[5, 61] although open biopsy findings are useful.[5, 36]

Whereas surgical intervention is indicated in Riedel's struma, the possibility of chronic fibrous thyroiditis must be kept in mind to limit the event of surgery if no malignancy is found. Surgical intervention is indicated on two grounds as follows: (1) to exclude carcinoma and (2) to relieve tracheal compression.[5, 36, 59] Operation is limited to excising a wedge of thyroid isthmus when the process is diffuse. Extensive resection is not indicated, particularly since Riedel's struma tends often to be benign and self-limiting. Moreover, such a procedure may add considerable risk of injury to adjacent involved vital structures within the neck, such as the carotid artery and recurrent laryngeal nerve.[5, 36, 59] Subtotal lobectomy may be performed, however, if the process is localized to one lobe of the thyroid gland.

After surgery, the disease sometimes subsides or takes a benign self-limiting course.[5, 36, 54] However, recurrences and progression have been reported, and the mortality rate varies from 6 to 10%. Spontaneous remissions without surgery may occur, and secondary surgery is only rarely required.[5, 36, 59]

Corticosteroids have been utilized in the treatment of this disorder but appear to be of variable value.[29, 31, 57] Thyroid hormone suppressive therapy has also been utilized and, although adequate assessment has not been carried out, it seems unlikely that this therapy will add much to the management of this rare disease.[59]

PROGNOSIS

As mentioned, the course of the lesion may be slowly progressive, may stabilize, or may

remit. Following surgery, there is a 16% recurrence rate.[15] The mortality rate ranges from 6 to 10%, and death is usually due to asphyxia secondary to tracheal compression or laryngospasm.[5, 36] Hypothyroidism is relatively uncommon,[3, 8, 30, 64] and hypoparathyroidism is rare.[8, 11] In many instances, the condition is self-limiting and improvement often persists after a wedge isthmic resection.[5, 36] The disorder may be further complicated by the occurrence of fibrotic lesions elsewhere in the body, as mentioned.

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13
Graves' Disease

ROBERT VOLPÉ

HYPERTHYROIDISM (PREAMBLE)

Hyperthyroidism may be defined as a group of disorders resulting from excessive tissue and circulating concentrations of thyroid hormones.[294] However, this definition is not adequate for three reasons. First, some of the conditions to be described are quite complex and have extrathyroidal manifestations. Secondly, hyperthyroidism may result from a variety of different causes. Thirdly, there is one condition characterized by excessive levels of thyroid hormone in tissues and blood, in which the patients are certainly *not* hyperthyroid and rather may be clinically hypothyroid or euthyroid; this is the rare condition of *resistance* to thyroid hormones (Refetoff's syndrome).[238] Thus not only does the thyroid hormone have to be excessive in amount, but cellular function has to be normal so as to permit *the action* of the hormones.

The causes of hyperthyroidism are listed in Table 13–1.

Graves' disease is the most common cause, but in Canada 15% of cases of hyperthyroidism will be caused by some form of thyroiditis.[322] Toxic nodular goiter accounts for only 5%. In

Table 13–1. Causes of Hyperthyroidism (Preamble)*

1. Graves' disease (Basedow's disease, Parry's disease, toxic diffuse goiter, autoimmune hyperthyroidism)
2. Toxic nodular goiter (toxic adenoma, Plummer's disease) or multinodular toxic goiter
3. The hyperthyroid phase of thyroiditis (subacute thyroiditis, painless thyroiditis, post-partum thyroiditis, or Hashimoto's thyroiditis)
4. Hyperthyroidism due to excessive human chorionic gonadotropin (hCG), produced by choriocarcinoma or hydatidiform mole
5. Hyperthyroidism associated with acromegaly
6. Hyperthyroidism in thyroid carcinoma due to a toxic carcinomatous nodule (very rare) or due to excess thyroid hormone from widespread metastases (function per gram of tissue not increased, but total mass very large)
7. Hyperthyroidism due to administration of excessive thyroid hormone
8. Hyperthyroidism due to excess pituitary thyroid stimulating hormone
9. Hyperthyroidism due to excess iodide (Jod-Basedow's syndrome)
10. Hyperthyroidism due to an autonomous struma ovarii
11. Hyperthyroidism related to polyostotic fibrous dysplasia

*Adapted from Cooper, D. S., Ridgway, E. C., and Maloof, F. Unusual types of hyperthyroidism. Clin. Endocrinol. Metab. 7:199–220, 1978 and Volpé, R. Thyrotoxicosis. Clin. Endocrinol. Metab. 7:1–2, 1978.

Europe and other parts of the world, toxic nodular goiter is much more common, whereas hyperthyroidism due to thyroiditis appears to be less common. Hyperthyroidism is usually characterized by increased production of both thyroxine (T_4) and triiodothyronine (T_3), but at least in a small percentage (about 5%) T_3 alone is increased ("T_3 thyrotoxicosis").[52]

GRAVES' DISEASE

Graves' disease may be defined as hyperthyroidism associated with a diffusely hyperplastic goiter, resulting from immunologic causes.[293, 295] Its various synonyms include exophthalmic goiter, toxic diffuse goiter, Basedow's disease, Parry's disease, primary hyperthyroidism, and autoimmune hyperthyroidism. While Graves[111] received credit for the first description of the disease and von Basedow[300] is often given this distinction in Europe, the first description was clearly that of Parry of Bath.[225] Certain extrathyroidal features occur in this disorder, including exophthalmos, ophthalmoplegia, pretibial myxedema, and acropachy. Some of these manifestations are quite uncommon, and all may be absent in a given patient. Graves' disease is said to occur with an incidence of about 23/100,000 overall population,[93] although Tunbridge and colleagues provide a much higher figure of 1% of the population.[285] The sex ratio is about 4 or 5 females to 1 male.

Etiology of Graves' Disease

Graves' disease is now generally accepted as an organ-specific autoimmune disorder, characterized by the presence of an antibody that stimulates the thyroid gland (thyroid stimulating antibody or TSAb).[293, 295] Although in the past, some thyroidologists suspected that the cause of Graves' disease was pituitary stimulation,[14] no increase in thyroid-stimulating hormone (TSH) could be demonstrated either in the pituitary gland itself or in the serum of patients with Graves' disease when it became possible to measure this substance. Indeed, TSH was found to be suppressed in Graves' disease as a result of the excessive amount of circulating thyroid hormone.[129]

In 1956, Adams and Purves[1] of New Zealand showed that in the serum of many patients with Graves' disease, there was a substance that stimulated the thyroid gland of guinea pigs for a much longer period than that for pituitary TSH. Some years later, the substance was proved to be an immunoglobulin G (IgG)[169]; it was termed "long-acting thyroid stimulator (LATS)." Using a mouse bioassay it was found to be present in approximately 50% of patients with Graves' disease.[188] However, the fact that LATS could *not* be demonstrated in the other 50% of patients made it suspect as the cause of the hyperthyroidism.[262]

Evidence has shown that the failure was caused by lack of sensitivity of the assay system. The use of human thyroid tissue in more recent assay systems now makes it possible to demonstrate the presence of an IgG thyroid stimulator (thyroid stimulating antibody) in virtually all patients with active untreated Graves' disease.[293, 295] Evidence is now overwhelming to indicate that this IgG is an antibody to the human TSH receptor on the thyroid cell membrane (Fig. 13–1).[295] Since the term LATS has become associated with the mouse bioassay, most observers now employ the term "thyroid stimulating antibody (TSAb)" or "thyroid stimulating immunoglob-

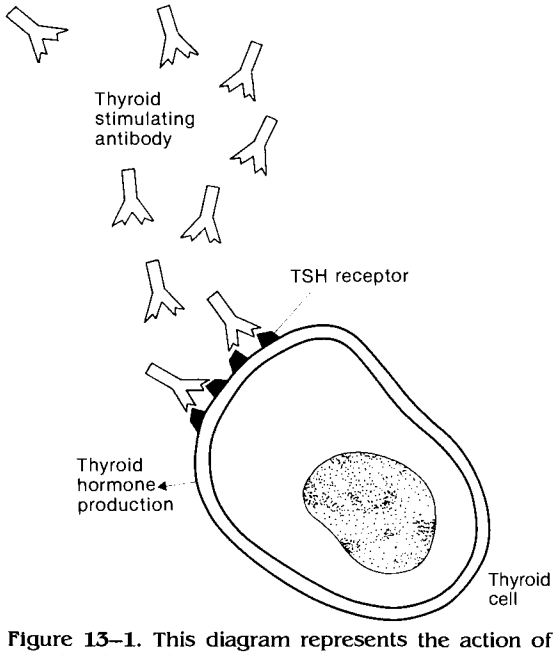

Figure 13–1. This diagram represents the action of thyroid-stimulating antibody (TSAb) on the thyroid cell. The antibody is directed to the TSH receptor, captures it in a manner indistinguishable from that of TSH, and causes an increase in adenylate cyclase and consequently cAMP. The net result is a stimulation of the thyroid cell, resulting in increased thyroid hormone secretion. The antibody, however, is capable of stimulating the thyroid cell for a much longer interval than does TSH.

ulin (TSI)" to describe the stimulators. However, there are other antibodies to the TSH receptor, i.e., thyrotropin receptor antibodies (TRAb), found in both Graves' disease and other forms of thyroid autoimmune disease that do not stimulate the thyroid gland. Both TSAb and these nonstimulating TSH receptor antibodies can be detected by a technique that measures the *prevention* of binding of TSH to its receptor, i.e., thyrotropin binding inhibitory immunoglobulin (TBII).[295] It is important to emphasize that TBII cannot necessarily be equated with TSAb. The reason for this statement is that some IgG samples, positive in the TBII assay, do not stimulate, and some actually *inhibit* the biologic effects of TSH, thus causing hypothyroidism. Thus, TRAb can be utilized as a generic term for all antibodies against the TSH receptor, whereas TSAb refers to only those that actually stimulate.[295]

It is now quite clear that the hyperthyroidism of Graves' disease is indeed caused by TSAb.[295] Following binding to the TSH receptor, this antibody acts as a TSH agonist, stimulating adenylate cyclase and cyclic AMP.[19] Aside from its prolonged duration of action, the cellular response is identical to that of TSH. Moreover, since TSAb crosses the placenta, when present in very large amounts it can cause passive transfer fetal and neonatal hyperthyroidism, which lasts only as long as the antibody remains in the circulation of the infant.[335] This is another point in favor of the causal relationship of the TSAb to hyperthyroidism (Table 13–2).

Since TSAb is obviously a product of bursa-equivalent (B) lymphocytes, it has been a matter of intense interest over the past generation to determine why this particular autoantibody should appear. In the past, it was assumed that an antigenic stimulus was needed for lymphocytes to produce such antibodies. However, there is no evidence for any antigenic alteration in the thyroid tissue of Graves' disease.[160, 301] Indeed, at least two studies have shown that thyroid tissue is unaltered in this disorder.[160, 301] It would appear that only the presence of the normal self antigen is required.

To a certain degree, this has been disputed by Bottazzo and associates[33] who have hypothesized that aberrant expression of HLA-DR is required on the thyroid cell to allow a thyroid autoimmune response. They have hypothesized that this expression of HLA-DR antigen on the basal cell membrane surface of thyroid cells may be a prerequisite for the initiation of autoimmune thyroid disease. These investigators have suggested that the initial precipitating event is some form of injury, possibly viral, to the thyroid cell that allows it to express DR antigen on its surface, to then permit direct antigenic presentation by the thyroid cell, and thus to precipitate a cascade of further events. In this hypothesis, it is still clear that there must also be a disorder of immunoregulation, since such HLA-DR expression on thyroid cells would not precipitate hyperthyroidism in a person not predisposed by a genetic defect in immunoregulation.

However, even later evidence suggests that this DR expression on thyroid cells may well be a secondary step after the initial immune assault, since it has been shown that T (thymic dependent) lymphocytes induce this alteration.[140] In any event, there does indeed appear to be an inherited specific defect in immunoregulation, and this defect appears to be a specific abnormality in a clone of thyroid-directed suppressor T lymphocytes.[293, 295, 295a, 298] Several reports now attest to the presence of such a disorder.[214a, 270a, 298] Such a defect in a specific clone of suppressor T lymphocytes would permit a "forbidden clone" of thyroid-directed, autoreactive, helper T lymphocytes (arising by normal random mutation but not suppressed because of this defect) to survive, to interact with its complementary antigen on the thyroid cell membrane (presumably the TSH receptor), and to set up a localized cell-mediated immune response within the thyroid gland (Fig. 13–2). Such T lymphocytes, sensitized to the thyroid antigen, would then direct and cooperate with groups of already present, appropriate thyroid-directed B lymphocytes, which consequently would produce the thyroid-stimulating antibody.

Table 13–2. Biologic Effects on Thyroid Shared by LATS, TSAb, and TSH*

Stimulation of
Uptake and discharge of ^{131}I *in vivo*
Release of ^{131}I *in vitro*
Colloid droplet formation in follicular cells
Glucose oxidation
Incorporation of ^{32}P into phospholipids
Adenyl cyclase activity
cAMP accumulation

*LATS = long-acting thyroid stimulator; TSAb = thyroid-stimulating antibody; TSH = thyroid-stimulating hormone. (Reproduced from Kendall-Taylor, P.: Comparison of the effects of various agents on thyroidal adenyl cyclase activity with their effects on thyroid hormone release. J. Endocrinol. 54:137–145, 1972, with permission.)

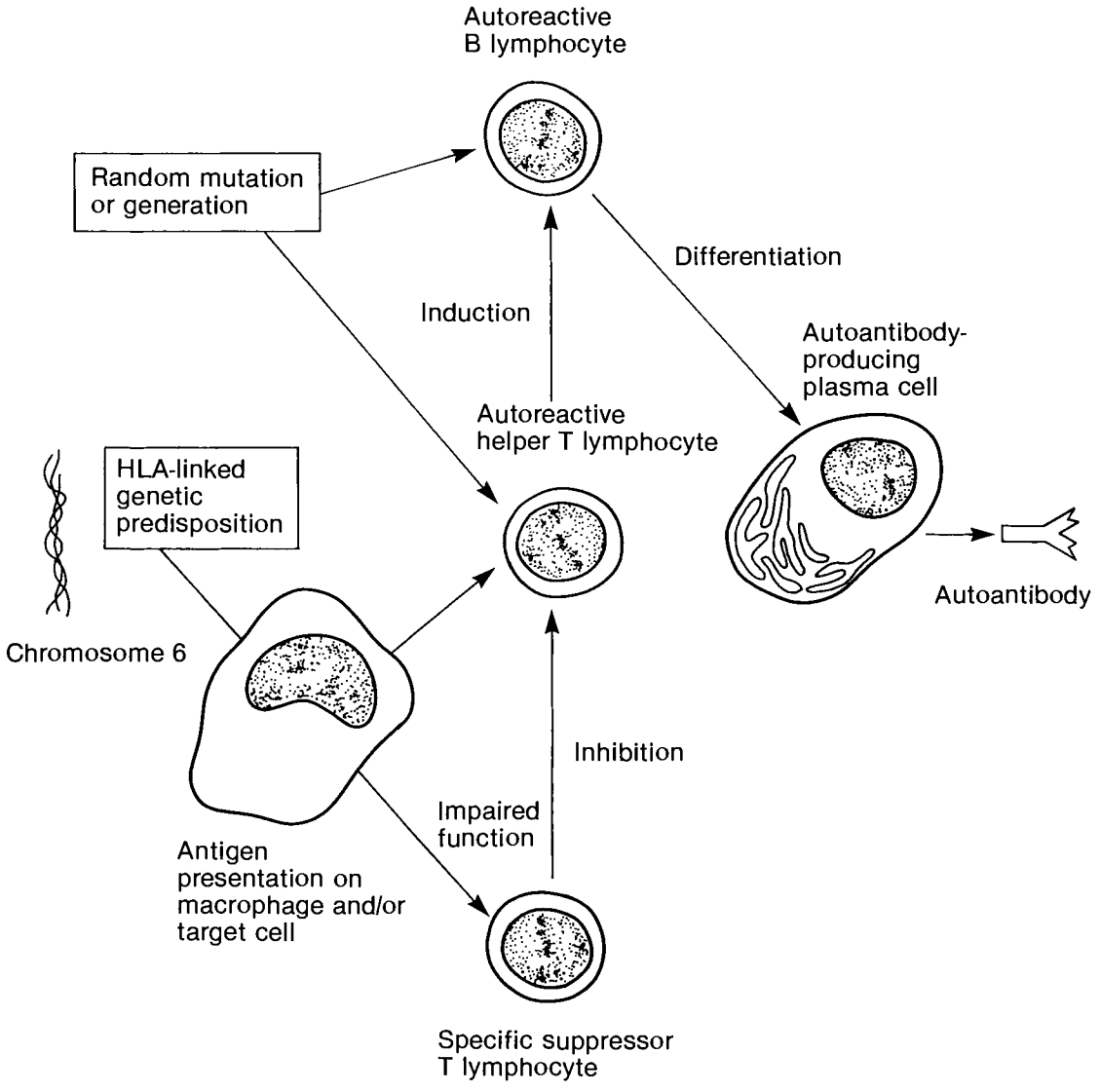

Figure 13–2. Schema for the hypothesis for organ-specific autoimmune endocrine diseases, particularly autoimmune thyroid disease. Human leukocyte antigen (HLA)–linked genetic predisposition appears to result in a specific defect in a clone of suppressor T lymphocytes that is specific for each organ specific autoimmune disease. Thus, clonal suppression of autoreactive T lymphocytes does not properly occur when the lymphocytes arise by normal random mutation. If these autoreactive helper T lymphocytes are not suppressed, they will interact with appropriate, specific, and already present autoreactive B lymphocytes that, in consequence, will produce the appropriate antibodies.

This sequence would certainly require the presence of antigen-presenting cells, which could be the thyroid cells themselves[33, 144] but in the initial phase most likely would be macrophages.[109, 125] Various chemical mediators would also be necessary for these events to occur, but this simple outline should suffice for an understanding of the process. There has been some confusion generated by reports that indicate that in active untreated Graves' disease, there is a reduction in the function and number of overall suppressor T lymphocytes.[298] These findings would not be expected if there was only an organ-specific defect in a clone or a few clones of suppressor T lymphocytes. However, further evidence suggests that hyperthyroidism per se can cause such a reduction in the number and proportion of generalized suppressor T lymphocytes, which would temporarily be additive to the antigen-specific disorder[102, 298]; this may add a self-perpetuating factor to the course of patients with Graves'

disease, tending to prevent them from experiencing remissions until this added element is brought back or returns to its previous levels, by normalizing thyroid function.[102, 295b, 298]

Other mechanisms of disordered immunoregulation have been suggested, such as the antiidiotypic network. In this hypothesis, it is proposed that when antibodies against self arise, there are further antibodies against autoantibodies (i.e., auto-auto antibodies or antiidiotypic antibodies), which would neutralize them.[80] However, this network has not been demonstrated to be a significant factor in autoimmune endocrine disease. It has further been suggested that TSAb might be an antiidiotype itself, i.e., an antibody to an anti-TSH.[139] But evidence on this point suggests that when such antiidiotypes do occur, they are transient in nature, are present in very low titer, and have a different binding pattern when compared with Graves' IgG.[139] They thus appear to be relatively inconsequential. Antibodies to TSAb also occur that have the effect of acting as antibodies to TSH, but likewise these are relatively uncommon in Graves' disease and do not appear to be of importance.[234a]

Genetic Factors. It is obvious that both Graves' disease and Hashimoto's disease aggregate in specific families and thus appear to be genetically induced.[273, 281, 293, 295] Indeed, these two disorders tend to occur in the same families and even may coexist within the same thyroid gland. Moreover, there are reports in which one homozygous twin has Graves' disease while the other has Hashimoto's thyroiditis. The increased incidence of other organ-specific diseases in patients with Graves' or Hashimoto's disease, as well as in their families, is now well known and is discussed subsequently.[293, 295]

The occurrence of Graves' disease in both siblings of dizygotic twins is reported to be about 3 to 9% and of monozygotic twins to be about 30 to 60%.[293, 295] Numerous case reports attest to the frequency of concordance of monozygotic twins for Graves' disease. The fact that monozygotic twins have a higher concordance rate than dizygotic twins is strong evidence for a genetic basis for Graves' disease, but genetic factors alone do not explain why some develop this disease while others do not, i.e., the lack of concordance in 40 to 70%. It is of further interest that in even highly selected case reports of twin studies of Graves' disease, the age of initiation of the disease varies greatly between the twins, even as much as 10 years in siblings under 18 years of age. Thus, it is clear that influences other than purely genetic ones must be present before the disease is expressed.

Studies of the age-specific incidence rates in this disorder have indicated that Graves' disease occurs at random in the genetically predisposed population with an ultimate penetrance that approaches unity.[293, 295] Studies of families of patients with Graves' disease indicate that Hashimoto's thyroiditis and Graves' disease seem to share a common inheritance, and it even has been suggested that the same gene predisposes to both disorders.[273, 281] At least, the two disorders must be very closely related genetically, although I believe that, ultimately, separate genes for Graves' as opposed to Hashimoto's thyroiditis will be proved (see subsequent discussion).

There are at least two genes that appear to be important genetically in Graves' disease. These include not only HLA genes[273, 281] but also genes related to the IgG heavy chain allotype, termed Gm.[273, 281, 286] In whites with Graves' disease, there is an increased incidence of HLA-B8 and HLA-D3, whereas in Japanese the appropriate HLA types have been those of HLA-Bw35 and Dw12.[79] Only about 56% of white patients with Graves' disease, however, are found to be positive for HLA-D3 versus about 20% of the general population. The fact that not all patients have the "appropriate" HLA gene, and that persons in the population with that particular HLA gene increase their relative risk only about five times that of the general population, shows clearly that these genes do not themselves cause the disease.[17] There has been controversy as to whether there is a true "disease susceptibility" gene, but it is evident that if there is such a gene, it is not identical to the aforementioned HLA genes. It may, however, lie in linkage disequilibrium with these histocompatibility genes; further studies with cDNA probes may elucidate this point.[17]

The influence of the Gm genes is separate from the HLA genes. An appropriate Gm marker would ensure that a particular person has the ability to produce the appropriate immunoglobulins.[17, 273, 281, 286] Thus, HLA genes or closely associated genes would perhaps determine the level of suppressor and helper T lymphocyte function in the production of TSAb in Graves' disease, whereas the Gm

marker would permit the production of the actual TSAb by the B lymphocytes.

Females exceed males in a ratio of about 4:1 in this condition. Whether this is an effect of estrogen on immunoregulation seems somewhat unlikely, since this female/male ratio pertains to all age groups, including females before puberty and after menopause. Another possibility is that the female/male ratio may be due to a chromosomal effect, whereby genes on the X chromosome or the Y chromosome influence events on chromosome 6 where the histocompatibility genes lie.[2]

A variety of other organ-specific autoimmune diseases occur in much greater frequency in these same patients and in their relatives. These include Type 1 diabetes mellitus, pernicious anemia, vitiligo, Addison's disease, myasthenia gravis, idiopathic thrombocytopenic purpura, rheumatoid arthritis, and chronic active hepatitis.[293, 295] Most, but not all, of these diseases are also HLA-D3 related.[17, 79, 270]

It is of interest that there are certain HLA differences between Graves' and Hashimoto's diseases, despite the results of the aforementioned family studies,[272, 281] which suggest that the two disorders share a common inheritance. In goitrous Hashimoto's disease, there tends to be an increased incidence of HLA-DR5 as opposed to the situation in groups of patients with Graves' disease where the increase is in HLA-DR3.[82, 279, 311] This difference as well as discrepancies in the incidence of exophthalmos, the frequency of thyroid stimulating antibody, and the precise frequency of association with other organ-specific autoimmune diseases makes me believe that Graves' and Hashimoto's disease, however closely related they are to one another, represent different entities and thus have some genetic differences.[293, 295]

Clinical Manifestations of Graves' Disease

Characteristically, although not invariably, Graves' disease is the most florid type of hyperthyroidism. The symptoms may commence precipitously or subtly. Sometimes they seem to follow specific stresses, such as an automobile accident, bereavement, infection, or psychologic disturbance; other precipitating factors include rigorous dieting and even thyroid hormone therapy.[293, 295] Because such stress may interfere with immune mechanisms, possibly through cortisol production, the induction of hyperthyroidism may prove to have a rational explanation. While there is considerable anecdotal evidence that such a relationship obtains, there has been no impeccable study that completely proves this notion. Indeed, most studies on this point indicate that the number and magnitude of the stresses encountered by patients prior to the onset of hyperthyroidism do not differ from those encountered by a normal control group who did not, of course, develop Graves' disease.[112] Such studies do not take into account the genetically based immune vulnerability of those predisposed to Graves' disease to a perturbation that would not affect a perfectly normal person. However, it is certainly my view that such a relationship does indeed exist.[295]

The symptoms of hyperthyroidism include nervousness, emotional lability, sweating, intolerance to heat, rapid heart beat, fatigue, weight loss associated with increased appetite, thirst, dryness of mouth, dyspnea, muscular weakness particularly of the "girdle" muscles (quadriceps and deltoids), tremulousness, frequency of micturition, and diarrhea.[64, 320] About half the patients develop ocular symptoms (discussed subsequently). Somewhat less common complaints include swelling of the ankles, falling out or thinning of the hair, male gynecomastia, loss of libido in the male, and oligomenorrhea in the female. Decreased fertility is common. Increased headaches, pruritus, and nausea and vomiting may be experienced.[64, 320] A few patients experience anorexia rather than increased appetite, whereas a small number of patients may actually gain weight as a result of their voracious appetite. Some patients become aware of their goiters and may even have some local discomfort or pain in the neck.

The "nervousness" of Graves' disease is associated with marked restlessness, difficulty in falling asleep, and insomnia.[310] Some patients are agitated and exhibit manic behavior. In some patients, the tremulousness and feeling of muscular weakness may be profound. A family history of thyroid disease, other organ-specific autoimmune diseases (see previous discussion), or both is frequently obtained.[293, 295]

Often, the diagnosis can be made on brief inspection (Fig. 13–3).[64, 320] These patients are usually thin, restless, hyperactive, nervous, and suffused. Eye signs are common but not invariably present; only about 50% of patients

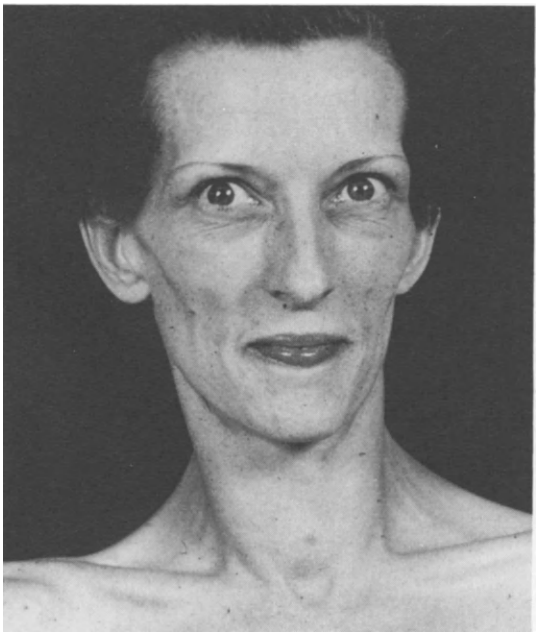

Figure 13–3. A patient with Graves' disease. Note the presence of exophthalmos, evidence of weight loss, and goiter.

with hyperthyroidism of Graves' disease will have clinically evident exophthalmos. Ocular manifestations are subsequently discussed separately. Enlargement of the thyroid gland is characteristic. The goiter is generally diffusely enlarged, although it may be asymmetric, the right lobe usually as the larger lobe. The enlargement may be minimal to marked. The gland is firmer than normal or may be even quite firm. There may be some lobulations, and in those glands in which there is coexistent Hashimoto's thyroiditis, the gland may be quite large, quite firm, and definitely lobulated. It has been claimed that in a very small proportion of patients, the gland is normal in size or even smaller than normal.[64] However, DeGroot[64] cautions that observation of a normal-sized thyroid should alert the physician to the possibility of some other cause of the hyperthyroidism. In a significant minority of patients, a bruit will be heard over one or both lobes of the thyroid gland. These may be continuous or systolic in timing and occasionally are associated with thrills. The trachea is not usually deviated.

The heart is generally of normal size, although it may be enlarged, particularly in older patients who have coincidental atherosclerotic heart disease unrelated to, but aggravated by, the hyperthyroidism.[62] Tachycardia is characteristic; the heart may beat with considerable force and may be a distressing symptom for the patient while in bed. Approximately 10% of patients have auricular fibrillation, which is more common in older patients. A bounding precordium is common, and on auscultation the heart sounds are heard most forcefully. A basal, ejection-type systolic murmur is very common and may be heard over the entire precordium. A grating pulmonic systolic sound with some of the aspects of a pericardial friction rub is sometimes heard over the sternum in the second left interspace, and the scratching sound is characteristic (Means-Lehrman "scratch").[64] The pulse pressure is wide, and bruits can occasionally be heard over the peripheral arteries. Peripheral pulsations are rapid and bounding, with elevations of the systolic blood pressure. Capillary pulsations are also often demonstrable.

When a patient presents with a tachyarrhythmia, hyperthyroidism must always be considered in the differential diagnosis. Generally, the electrocardiogram will show only tachycardia or increased voltage with occasional prolongations of the PR interval.

Some patients may be found to be in congestive heart failure and if this condition is present, it will be resistant to the usual dosages of digitalis.[110, 250] The various effects on the cardiovascular system as previously noted may be due in part to the increased sensitivity to circulating epinephrine in hyperthyroidism.[62]

Although the liver is occasionally enlarged, the spleen is clinically palpable in about 5% of patients, which may be a concomitant of the immunologic disturbance. Similarly, lymph node enlargement is occasionally observed.[64, 320]

Integumentary changes are usually evident.[91] The skin is warm, smooth ("velvety"), and moist. Palmar erythema, arteriolar cutaneous "spiders," and onycholysis (Plummer's sign) are fairly common. Patchy hyperpigmentation on the face and neck is frequently seen. Conversely, patchy vitiligo is found in about 7% of patients with Graves' disease, evidence of an associated autoimmune disorder directed against melanocytes.[198, 222] The hair tends to be fine and soft. Rarely, alopecia areata may be found in association with Graves' disease.[198]

Clubbing of the fingers associated with thickening of the distal phalanges (thyroid acropachy) occurs in about 5% of these patients (Fig. 13–4).[64, 320] This may be associated with pretibial myxedema (to be discussed separately). Gynecomastia in the male may be present. Neurologic disturbances include fine

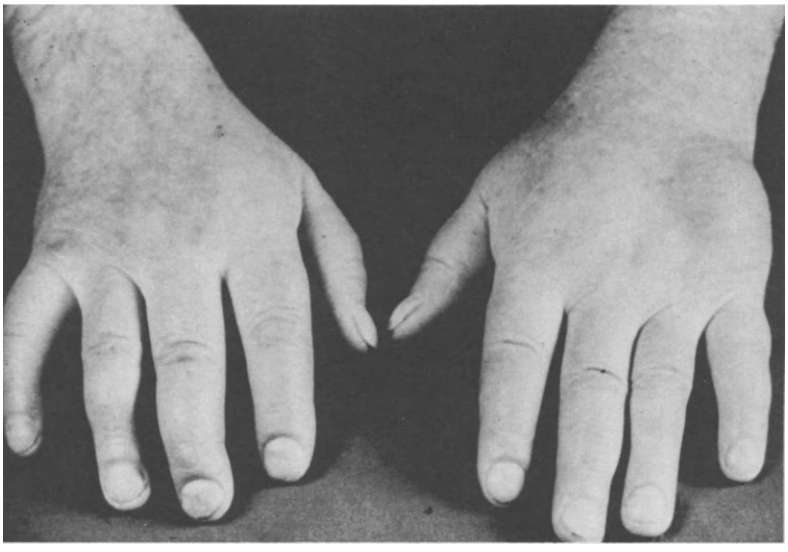

Figure 13–4. Thyroid acropachy. Also note the Graves' dermopathy on the dorsum of one hand.

tremor, hyperkinetic reflexes, and moderate to marked weakness of the girdle muscles.

The altered pathophysiology associated with many of these physical changes is discussed subsequently. It should be emphasized that other organ-specific autoimmune diseases, as mentioned, may occur in these patients much more frequently than chance alone dictates.

Pathology of the Thyroid

Graves' disease is characterized by hyperplasia and hypertrophy of the thyroid parenchyma.[320] There is increased height of the epithelium from the normal cuboidal form to a columnar form; there is increased infolding of the papillae, because of redundancy of the follicular lining, resulting from increased stimulation of the thyroid follicular cells (Fig. 13–5). Indeed, this increased stimulation was characterized by evidence of increased activity of the individual cell with increased numbers of mitochondria, hypertrophy of the Golgi apparatus, and vacuolization of the colloid. This picture is certainly not unique for Graves' disease but can occur in any situation where the thyroid gland is being stimulated. Characteristically, however, there is also infiltration of the thyroid gland by lymphocytes and plasma cells, and there even may be lymphoid germinal centers. Occasionally, histologic Hashimoto's thyroiditis and Graves' disease coexist. (The pathologic abnormalities of extrathyroidal tissues involved in Graves' disease are discussed separately.)

Pretibial Myxedema (Graves' Dermopathy)

About 5% of patients with Graves' disease have localized (pretibial) myxedema, a unique swelling of the skin, and subcutaneous tissue found commonly over the tibia of one or both legs (Fig. 13–6).[64, 103, 320] Occasionally, however, the lesion is evident on the dorsum of the hand. Because the disorder is actually widespread, even when it appears to be localized clinically, a preferable term would be "Graves' dermopathy." Many of these so-affected patients have redundant subcutaneous tissue, appearing as large jowls in their cheeks and neck; this condition may result in a "frog-like" appearance. Histologically, the cutis is edematous and deposited between the collagen bundles is a material that stains like mucin.[161, 333] This microscopic finding and the gross appearance give rise to the term pretibial myxedema, although it has no relationship to hypothyroidism. Patients with pretibial myxedema tend to have the most severe immunologic disturbances, and it has been suspected that deposits represent an immunologic reaction.[293, 295] This change is found in a patient who also has some degree of exophthalmos; often the same patient also has thickening of the distal phalanges of the fingers associated with clubbing of the fingers (thyroid acropachy) (see Fig. 13–4).[64] Furthermore, TSAb levels are almost invariably high.[257, 293, 295]

The spontaneous course of pretibial myxedema is variable. In some patients the lesions gradually disappear over several years.[64, 320] In

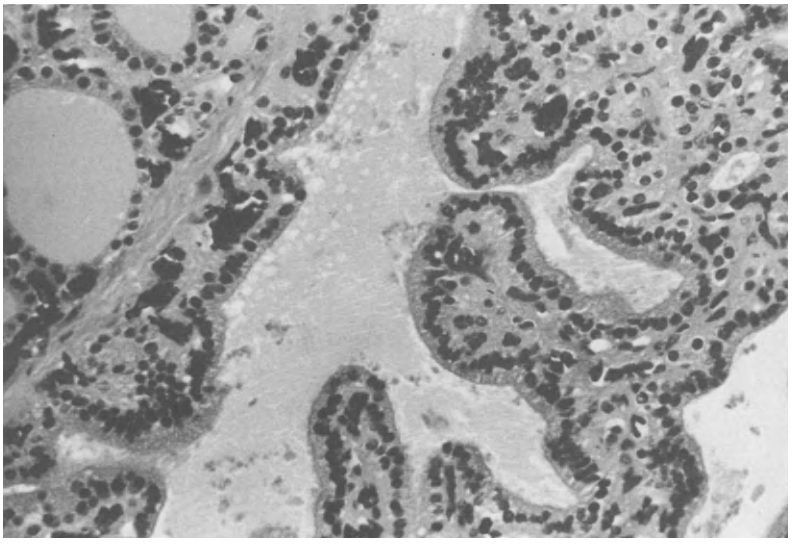

Figure 13–5. Pathology of the thyroid in Graves' disease. Note the high columnar epithelium associated with redundancy and infolding.

others, they are progressive, leading to widespread lymphatic obstruction and fibrosis. In extreme cases, the appearance resembles marked lymphedema. No therapy cures pretibial myxedema, although large doses of systemic corticosteroids or local corticosteroid ointment or injections bring some relief. The skin of the region may be coated with the ointment and the leg wrapped in saran.[91] Others prefer to employ local injections of steroid into the lesions.[178] These treatments may result in considerable improvement if commenced early enough. There may be occasional spontaneous remission of this disorder, as mentioned.

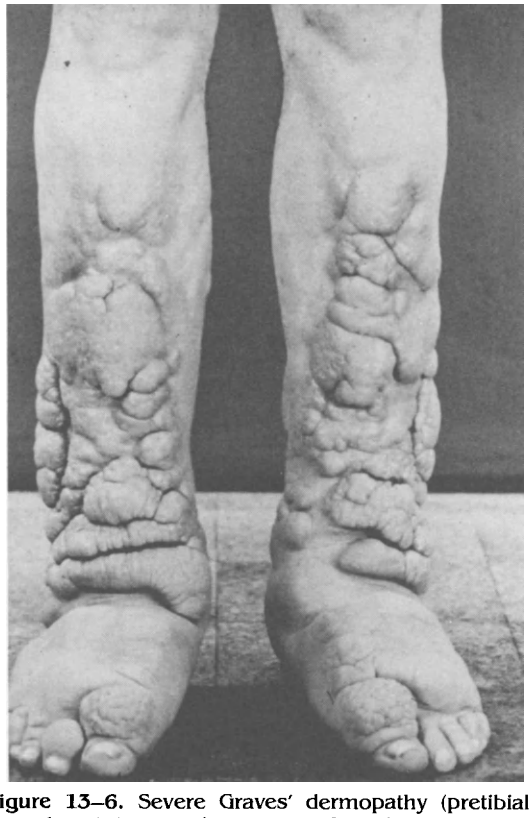

Figure 13–6. Severe Graves' dermopathy (pretibial myxedema) in a patient years after ablation of the thyroid gland for Graves' disease.

Ocular Manifestations

About 40 to 90% of patients with Graves' disease will have some ocular manifestations, which can be either overt or occult,[7] but severe Graves' hyperthyroidism may occur without any eye changes (see Fig. 13–5).[16]

Conversely, endocrine exophthalmos may occasionally develop without hyperthyroidism ("euthyroid ophthalmic Graves' disease"), occurring with Hashimoto's thyroiditis or even idiopathic myxedema; exophthalmos may also occur when thyroid functional abnormalities are more occult, or it may occur when there is *no* thyroid dysfunction whatever.[108, 141, 183, 215, 293, 295] The eye changes, which vary considerably from patient to patient and even from time to time in a given patient, may include those of physical appearance, i.e., undue prominence of one eye or both; excessive lacrimation; pain or discomfort; recurrent infections; frequent headaches; photophobia; aching; blurring of vision; loss of vision; and diplopia or occasional prolapse.[108] The minimal clinical eye sign in Graves' disease is proptosis,

a staring expression, and widening of the palpebral fissures. There is weakness of convergence, and lid lag is found when the eyes are rotated downwards. The various ocular signs are listed in Table 13–3 and Table 13–4.

The ocular manifestions are commonly divided into the two following types: (1) those characterized by protrusion and (2) those in which infiltrative manifestations are more pronounced.[108] In both types, marked periorbital edema, even without measurable proptosis, may be present. Proptosis can be measured by a variety of exophthalmometers. It may be considered abnormal in whites when protrusion is greater than 20 mm. Infiltrative exophthalmos is characterized by considerable orbital edema, congestion, chemosis, and conjunctival injection. Discomfort may be pronounced in this type; lacrimation and a feeling of sand in the eyes are very common. If the exophthalmos becomes very marked (malignant exophthalmos) the cornea may ulcerate (Fig. 13–7). Panophthalmitis may then occur, and blindness may result. Visual impairment and blindness may also occur as a result of pressure on the optic nerve from an enlarged medial rectus muscle. Of the variety of all the extraocular muscle palsies (ophthalmoplegias) that may occur, weakness of convergence is most common and least important. Failure of upward gaze due to superior rectus muscle weakness is serious, and other extraocular muscles may be variably affected. The diplopia that results can be very incapacitating.

Table 13–3. Ocular Signs in Graves' Disease*

Ophthalmic phenomena reflecting thyrotoxicosis per se and apparently resulting from sympathetic overactivity
Lid retraction
Wide palpebral aperture (Dalrymple's sign)
Lid lag (von Graefe's sign)
Staring or frightened expression
Infrequent blinking (Stellwag's sign)
Absence of forehead wrinkling on upward gaze (Joffroy's sign)
Ophthalmic phenomena unique for Graves' disease and caused by specific pathologic changes in the orbit and its contents
Inability to keep the eyeballs converged (Möbius's sign)
Limitation of movement of the eyeballs, especially upward
Diplopia
Blurred vision due to inadequate convergence and accommodation
Swelling of orbital contents, puffiness of the lids
Chemosis, corneal injection, or ulceration
Irritation of eye or pain in the globe
Exophthalmos (also mechanically produces a wide palpebral fissure)
Visible and palpable enlargement of the lacrimal glands
Visible swelling of the lateral rectus muscles as they insert into the globe and injection of the overlying vessels
Decreased visual acuity due to papilledema, retinal edema, retinal hemorrhages, or optic nerve damage

*From Werner, S. C.: Classification of the eye changes of Graves' disease. J. Clin. Endocrinol. Metab. 29:782, 1969.

Computerized axial tomographic and ultrasonographic examinations demonstrate the markedly increased size of the extraocular muscles, particularly the medial rectus.[80, 88, 89, 108, 141, 282, 318] Pressure by this enlarged muscle on the optic nerve may result in visual impairment or blindness. These techniques may be used to demonstrate abnormalities in the eyes of many patients with Graves' disease who have no *clinical* evidence of exophthalmos. Indeed, the measurement of intraocular pressure on upward gaze, which is elevated in exophthalmos, is useful to demonstrate subclinical ophthalmopathy in many patients with Graves' disease who have no other evidence of ocular involvement.[97, 108] There remains a small residual group of Graves' patients who still have no evidence even by these sophisticated techniques of any ocular involvement.[183]

Patients with exophthalmos have an increase in muscle fiber bulk, with degeneration of muscle fibers; infiltration of lymphocytes and macrophages; and increased acid mucopolysaccharide, fat, and water content. The lymphocytic infiltration of the muscles may be extreme (i.e., lymphorrhages); the inflammatory changes in the extraocular muscles lead to marked muscle thickening, which may lead to later fibrosis and shortening and thus ophthalmoplegia.[108, 141, 241] The cause of both the proptosed and infiltrative ocular lesions remains unclear. The exophthalmos appears to be caused by an immune process that, in my opinion, is closely related to, but not identical with, the immune process that causes the hyperthyroidism of Graves' disease.[293, 295] Sensitization of circulating T lymphocytes of these patients in response to retroorbital muscle antigen has been demonstrated, which does not correlate with sensitization against thyroid antigen.[219] Three groups have identified antibodies to ocular muscle in patients with exophthalmos that are absent in patients with Graves' hyperthyroidism but no eye signs.[83, 157, 162] Their results suggest that exophthalmos is not due to the thyroid disease and is separate

Table 13–4. Classification of the Ocular Changes in Graves' Disease*

Classes (0 to 6)	Grades (0, a, b, c)	Ocular Symptoms and Signs
0		No signs or symptoms
1		Only signs, no symptoms (signs limited to upper lid retraction and stare, with or without lid lag and proptosis). Proptosis associated with class 1 only (specify difference of 3 mm or more, grade 0 included).
	0	Absent (20 mm or less—normal)
	a	Minimal (21 to 23 mm)
	b	Moderate (24 to 27 mm)
	c	Marked (28 mm or more)
2		Soft tissue involvement (symptoms of excessive lacrimation, sandy sensation, retrobulbar discomfort, photophobia, but not diplopia).
		Objective signs as follows:
	0	Absent
	a	Minimal (edema of conjunctiva and lids; conjunctival injection and fullness of lids, often with orbital fat extrusion; palpable lacrimal glands or swollen extraocular muscle palpable laterally beneath lower lids)
	b	Moderate (same as minimal plus chemosis, lagophthalmos, lid fullness)
	c	Marked
3		Proptosis associated with classes 2 to 6 only (specify if inequality of 3 mm or more between eyes or if progression of 3 mm or more under observation)
	0	Absent (20 mm or less)
	a	Minimal (21 to 23 mm)
	c	Marked (28 mm or more)
4		Extraocular muscle involvement (usually with diplopia)
	0	Absent
	a	Minimal (limitation of motion, evident at extremes of gaze in one or more directions)
	b	Moderate (evident restriction of motion without fixation of position)
	c	Marked (fixation of position of a globe or globes)
5		Corneal involvement (primarily due to lagophthalmos)
	0	Absent
	a	Minimal (stippling of cornea)
	b	Moderate (ulceration)
	c	Marked (clouding, necrosis, perforation)
6		Sight loss (due to optic nerve involvement)
	0	Absent
	a	Minimal (disc pallor or choking or visual field defect 20/20 to 20/60)
	b	Moderate (disc pallor or choking, visual field defect 20/60 to 20/200)
	c	Marked (blindness, i.e., failure to perceive light, vision less than 20/200)

*Note that in addition to classification by type of involvement, there is also a grading according to severity. From Werner, S. C.: Classification of the eye changes of Graves' disease. J. Clin. Endocrinol. Metab. 29:782, 1969.

from it. The marked overlapping between the thyroid disorder and the eye disease may be due, first, to very close genetic and pathogenic pathways and, second, to a possible deleterious effect of the hyperthyroidism per se on an already precarious and vulnerable immunoregulatory system.[31, 102, 293, 295, 298] Some workers still contend that the pituitary may contribute to the genesis of these ocular manifestations,[80–86] even though severe exophthalmos can occur in patients who have undergone hypophysectomy.[94] Moreover, TSH and its subunits are quickly suppressed in hyperthyroidism.[167, 223] This fact suggests that TSH has no role in the cause of the exophthalmos. Conversely, hypothyroid patients with markedly elevated TSH values do not develop exophthalmos.[183] In my view, therefore, the pituitary may be excluded as a factor in the pathogenesis of exophthalmos.

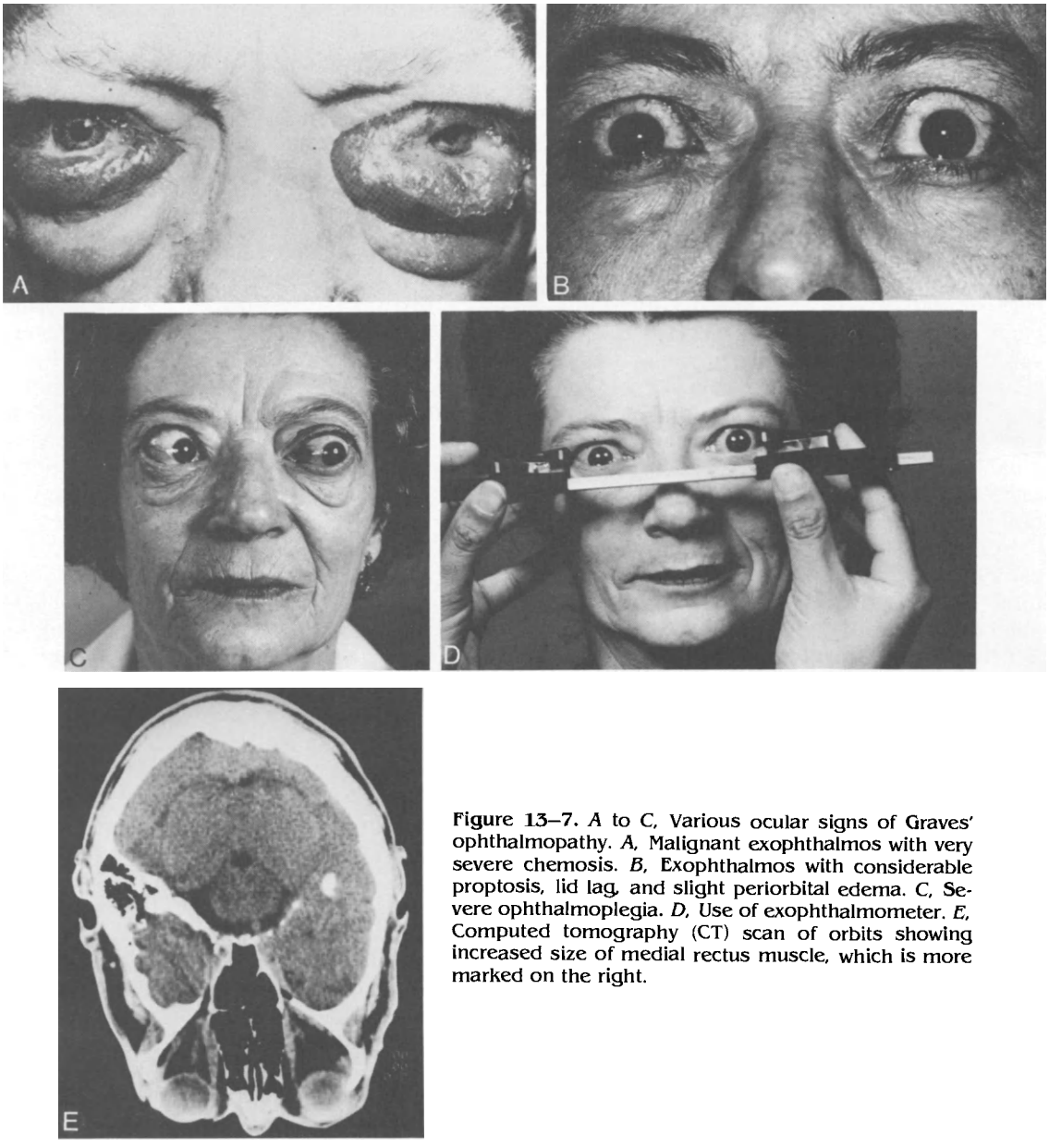

Figure 13–7. *A* to *C*, Various ocular signs of Graves' ophthalmopathy. *A*, Malignant exophthalmos with very severe chemosis. *B*, Exophthalmos with considerable proptosis, lid lag, and slight periorbital edema. *C*, Severe ophthalmoplegia. *D*, Use of exophthalmometer. *E*, Computed tomography (CT) scan of orbits showing increased size of medial rectus muscle, which is more marked on the right.

Still another theory suggests that the burden of lymphocytes, thyroglobulin, and thyroglobulin-antithyroglobulin complexes reaches the orbit from the lymphatics in a retrograde flow from the hyperactive thyroid itself.[168, 199] It has been proposed that these elements may cause the exophthalmos. However, the presence of immune complexes does not correlate with the presence or severity of exophthalmos.[141, 293, 295] Moreover, the previous suggestion that eye muscle contains thyroglobulin has not been confirmed.[163, 218] Indeed, lymphocyte-induced damage to human eye muscle cells *in vitro* occurs independently of thyroglobulin.[26] Moreover, this theory cannot account for the fact that exophthalmos may predate the hyperthyroidism or follow it years later, and it cannot account for those cases of exophthalmos that have no thyroid disease whatever. Thus, the proposal that exophthalmos and hyperthyroidism are very closely related but separate, overlapping autoimmune entities seems the most acceptable.

The treatment of exophthalmos remains em-

piric.[108, 141, 168, 199, 306] Fortunately, in some patients, the exophthalmos improves spontaneously or may need no treatment. In others, the infiltration becomes pronounced and even intolerable. Local steroid-antibiotic ointments and systemic corticosteroids in large doses may improve the symptoms associated with such infiltrations. As well as large-dose corticosteroid therapy, immunosuppressive agents (azathioprine, cyclophosphamide) have also proved useful either alone or in conjunction with steroids in severe cases of vision-threatening ophthalmopathy.[108, 141, 168, 199, 306] Some workers have advocated injection of corticosteroids into the orbit. Artificial teardrops (e.g., methyl cellulose) may add some symptomatic relief. Total surgical removal or radioablation of the thyroid has been proposed to relieve the exophthalmos, on the assumption that this may reduce the thyroid antigen—the putative cause, according to those investigators, of the exophthalmos.[18, 45, 321] However, total thyroid ablation,[148, 226, 296, 319] pituitary ablation, and irradiation of the pituitary[199] have not been shown to be effective. Radiation to the orbital tissue has achieved some success as treatment for active exophthalmos.[141, 199] Presumably, the orbital radiation destroys the lymphocytes and reduces the infiltration. The use of plasmapheresis has met with limited success.[59, 104, 153, 174, 253, 332] Some reports have suggested a beneficial role for cyclosporin A, for which a randomized control study would be of great interest.[287, 308] Other reports have shown no benefits from this agent.[34, 135, 153]

Transantral decompression of the orbits or other direct operations on the eye may provide cosmetic and physical relief.[108] However, these procedures may aggravate ophthalmoplegia, thus producing or aggravating diplopia. Operations on the eye muscles to relieve diplopia may bring about considerable improvement but rarely produce a complete "cure."[108] Because hypothyroidism may aggravate but of itself does not cause exophthalmos, thyroid replacement therapy for that condition is most important. However, it should be administered to only those patients who are actually hypothyroid; it will not ameliorate ophthalmopathy in euthyroid patients.

Neuromuscular Manifestations

In addition to nervousness and tremulousness, hyperthyroid patients may have profound muscular weakness that may even simulate progressive muscular atrophy.[64, 320] On the one hand, this weakness may disappear when thyrotoxicosis is treated; on the other hand, weakness due to myasthenia gravis, which occasionally coexists with hyperthyroidism, does not disappear when hyperthyroidism is treated.[70, 74, 120, 189, 200, 309] Some Oriental patients may have "thyrotoxic periodic paralysis," manifested by profound muscular weakness; this condition is associated with low potassium concentrations and becomes evident only when the patient is hyperthyroid.[75, 76, 258] Cure of the hyperthyroidism also corrects the state of hypokalemia and muscle weakness. This disorder is due to the presence of a genetic trait for the muscle disorder, which can be expressed only if the patient is hyperthyroid due to a second, unrelated genetic trait. The first genetic trait is confined almost entirely to Oriental populations.[334]

Thyroid Storm

Crises that result from severe hyperthyroidism chiefly occur following subtotal thyroidectomy or other operations, as well as other types of stress that aggravate preexisting hyperthyroidism, e.g., infections, trauma, and dehydration.[137, 246] Thyroid storm is seen most commonly in those who have been very ill and are suddenly exposed to marked hyperthyroxinemia. The patient develops marked hyperpyrexia; the body temperature sometimes reaches or exceeds 41°C.[137, 246] The skin is often dry, and the patient generally has marked tachycardia, prostration, restlessness, and, rarely, apathy or delirium. Fortunately, the thyroid storm is rare and usually can be avoided by administering antithyroid drugs before operation or other stress. Further specific therapy is discussed subsequently.

Pathophysiology of Manifestations of Graves' Disease

The nervous and mental disturbances may be a manifestation of increasing sensitivity to circulating epinephrine, although this proposal has not been established beyond doubt.[182] Levels of epinephrine and catecholamine excretions are actually within normal limits.[320] However, beta-adrenergic blocking agents seem to yield some benefit in terms of the nervous system symptoms.[182]

In animals, thyroxine decreases the threshold to convulsive stimuli, and electroencepha-

lograms show increased fast wave activity and some bursts of tall spike waves. Sensenbach and colleagues[256] have shown the cerebral blood flow to be increased and the cerebral vascular resistance to be decreased; there is a decreased arteriovenous oxygen difference, but cerebral oxygen consumption is said to be unchanged in hyperthyroidism.

The muscle weakness described in this condition appears to be a catabolic disturbance.[331] Creatine excretion is elevated, as associated with the muscle wasting.[280, 331] Creatinine excretion is initially higher in the acute phase of hyperthyroidism, but ultimately creatinine excretion declines in the urine, as muscle mass is markedly diminished.

Studies of cardiac function in hyperthyroidism have shown greater heart rate and stroke volume, greater cardiac output, and reduced circulation time.[62, 110, 250] Coronary blood flow and myocardial oxygen consumption per stroke are increased. Circulating plasma volume is elevated. The cardiac output in response to exercise is excessive in relation to the amount of oxygen consumed. There is also dilatation of superficial capillaries. These cardiac effects may be due to the additive or synergistic effects of thyroid hormone and epinephrine.[182, 320]

Hematologic changes may be noted in hyperthyroidism.[87] Normocytic anemia is often seen and may relate to the catabolic state.[87, 126, 242] A greater incidence of occult or overt pernicious anemia is seen in patients with Graves' disease.[293, 295] In addition, the glucose-6-phosphate dehydrogenase activity of erythrocytes is increased in hyperthyroidism.[87] The relative lymphocytosis, as commonly noted in the peripheral blood of patients with Graves' disease, may be due in part to the immunologic disturbance.[293, 295] However, the total lymphocyte count is often not affected, and thus the relative lymphocytosis may be due to an absolute diminution of neutrophils. This reduction in neutrophils may in turn be due to antineutrophil autoantibodies.[312]

Occasional patients with Graves' disease and thrombocytopenic purpura have been described, a coincidence of two autoimmune diseases.[295] Platelets may have a reduced life span in hyperthyroidism in any event.[320]

Gastrointestinal changes also occur in hyperthyroidism. Weight loss is due to increased catabolism.[320] There is an increase in peristaltic activity in this disorder, and the rate of absorption from the intestinal tract is accelerated.[39, 146, 254, 259] Liver enzymes, most particularly alkaline phosphatase, may be elevated, which may be due to relative hepatic hypoxia if the metabolic demand for oxygen exceeds the supply.[101, 227] It has been suggested that in certain thyrotoxic patients, there is a great rise in the metabolites that must be detoxified by the glucuronyl transferase system, thus resulting in a reduced rate of conjugation of various substrates.[320] This would appear to account for the increased levels of free estradiol in hyperthyroid patients,[53] which in turn would account for the findings of oligomenorrhea, palmar erythema, arteriolar cutaneous "spiders," and gynecomastia. In a male, there is often an increase of gonadotropins and testosterone.[53] As noted previously, there is also a higher incidence of associated autoimmune hepatitis, which will not respond to the treatment of the hyperthyroid state.[295]

It is obvious that basal oxygen consumption is increased in hyperthyroidism.[320] However, the greater rate of metabolism is associated with a less efficient coupling of oxidation and energy utilization than would be expected, and thus muscular work is inefficient.[38a, 232] Intermediary metabolism is also accelerated with increased rates of absorption, utilization, and degradation of all dietary elements.[320] Hyperthyroidism also increases the requirement for insulin[191] and the turnover rate of cortisol.[96] Serum lipid values are depressed in hyperthyroidism as a result of higher rates of degradation of lipids.[171, 284] The catabolism of proteins occurs for similar reasons.[159, 236, 260] This catabolic state accounts for the weight loss and muscular weakness, as observed in hyperthyroidism, and can be a factor in producing thyrotoxic cardiomyopathy.[320]

Natural Course

Graves' disease is now rarely left untreated; however, the early medical literature pointed out that some patients go into spontaneous long-term remission without specific therapy.[220, 251] Such remissions may be lifelong but in some instances they are only temporary. Some patients have recurrent remissions and exacerbations. In the majority, the disease remains constant and unremitting if untreated. A significant minority may even go on to spontaneous hypothyroidism as a result of continuing autoimmune thyroiditis.[65, 142, 176, 328, 329]

Because we cannot accurately predict the natural course in any one patient and because

the untreated disease often tends to be self-perpetuating,[220, 251] Graves' disease should at least be suppressed in all patients. The nature of the remissions that do occur spontaneously or after antithyroid drug therapy is of considerable interest.[293, 295] The presence of the histocompatibilty gene HLA-Dw3 has been associated with a high frequency of relapse, whereas the absence of the gene is more commonly associated with long-term remissions.[20, 138, 201, 278] This finding, however, has been challenged.[5] In many patients in long-term remissions, all immunologic stigmata of the disease disappear, including thyroid stimulating antibody.[201, 205, 293, 295] Thus, this form of remission may be considered an immunologic remission.[293, 295] It is possible that nondestructive treatment of the hyperthyroid state, such as with antithyroid drugs, which renders the patient euthyroid also reduces the "stress" of the disorder.[102, 298] Although antithyroid drugs may exert an immunosuppressive effect (see subsequent discussion), it is unlikely that long-term remissions are brought about by this effect. The action of the drug is over within hours yet long-term remissions last for weeks, months, or years. Thus, it seems more likely that return to the euthyroid state seems to relieve the effect of the hyperthyroidism itself on the immune system and may restore the immune system to its former competence.[102, 295b, 298] In other patients, the remission may be caused by associated continuing thyroid damage due to autoimmune thyroiditis, which interferes with the gland's ability to respond to the still present thyroid stimulating immunoglobulin. This group of patients will be "cured" by virtue of the thyroidal damage, which may go on further to spontaneous hypothyroidism.[295]

It is a curious observation that in a very few patients, the reverse sequence may be observed. A few patients who have suffered from primary hypothyroidism due to autoimmune thyroiditis have recovered from this condition and ultimately have suffered hyperthyroidism due to Graves' disease.[37, 65, 92, 98, 105, 107, 130, 142a, 187, 235, 297] The mechanisms that underlie this curious sequence have not yet been elucidated.

Diagnosis of Graves' Disease

Frequently, the diagnosis of Graves' disease is readily made. The classic features of this disorder are so widely known amongst physicians that when the condition is in full bloom, it is difficult to avoid recognition. However, it must be conceded that there are many patients who do not present with the classic picture of Graves' disease; when such patients have no exophthalmos and when the goiters may not be readily detectable, the symptoms may lead one to erroneous, often functional diagnoses. However, once the diagnosis of thyrotoxicosis has been entertained, the performance of appropriate laboratory procedures usually quickly elucidates the diagnosis. While thyroid function tests are considered in detail elsewhere in this text, some remarks are made here about the approach to be taken to the laboratory-based diagnosis of Graves' disease.[150]

In past generations, estimations of the basal metabolic rate (BMR) were commonly carried out, since the BMR is elevated in Graves' disease. However, the procedure is nonspecific and there are many methodologic problems, particularly the difficulty in achieving a "basal" state; thus, this procedure has been largely abandoned. Likewise, the Achilles reflex time, which is usually shortened in hyperthyroid patients, is far too insensitive to be of value and is not utilized generally.

The serum thyroxine (T_4) concentration is the most important and widely available test of thyroid function, and this test combined with a T3 resin uptake or some other measure of thyroxine-binding globulin (TBG) is the most common means of at least "screening" suspected patients of having hyperthyroidism. As procedures for performing the free thyroxine directly become available, these may replace the free thyroxine index as a first procedure.

In addition, the total serum triiodothyronine (T_3) will be useful in Graves' disease, since there is usually a markedly increased T_3/T_4 ratio.[52, 179, 302] The total T_3 is often disproportionately higher relative to the total T_4, owing to a disproportionate thyroidal production of T_3, and may account for the severity of hyperthyroidism in many patients with Graves' disease. This is not the case in hyperthyroidism secondary to thyroiditis, in which the T_3/T_4 ratio approximates that normally found, i.e., both hormones are *proportionately* elevated. Nearly all patients with hyperthyroidism have elevations of both serum T_4 and T_3 concentrations. In occasional patients with hyperthyroidism, the serum T_4 concentration will be found to be within normal limits, whereas the serum T_3 concentration is elevated (T_3 thyrotoxi-

cosis). T_3 thyrotoxicosis may occur in instances when there is limited iodide intake.

The clinical manifestations of T_3 thyrotoxicosis are similar to those of hyperthyroidism in general, but it cannot be regarded as a separate entity. The frequency of this variant clearly changes from region to region, and in Toronto, Canada, it is on the order of 5%. The converse situation, i.e., clinical hyperthyroidism with an elevated T_4 but normal serum T_3 levels ("T4 thyrotoxicosis"), has also been described.[147] These so-affected patients apparently were so ill that extrathyroidal T_4 to T_3 conversion was reduced sufficiently such that serum T_3 concentrations had declined to within the normal range. It is therefore possible that T_4 thyrotoxicosis, a rare finding, occurs in patients with hyperthyroidism complicated by other severe illnesses and primarily in elderly patients. Since there are other causes of elevated serum thyroxine and normal total serum triiodothyronine levels aside from hyperthyroidism, e.g., dysalbuminemic hyperthyroxinemia, antibodies to T_4, elevated TBG, and exogenous thyroxine therapy, it will be necessary to investigate such a patient more thoroughly to determine true thyroid status, as mentioned further subsequently.

The converse situation, i.e., normal serum T_4 and T_3 concentrations, may be found in a patient with TBG deficiency who is actually hyperthyroid.[173] Such a person would have low serum T_4 and T_3 concentrations when euthyroid but normal values when hyperthyroid. However, the T_3 resin uptake is elevated, and estimations of free T_3 and free T_4 indices would clearly demonstrate high values consistent with hyperthyroidism. Direct measurements of free T_3 and free T_4 would show similar elevated results.

The serum TSH determination is also useful to demonstrate hyperthyroidism. Heretofore, a low TSH value would not be sufficient of itself to indicate suppression of the thyroid gland by excess circulating thyroid hormones, since an undetectable TSH level by conventional radioimmunoassays is sometimes seen in completely normal persons.[150] At present, however, using double monoclonal antibody technology, a new method for determining TSH has been developed, termed an immunoradioabsorbent (IMRA) procedure.[214] This test is so sensitive that values below 0.3 milliunit/L may be considered low and consistent with thyroid gland suppression.

Utilizing even the conventional radioimmunoassay, it has been possible to determine that TSH is truly suppressed. The means of demonstrating this point is by utilizing the TSH response to TRH.[150] In normal persons, TSH responds two or threefold to the intravenous injection of 400 μgm of TRH. In hyperthyroidism, by comparison, the response is flat. The TSH response is also low or absent in a normal person who is given small doses of exogenous thyroid hormones. The response is also similarly flat in patients with autonomously functioning thyroid adenomas, despite normal levels of serum thyroxine and triiodothyronine. These observations are merely a reflection of the sensitivity of the pituitary TSH-secreting cells to inhibition by even the most minute increment of thyroid hormone.

Another means by which thyroid autonomy may be shown is by a thyroid suppression test. The demonstration of autonomy in this fashion is not a demonstration of hyperthyroidism per se but of merely the fact that some or all of the thyroid tissue is not under the normal control of pituitary TSH and, therefore, cannot be suppressed by inhibiting TSH.

The thyroid suppression test is usually done by determination of thyroidal radioactive iodine uptake before and after administration of 75 or 100 μgm of T_3 daily for 7 to 10 days. It would be normally expected that the uptake would fall by 50% or more after this administration. A lesser fall in uptake willl be indicative of thyroid autonomy, but this is not necessarily proof of hyperthyroidism.

Although estimations of thyroid stimulating antibody (TSAb) are not yet generally available, they are indeed useful in confirming that hyperthyroidism is specifically due to Graves' disease. Thus, if TSAb results, when determined by a bioassay that measures the generation of cAMP in thyroid cell membranes that are positive, it can be stated with assurance that that patient does indeed have Graves' disease.[293, 295] This procedure's results have been found to be positive in approximately 90 to 95% of patients with active untreated Graves' disease, although these results are certainly influenced by treatment.[295] The test is useful in following patients with Graves' disease in pregnancy, in neonatal Graves' disease, and in antithyroid drug therapy.

***In Vivo* Isotopic Tests.** In most forms of hyperthyroidism there is an increased thyroidal uptake of radioactive iodine. The 2-, 4-, and 24-hour radioactive iodine uptake values are

usually, although not invariably, elevated in Graves' disease. The test can be sharply influenced, however, by iodine intake, including radiographic contrast dyes, and by some drugs. Elevated radioactive iodine uptakes may be found in patients with other thyroid disorders, such as Hashimoto's thyroiditis; patients with enzymatic defects, and patients in the recovery phase of subacute thyroiditis. The radioactive iodine uptake in the thyroid suppression test has been discussed.

Thyroid scans are useful in confirming that the entire gland is involved in Graves' disease. In cases that are clinically obvious, in which a diagnosis can be made readily without such thyroid scanning, there is no great merit in doing such a procedure routinely.

Hyperthyroidism in Pregnancy

Hyperthyroidism occurs in about 2/1000 pregnancies and has been associated with a significant increase in the frequency of low birth weight infants.[41, 42] Thyrotoxicosis during pregnancy is more difficult to diagnose, and there are certain problems relative to treatment that are discussed in this chapter in the Management of Hyperthyroidism. Problems in diagnosis relate to the fact that the euthyroid pregnant woman may manifest goiter, sweating, and heat intolerance; moreover, test findings of thyroid function are appropriately disturbed, owing to the increase in thyroxine-binding globulin secondary to hyperestrogenism in normal pregnancy. Signs of true hyperthyroidism, such as weight loss, may be obscured by the weight gain of pregnancy.

Test results of thyroid function, as mentioned previously, may be disturbed in normal pregnancy. Thus, the BMR is about 20% greater in normal pregnancy. Moreover, the serum thyroxine and total serum triiodothyronine concentrations are elevated, whereas the T_3 resin uptake is normally reduced. Free thyroxine values should be normal.

In *hyperthyroid* pregnant women, the serum thyroxine and total serum triiodothyronine levels are often very markedly elevated, owing to the combined effects of increased TBG and the hyperthyroid state itself. The T_3 resin uptake is not usually elevated but compared with its normal low value in pregnancy, it is relatively higher and is often in the normal range.

It is of interest that Graves' disease often seems to be precipitated in the first trimester of pregnancy so that there may be a greater incidence of hyperthyroidism at this particular time. It is not clear what the factor or factors are that cause this precipitation of Graves' disease during the first trimester. In any event, during the *third* trimester, there is an amelioration of immune processes such that all antibodies decline during this period of gestation.[297] Thus, TSAb also usually declines, making it easier to control most patients with antithyroid drugs under these circumstances (see Antithyroid Drugs). In a small proportion of pregnant women with Graves' disease, however, the levels of TSAb, while perhaps lower in the third trimester, are still exceedingly high. Since these antibodies are IgGs, they traverse the placenta without difficulty and, when present in high titer, can cause fetal and neonatal hyperthyroidism.[293, 295, 297] (This problem is dealt with under Management of Hyperthyroidism). Following delivery, TSAb begins to rise, and there are often relapses that occur a few months after the end of gestation. (The nature of this relapse is discussed elsewhere in this text in relation to post-partum thyroiditis. Further maternal and fetal considerations are discussed under Management.)

Management of Hyperthyroidism

The availability of many methods for the management of hyperthyroidism permits a wide variety of therapeutic regimens, all of which result in either a temporary or permanent cure of the disease. There is a considerable difference of opinion amongst thyroidologists concerning the correct approach to treating Graves' disease and this should be kept in mind as the following recommendations for therapy are discussed.[70a]

Antithyroid Drugs

Antithyroid drugs can be employed as long-term therapy for hyperthyroidism or as interim therapy prior to thyroid destructive therapy surgery or radioactive iodine. While antithyroid drug therapy can be employed in forms of hyperthyroidism other than Graves' disease, there are special considerations in the latter that must be taken into account. In general, antithyroid drugs interfere with the production of thyroid hormone by the gland, and with appropriate dosage suppression of the disease can be effected.[54, 193, 331]

The drugs formerly or currently in use to treat hyperthyroidism are the following thioureylenes

of various types: the thiouracils—propylthiouracil (6-propyl-2-thiouracil, PTU), methylthiouracil (6-methyl-2-thiouracil, MTU), and thiouracil (2-thiouracil, TU)—and the imidazoles—methimazole (1-methyl-2-mercaptoimidazole, MMI) and carbimazole (1-carboxy-3-methyl-thioimidazole). They are structurally classified as thionamides (Fig. 13–8). Those drugs currently employed in North America include PTU and MMI. Propylthiouracil is available in 50-mg and 100-mg tablets and methimazole in 5-mg and 10-mg tablets. Propylthiouracil has the following actions:

1. It inhibits the peroxidase enzyme system of the thyroid gland, thus preventing oxidation of trapped iodide and subsequent incorporation into iodotyrosines and ultimately iodothyronine.
2. It inhibits coupling with iodotyrosines.
3. It inhibits the conversion of L-thyroxine (T_4) to 1-triiodothyronine (T_3) in peripheral tissues.[54, 193, 331]
4. It has a mild immunosuppressive effect as demonstrated experimentally.[202, 204, 206, 239, 305, 307] Indeed, Weetman and coworkers[307] have argued that an immunosuppressive effect is central to the action of antithyroid drugs, although further evidence suggests that this may not be of importance under actual clinical circumstances.[143, 145, 245, 281b, 295b, 299a, 314] The imidazole group lacks any peripheral effect on conversion of T_4 to T_3.[54, 193] Otherwise, the actions of these two drugs are similar.

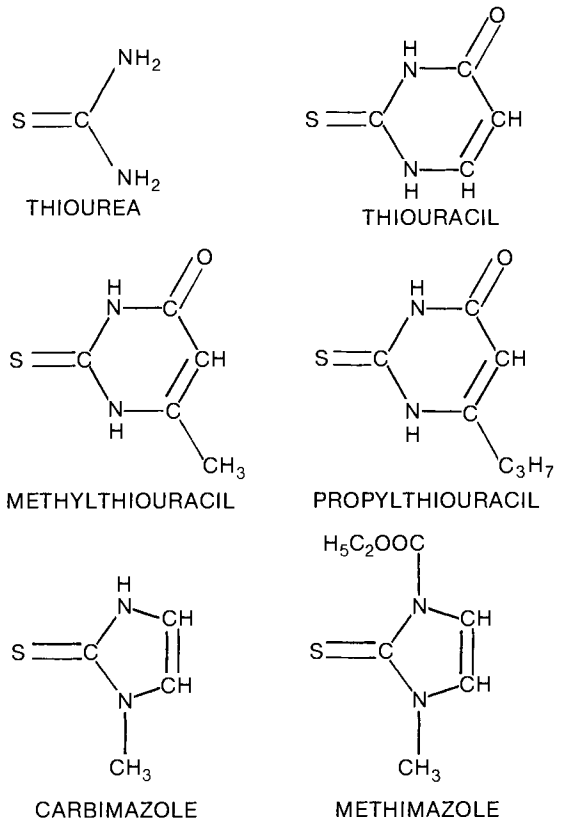

Figure 13–8. Structure of antithyroid drugs in common use.

The use of such agents as antithyroid drugs had its origin in 1941. In that year and in 1943, MacKenzie and associates[208, 209] noted that rats that were administered sulfaguanidine developed large goiters. The subsequent historical sequence of usage of thiourea and thiouracil by Astwood[10, 11] has been reviewed by Landau.[177] Subsequently, extensive studies of aromatic antithyroid compounds indicate that a free aromatic hydroxyl moiety is necessary for maximal antithyroid action.[275] Most experimental evidence supports the theory that antithyroid drugs act through their reducing action. Pitt-Rivers[231] states that the antithyroid action of any compound may be expressed as a function of its reducing power and its preferential reactivity with iodine, thus inhibiting the formation of thyroid hormone. However, other reducing substances, such as cysteine and glutathione, do not interfere with thyroid hormone synthesis, although their ability to reduce iodine is similar to that of thiourea.[11]

Studies with purified enzymes have shown that the thionamides are inhibitors of specific enzymes, such as thyroid peroxidase, and thus prevent organic oxidative iodination in this fashion. Taurog[274] has proposed that these drugs inhibit the formation of a peroxidase-iodinium complex and that this inhibition is competitive with iodide over a limited range of drug concentrations. These results suggested that the thionamide drugs bind to the enzyme either at the same site as iodide or at a nearby site and that this binding interferes with the binding of iodide. There is also evidence that the thyroid peroxidase responsible for iodotyrosine synthesis also functions in the coupling of iodotyrosine to form iodothyronines. This reaction is also inhibited by PTU and MMI. There is further evidence that the thionamides inhibit the sulfenyl-iodide iodinating intermediate, thus inhibiting thyroid iodinating reactions. However, there is no definite proof at present that a sulfenyl-iodide is involved in the iodination of thyroglobulin.[274]

In addition to its primary effect of blocking oxidative iodination in the thyroid, PTU but not MMI interferes with the peripheral deiod-

ination of T_4 to T_3. This inhibition of peripheral deiodination is associated with a decrease in the biologic effectiveness of administered T_4. The question of an immunosuppressive effect is discussed subsequently.

Absorption and Distribution in Humans. The absorption of thionamides appears to be very rapid and virtually total. The plasma half-life of MMI was found to vary between 6 and 9 hours, whereas that of PTU was between 1 and 2.5 hours.[192] Both of these agents were found to localize within the thyroid gland.

The duration of action of a single 5 mg dose of MMI in humans is longer than a 500 mg dose of PTU.[266] This finding may be explained partly by the shorter plasma life of PTU compared with MMI and its rapid conjugation with glucuronic acid.[193] Greer and colleagues[113–116] have proposed that a once daily administration regimen of PTU to a thyrotoxic patient is quite effective; while this may be surprising in the light of the aforementioned considerations, the important point has to do with the duration of action in the thyroid itself rather than the half-life in the blood. There is now sufficient evidence that a once daily regimen of PTU is indeed quite effective. Previous data, employing acute suppression of radioactive iodine uptake as an index, suggested that MMI was 100 times as potent as PTU. Subsequent clinical trials indicated that the potency rate might be in the order of 10:1.[54, 192]

Indications for Use of Antithyroid Drugs

1. Primary long-term therapy in an effort to bring about long-term remission.
2. Treatment for hyperthyroidism during pregnancy or during childhood and adolescence prior to making a decision regarding ablative therapy.
3. Treatment of hyperthyroidism so as to control the disease initially in a patient who will definitely require surgical or radioactive iodine therapy.
4. Treatment of neonatal Graves' disease until the TSAb level declines.

Each of these indications is discussed further.

It is evident that the thionamide drugs will bring about remissions far more commonly than will beta-adrenergic blockers. Remissions after antithyroid drug administration have been reported in incidences between 31 and 77%.[192] Clinically, one may predict that patients who are most likely to go into remission are those with small goiters and those with initial precipitations of hyperthyroidism by stress.[293, 295] A reasonably good correlation has been reported between a high remission rate and an absence of HLA-DR3,[20, 113, 201, 205, 278] although this finding has recently been challenged.[5] Moreover, if patients still manifest thyroid-stimulating antibodies at the end of their treatment period with antithyroid drugs, relapses are almost certain to occur.[27, 28, 41, 85, 136, 151, 173, 201, 204, 205, 207, 210, 211, 217, 224, 237, 269, 293, 295] In our clinic, therefore, patients under the age of 50 years with small goiters are selected for long-term antithyroid drug therapy. The mode of administration, follow-up, and duration is discussed subsequently, as is the possible nature of the remissions that occur in this disorder.

The management of hyperthyroidism in pregnancy is discussed separately. Children and adolescents are treated with antithyroid drugs for prolonged periods of years if there is no problem with compliance, side effects or response.[15] The objective would be to maintain them in a euthyroid state with this medication until remission occurs or until early adult life, since the ablative treatment of choice in this medical center is radioactive iodine. This is not employed for empirical reasons before the age of 20 years (see subsequent discussion). Otherwise, if there are problems in managing children or adolescents with antithyroid drug therapy (see Complications), subtotal thyroidectomy should be undertaken.[15] Treatment of passive-transfer neonatal Graves' disease is briefly considered subsequently.

In patients who have large goiters, it has been the experience of many[20, 201, 205, 278] that remissions are rare after antithyroid drug therapy. However, in our view there are theoretic reasons for utilizing this modality of therapy for at least several months before ablative measures are taken. This approach is based upon our perspective of the pathogenesis of exophthalmos, which is considered by our group and others to be a very closely related, overlapping, but *separate* organ-specific autoimmune disease.[183, 293, 295] If this is so, then it too would be aggravated by adverse influences on suppressor T lymphocytes. Since hyperthyroidism itself may represent such an adverse influence, it is wise to treat the hyperthyroidism with the objective of normalizing thyroid function. The objection to the use of radioactive iodine at an early juncture is that it appears to stimulate autoantibody production, in contrast to thionamides that appear to reduce

antibody production, perhaps by increased thyroid antigenic presentation by the disintegrating thyroid cells or by a direct but deleterious effect on suppressor T lymphocytes.[203, 229] It has thus become our practice to use antithyroid drugs for several months before radioactive iodine is administered in the hope that this may reduce the tendency to develop exophthalmos[320] or may reduce the progressive nature of the exophthalmos, although this proposal remains highly theoretic and unproven.

Patients over the age of 50, whether HLA-DR3 positive or negative, are treated in the same manner, with a few months of antithyroid drug treatment followed by radioactive iodine therapy. The same is done for patients with other associated disease, e.g., heart disease and diabetes mellitus, even for those under the age of 50. Patients who might go into a remission after a course of thionamide may relapse later, and elderly patients and those with other associated diseases may suffer further and severely with such relapses. Moreover, while post-^{131}I thyroiditis is rarely of clinical significance, the hyperthyroxinemia associated with the thyroiditis is avoided by prior antithyroid drugs therapy. In the categories of patients under consideration, even mild thyroiditis might aggravate associated diseases. It is for these reasons that patients in these categories are first treated with antithyroid drugs and then, ultimately, treated in a definitive manner with ablative therapy.

Dosage and Selection of Antithyroid Drugs. The usual starting dosage of methimazole or carbimazole is generally 30 mg daily. While this certainly can be given in divided dosages, Greer and associates[113–116] have shown that there is no benefit to be derived by such a regimen and recommends a single daily dose of this medication. Since the metabolism of this agent, as stated, is much slower than that for propylthiouracil, there is no benefit to be derived by dividing the doses during the day. Seidel and coworkers[255] have advocated even lower starting and maintenance dosages of methimazole than those usually recommended.[255]

The general practice with propylthiouracil is to give divided doses, particularly at the outset. The usual initiating dose is 100 mg, four times daily, although this can be varied, depending on the severity of the illness and the size of the patient. Dosages as high as 1200 mg/day have been employed, although it is my practice to initiate the medication with a standard daily dosage of 400 mg/day, to determine whether it is effectual, and to vary the dose accordingly. Even with propylthiouracil, however, Greer and colleagues[113–116] have recommended that it may also be administered on a once daily basis and that clinical results do not differ from a regimen based on divided dosage. Nevertheless, most physicians are more comfortable with multiple daily doses, although this is clearly unnecessary with methimazole. In any event, as the patient improves and as the thyroid function test results indicate high normal values, the dosage of the drug is reduced in a step-wise fashion. Quite often it is possible to maintain patients in a euthyroid state with as little as 50 or 100 mg of propylthiouracil once daily or 5 mg of methimazole once daily. Indeed, if patients cannot be readily managed in this fashion and continue to require high dosage regimens remissions are not to be anticipated, and ablative therapy is the ultimate therapy.

Carbimazole is rapidly converted to methimazole both *in vitro* and *in vivo*. It is for this reason that carbimazole is no longer to be found in the pharmacopeia of some countries.

Complications of Antithyroid Drug Therapy. Complications of antithyroid drug therapy relate to the following: (1) difficulties in controlling the hyperthyroidism of a few patients with reasonable dosage schedules, (2) problems with compliance, and (3) untoward reactions.[54, 193, 283, 331] We will deal only with the last item in this section.

Generally, toxic reactions will be observed in a very small proportion (about 4%) of patients, within 8 to 12 weeks of the initiation of a course of treatment and are very rare during prolonged therapy. However, they may be seen more commonly if the medication has been discontinued, only to be restarted several weeks or months later; the incidence of untoward side effects in the second or later courses is certainly much greater than in the initial course.

The most important complication is that of agranulocytosis.[54, 193, 283, 331] The incidence of this side effect is very low and may be on the order of 0.5%. This rare problem does not appear to be dose related and occurs quite precipitously. There is thus no point in performing weekly leukocyte counts on patients who take this medication. Generally, agranulocytosis is completely reversible if the medi-

cation is discontinued early after symptoms occur. It is thus very important to warn the patient about the symptoms, such as a severe sore mouth or throat associated with a fever. Even more rarely aplastic anemia and thrombocytopenia have been reported.

Other somewhat more common but less severe side effects include pruritus, urticaria, maculopapular rashes, arthralgia, and fever; rare effects include hepatitis and a transient form of apparent disseminated lupus erythematosus.[8, 271] Chevalley and associates[51] have found a total incidence of 4.3% of toxic side effects with methimazole therapy.

Instructions to Patients and Follow-up. Because of the aforementioned complications, it is very important to instruct patients what to expect from the thionamide drugs. Thus, patients must be instructed to watch for acute symptoms, which may be related to the complications described. These would include a severe sore mouth and throat, fever, joint pains, or rash. If they suffer any of these symptoms the medication must be discontinued at once and appropriate laboratory tests carried out. Most important, as mentioned, would be a leukocyte count, since agranulocytosis is the most severe and dangerous complication of these drugs, albeit a rare side effect.[154, 193, 283, 331] Incidentally, it should be emphasized that moderate granulopenia and leukopenia are common in active, untreated Graves' disease and are of no real concern.[312] It is only when the granulocytes virtually disappear that the condition is manifest.

If the agent is quickly discontinued, most patients recover without any specific measures. This is likewise true for the various other complications listed. The agent must not be started again on any account. Furthermore, while there is not complete cross-reactivity between the various thionamides, there is still significant chance that by changing from methimazole to propylthiouracil or vice versa, the same complication will ensue. Thus, when severe complications occur with thionamide drugs, further use of these agents should be eschewed.

Fortunately, most patients do not have any complications with these drugs and all but a few respond quite favorably in terms of improvement of the hyperthyroid state. Patients are monitored at first at monthly intervals and as improvement continues, at longer and longer intervals.

There is no established period of time that has been proved to be optimal in a course of antithyroid drug therapy. Remissions have been known to occur within several weeks,[114, 115] although it has been suggested that much longer periods up to 2 years provide the most optimal percentage of remissions.[272] It is generally found that half or less of all patients who satisfactorily complete a 1-year course have permanent remissions. The remainder need repeated courses of antithyroid drug therapy, need to be maintained on the drug indefinitely, or need to be provided with some other form of treatment.

Patients are initially seen every 4 weeks, examined, and samples for tests of thyroid function drawn. As clinical and biochemical improvement is observed, in our clinic it is customary to reduce the dosage of the thionamide so as to titrate dosage against response. This is in accord with the basic pharmacologic principle to use the minimal dosage of an agent that will cause appropriate clinical benefit. Thus, we are not in accord with the view that large doses of thionamide should be continued while thyroxine is added so as to prevent hypothyroidism.[143, 205, 244] If the patient does become hypothyroid the dosage of antithyroid drug should be reduced further.

If the objective is to attempt to keep patients on antithyroid drugs until they are in remission, the duration of treatment is subject to only arbitrary guidelines. Greer and associates[113–116] use only a short course of about 3 months and then stop the medication abruptly. In our clinic the medication is continued for 1 to 2 years, albeit at a low daily dosage. Ease of management with small doses; a reduction in the size of the goiter; a change in TSAb from positive to negative; and an absence of HLA-DR3, according to some workers, are all good prognostic signs favoring an immunologic remission.

After the patient has taken the thionamide for approximately 1 year, the medication is gradually withdrawn, and the patient is monitored at intervals thereafter. In some clinics a triiodothyronine-thyroid uptake suppression test is performed at the conclusion of the period of therapy. If the thyroid behaves normally that is evidence that the thyroid is under physiologic control and that immunologic remission has occurred. Most relapses are seen within 6 months, although some patients develop recurrent hyperthyroidism after several

years of remission (Fig. 13–9). The T_3 suppression test findings, if normal, certainly indicate at least a short-term remission but do not necessarily favor a permanent remission.[54, 193, 331]

Effect of Thionamides on Thyroid Autoantibody Formation. Antithyroid drugs bring about effects on the immune manifestations which are both direct and indirect. Long-term antithyroid drug therapy will cause the lymphocytes within the thyroid gland to decline in number or to disappear altogether.[21, 22] Furthermore, Pinchera and associates[229, 230] have noted a decline in long-acting thyroid stimulator (LATS) activity and in the thyroid autoantibodies in many cases of Graves' disease during the course of antithyroid drug therapy. A decline in thyroid antibodies associated with such therapy has also been noted by Feldt-Rasmussen and coworkers[185] and by Marcocci and coworkers.[194] Using thyrotropin-binding inhibitory immunoglobulin (TBII) or TSAb assays, similar results have been reported by other investigators (Fig. 13–10).[27, 28, 41, 85, 86, 136, 151, 173, 201, 205–207, 210, 211, 217, 224, 237, 269, 277] In these various series there was a trend towards normalization of results beginning as early as 1 month after the initiation of therapy. However, in all these series many patients showed no declines in these values during thionamide treatment, although this proportion varied from 16%[224] to 50%.[210, 211] Similar observations have been made with respect to cell-mediated immunity, using the migration inhibition factor (MIF) procedure.[133]

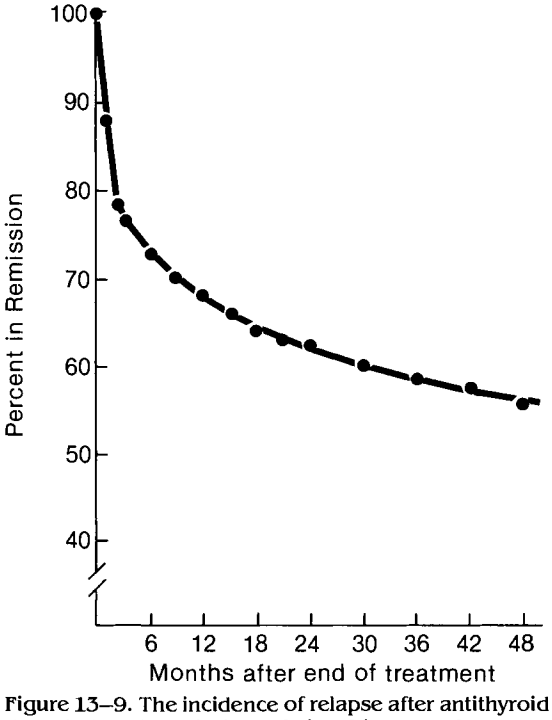

Figure 13–9. The incidence of relapse after antithyroid drug–induced remissions. It is of interest that most relapses occur within a few years after the initial remission. (Redrawn from Solomon, D. H., et al.: Prognosis of hyperthyroidism treated with antithyroid drugs. J.A.M.A. 152:201–205, 1953, with permission.)

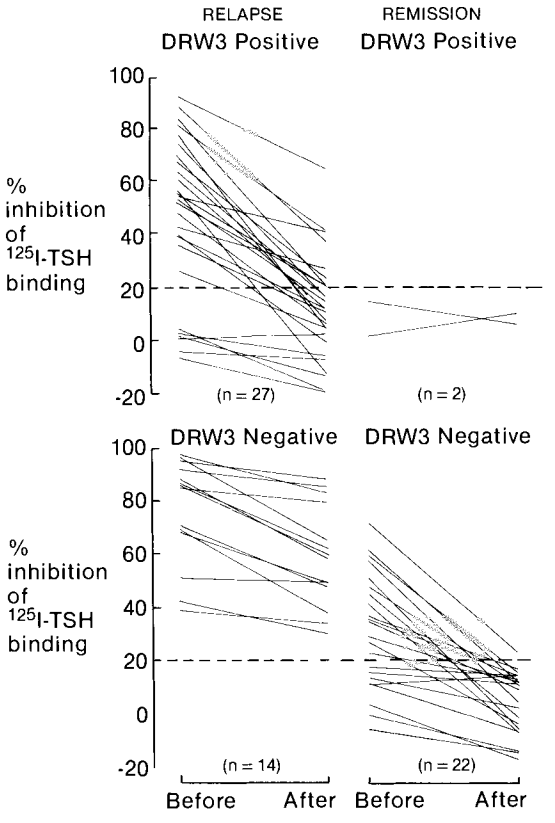

Figure 13–10. Response of the thyrotropin binding inhibitory immunoglobulin to long-term antithyroid drug therapy. Note the gradual reduction of these titers in time. Positive assays are lower than 80. Negative results are greater than 80. (From McGregor, A. M., et al.: Prediction of relapse in hyperthyroid Graves' disease. Lancet 2:1101–1103, 1980 (with permission.)

There is evidence that the antithyroid drugs are, at least weakly, immunosuppressive.[106, 122, 202, 206, 207, 239, 303, 305] McGregor and Weetman and associates,[201, 202, 204, 205–207, 305, 307] have suggested that this effect may account for the decline in TSAb and thyroid autoantibody titers and have argued that this effect is responsible for the remissions that occur after a course of antithyroid drugs (ATD).[307] Rennie and associates[239] have experimental evidence that methimazole reduced the inflammatory reaction in induced autoimmune thyroiditis. This group has also

shown evidence that this effect is mediated by preventing oxidative reactions within the macrophages (antigen-presenting cells).[305] This evidence strengthened their contention that the therapeutic effect noted in Graves' disease from antithyroid drugs is mediated by the immunosuppressive effects of these agents. However, such an explanation would not account for those patients who manifest no declines, although their thyroid hyperactivity may be simnilarly well controlled. Moreover, it would not suffice to explain the long-term remissions, which may persist following cessation of such therapy since the pharmacologic and immunologic effects would not persist after the drug was discontinued.[133] Moreover, Wenzel and Lente[314] have shown a decline in thyroid stimulating antibody during the treatment of Graves' disease with potassium perchlorate, which is a drug not known for any immunosuppressive activity, similar to that observed with thionamides. Furthermore, the intrathyroidal concentration of methimazole was considered too low by Jansson and colleagues[143] to have such an immunosuppressive effect on lymphocytes.

Two studies have shown that antithyroid drug causes no reduction in thyroid autoantibodies in patients with euthyroid Hashimoto's thyroiditis,[145, 245] thus contradicting an earlier study by McGregor and coworkers[202] on this point. Moreover, there is a reduction of HLA-DR (Ia) bearing activated T lymphocytes that occurs when patients take antithyroid drug for treatment of Graves' disease; this action of the antithyroid drug appears to be indirect (mediated through effects on the thyroid cell), since antithyroid drug applied to T lymphocytes *in vitro* has no such effect.[281a] Thus, while not ruling out an immunosuppressive effect of thionamides which may well occur, I feel that it is more likely that normalization of the thyroid status is a more important element in favorably influencing the immunologic disturbances in appropriate, susceptible patients. The complex manner by which antithyroid drugs might bring about remissions through their effects on the thyroid cells is depicted in Fig. 13–11.[281b, 295b, 299a] The effect of excessive thyroid hormone concentration on the immune system has been dealt with elsewhere,[102, 293, 295, 298] and it appears to be consistent with a direct or indirect adverse effect of hyperthyroidism per se on suppressor T lymphocyte function.

In most of the aforementioned series, the experience was that if a patient was still TBII or TSAb positive after prolonged antithyroid drug therapy despite a euthyroid state while on the medication, cessation of the drug was virtually always associated with almost immediate recurrence. When TSAb remained in the normal range, remissions were usually maintained, although some patients in this group would experience relapses.[224] Certainly, if a patient who is TSAb negative subsequently becomes TSAb positive, relapse will follow.[277, 278] Thus, these tests are of value for predicting remissions, but the value of the procedure seems to vary from laboratory to laboratory. Two notable exceptions were the study of Docter and colleagues,[69] which did not find the TBII to be of predictive value, and the study of Romaldini and colleagues[244] of TSAb, which reached similar conclusions.

McGregor and coworkers[201, 205] have confirmed the observation of Bech and coworkers[20] (Table 13–5) that those patients with Graves' disease with HLA-DRw3 are very much more likely to relapse after cessation of antithyroid drug therapy when compared with those without this gene. Of patients in remission (13 of 40) following such treatment, none was positive for HLA-DRw3 and all showed declines in TBII. Of the 27 patients in their study who relapsed, 22 (81%) were HLA-DRw3 positive, and the remaining five had persistent elevation of TBII activity at cessation of therapy (see Fig. 13–10). This group concluded that analysis of these two indices allowed prediction of relapse. Similar observations were subsequently made by Teng and associates.[277, 278] However, Allanic and coworkers[5] have found that HLA studies were of no predictive value in respect to remissions. (The relationship of remissions to the HLA-B and D genes is further discussed subsequently.)

The Nature of Remission in Graves' Disease

Even in the early accounts of Graves' (Basedow's) disease, it was recognized that the disorder was one of remissions and exacerbations.[220, 251] However, the nature of such remissions has been unclear heretofore and only recently has it been possible to at least formulate a hypothesis regarding the mechanisms involved.

It is clear that in Graves' disease there may be several forms of clinical remission.[40] One may be due to ^{131}I ablation or surgical removal of sufficient tissue to prevent recurrence by

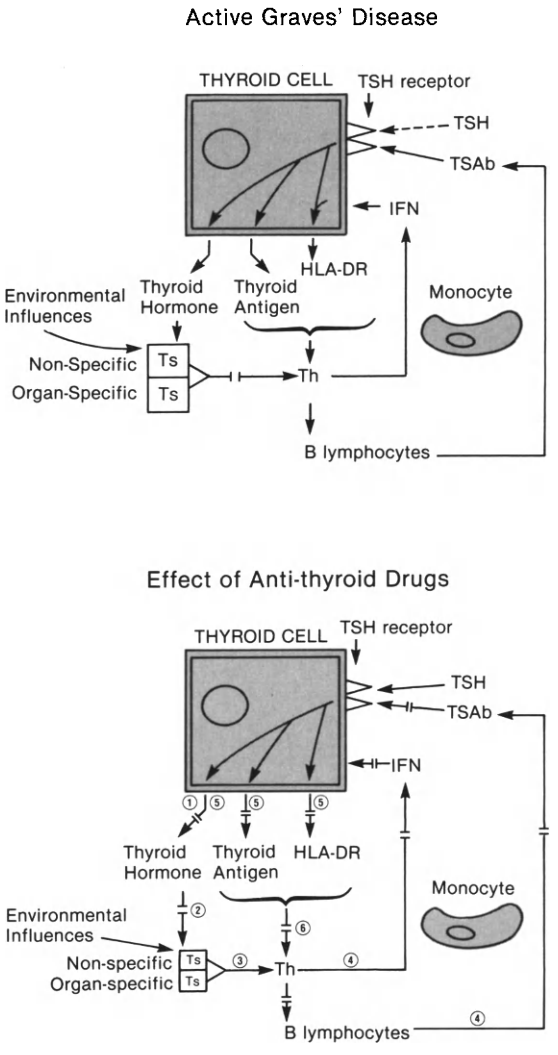

Figure 13–11. *A*, Hypothesis for the pathogenesis of Graves' disease (GD). The basic factor necessary for the development of GD is considered to be the human leukocyte antibody (HLA)–related, genetically induced, organ-specific defect in suppressor T lymphocyte (Ts) function. Precipitating factors from the environment (e.g., stress, infection, drugs, trauma) may cause a reduction in generalized Ts function and number, which are additive (superimposed) on the organ specific Ts defect. The resultant is to reduce suppression of a thyroid-directed helper T lymphocyte (Th) population. The specific Th will then in the presence of monocytes and, of course, specific antigen produce interferon gamma (IFN_γ) and will also stimulate specific B lymphocytes to produce thyroid stimulating antibody (TSAb). TSAb, similar to thyroid-stimulating hormone (TSH) stimulates the TSH receptor and will result in increased thyroid hormone production and increased thyroid antigen (e.g., microvillar antigen) expression. IFN_γ causes HLA-DR expression on the thyroid cell surface, and this effect is enhanced by TSAb and TSH. Thus, antigen presentation by the thyroid cell occurs directly, requiring the presence of the antigen and HLA-DR expression; this activates and stimulates the specific Th further and the cycle is repeated. Moreover, excess thyroid hormone acts on generalized suppressor T lymphocytes, reducing their number and function, further stimulating Th and adding to the cycle and, thus, perpetuating the disease. Without the specific Ts abnormality, however, continued cycling does not occur and the process would soon end. *B*, Induction of remissions in GD with antithyroid drugs (ATD). The sequence depicted in *A* is interrupted by ATD acting on the thyroid cells directly. The primary action is to reduce thyroid hormone production (1). This normalization of thyroid function releases the inhibition of nonspecific Ts and that function returns to normal (2); the additive effect on the basic organ specific Ts defect is thus lost. Suppression of the specific Th population is brought about (3) in that subset of patients who do not have severe organ-specific Ts defect. This results in a reduction of IFN_γ production and reduced Th stimulation of B lymphocytes to produce TSAb (4). The combined reduction of TSAb and IFN_γ will decrease thyroid antigen and HLA-DR expression, i.e., antigen presentation by the thyroid cell; there will also be reduction of thyroid cell hormone production (5). The reduced hormone production will further lessen Ts inhibition, decreasing the Th activity, and similarly reduced antigen presentation will have the same effect (6). This beneficial cycle will then repeat itself but will not occur in a patient with a severe organ specific Ts defect.

Table 13–5. The Association Between HLA-B8 and Dw3 and Remissions and Relapses after Antithyroid Drug Therapy in Patients with Graves' Disease*

Patients or Controls	HLA-B8		HLA-Dw3		Numbers
	Present	*Absent*	*Present*	*Absent*	
Normals	23.7%	76.3%	21%	79%	
Untreated Graves' disease	40 (47%)	46 (53%)	44 (51%)	42 (49%)	86
Patients with relapse after antithyroid drugs	26 (54%)	22 (46%)	31 (65%)	17 (35%)	48
Patients without relapse	5 (27%)	13 (73%)	6 (33%)	12 (66%)	18

*Reproduced from Bech, K., Lumholtz, B., Nerup, J., et al.: HLA antigens in Graves' disease. Acta Endocrinol. 86:510–516, 1977, with permission.

virtue of an insufficient thyroid remnant. However even without such destructive therapy, continuing spontaneous thyroid damage may bring about remission, probably as a result of a continuing immunologic process.[65, 142, 176, 328, 329] Other possibilities in the context of continuing immune processes include an alteration of TSAb to another, nonstimulating form of TBII, even with the TSH blocking propensities as described by Endo and associates[73] and Konishi and associates.[166] This sequence of events has not yet been proven to actually occur, but at least is within the realm of possibility. Another possibility, suggested by Van der Heide and coworkers[289] and Feldt-Rasmussen and coworkers,[85] is that immune complexes may arise that interfere with the ability of the antibody to produce its effects.

Another important form of remission is one in which all immunologic stigmata of the disease disappear, including thyroid autoantibodies, TBII, TSAb, and evidence of sensitization of T lymphocytes.[27, 28, 61, 86, 121, 136, 151, 173, 194, 201, 204, 206, 217, 224, 229, 230, 237, 269, 277, 278, 293, 295] It seems possible that this form of remission can occur only in a patient with a *partial* defect in immunoregulation, i.e., a partial defect in a single antigen-specific clone of suppressor T lymphocytes, a defect susceptible to further depression by "stress" and which is therefore reversible when that circumstance is overcome (see Fig. 13–11). There is also evidence that prolonged and severe hyperthyroidism per se has an adverse effect on suppressor T lymphocytes,[102, 293, 295, 295a, 298] thus acting as a self-perpetuating factor, after stress has initiated the disease possibly by the mechanism just described. The restoration of a euthyroid state by whatever means (e.g., antithyroid drugs, ^{131}I, surgery) should relieve the situation, since the possible effects of excessive thyroid hormones themselves directly on the immune system, as well as on the adrenocortical system, would be reversed under these circumstances. Similarly, social factors; rest; passage of time; clearing of infection; use of sedation and other nonspecific measures, such as beta-adrenergic blockers, would each serve to reduce "stress," thus allowing the partially defective immunoregulatory system to be restored to its previous functional capacity; thus, the thyroid-directed "forbidden" clone of helper T lymphocytes would again be suppressed and the disease would go into *immunologic* remission. Such mechanisms could also account for the spontaneous remissions that were observed long before any specific therapy became available.[220, 251] Of course, such patients would be prone to recurrence if similar "stressful" events were experienced later. Stress in this sense is used in a biologic (immunologic) sense, rather than in a purely emotional sense. Clearly "stress" is important only as it is perceived by the organism and in relation to the physiologic (e.g., hormonal, immunologic) response to it. What is required to prove this aspect is some sensitive means of measuring suppressor T lymphocyte function in response to such events in appropriate genetically predisposed persons.[3]

The person with a presumed *complete* defect would not be expected to go into immunologic remission, no matter how long antithyroid drug was continued or what form of therapy was employed. Only those remissions associated with continuing immunologic activity directed to the thyroid, thyroid destruction, or both would occur in this group. This observation would be in accord with the continuing evidence of humoral and cell-mediated immunity in some patients following antithyroid or other forms of therapy.[133]

There is some genetic evidence, previously mentioned, that is consistent with this concept. Irvine and colleagues[138] first showed in 1977 that those patients with Graves' disease who lack HLA-B8 are more likely to go into remission, whereas those patients who demonstrate HLA-B8 are likely to relapse. This was also confirmed by Stenszky and coworkers.[268] Similarly, Bech and associates[20] have shown that the presence of HLA-Dw3 is found in significantly higher numbers in those who relapse, and conversely in much lower numbers in those who remit (see Table 13–5). This finding, as previously noted, has been confirmed by McGregor and coworkers[201, 205] and Teng and coworkers,[277, 278] but not by Allanic and coworkers.[5] Thus, there may be a genetic basis for the ability to remit or relapse, which is also consistent with the aforementioned hypothesis for remission. Those with the presumed defect in immunoregulation may be more closely related to HLA-B8 and Dw3 (DR3), when compared to those who have only a partial defect and hence the possibility of undergoing immunologic remission. However, this point is now controversial and awaits further clarification.[5] The effect of hyperthyroidism on the immune system and the nature of the remission are more fully discussed elsewhere[102, 293, 295, 295a, 298, 299a]

Incidence of Remissions After Antithyroid Drug Therapy

Marchant and coworkers[192] have summarized the incidence of remission following thionamide drugs and have documented a range of between 31% and 77% from 1950 to 1973. Greer and colleagues[113–116] use a short-term antithyroid drug regimen and have likewise shown a 46% remission rate, which is virtually the average for the reported series.

This rate of remission exceeds that seen with propranolol quite significantly, where the remission rate has been reported to between 22 and 36%.[185, 196, 212, 228] For reasons already stated, it is unlikely that the thionamides will bring about remission by virtue of their immunosuppressive effects; normalization of thyroid function seems to be the more likely route by which remissions are effected.[299a] Thus, the difference between the remission rate brought about by thionamide and that by propranolol therapy may be the result of the normalization of thyroid function with thionamides which does not occur with beta-adrenergic blockers (see Fig. 13–11).

Relapses after remissions occur in about half of patients. Solomon and colleagues[261] have observed that in patients who were in definite remission following a single course of antithyroid drugs, 23% had a return of hyperthyroidism by the end of 3 months. However, after 3 months, the rate of relapse progressively declined and by the end of 4 years, 57% were still well (see Fig. 13–9). As mentioned, it is possible that relapses may be expected in patients who possess the HLA-DR3 antigen, thus providing a genetic basis for relapses versus continued remission.

Other Antithyroid Drugs

Lithium. Lithium salts have been found to have significant goitrogenic and antithyroid effects. These agents have been noticed to have calming effects in manic patients and are utilized widely in psychiatric practices. Lithium owes its antithyroid action in part to direct inhibition of thyroid hormone release,[100, 276] an action qualitatively and quantitatively similar to that of iodine.[276] *In vitro* studies have shown that lithium inhibits colloid droplet formation stimulated by cAMP, a critical step in hormone secretion.[255] It appears to prolong the fractional rate of disappearance of T_4 from the extrathyroidal pool, possibly by inhibiting T_4 to T_3 conversion.[155, 158, 263, 276] It has thus been suggested that lithium might be employed instead of thionamides when a rapid reduction of excessive secretion of thyroid hormone is required, such as in thyroid storm.[100, 158, 180, 263] However, a controlled trial has shown no advantage of lithium over moderate doses of thionamides.[170] Furthermore, many of the patients treated in this manner developed severe side effects, thus making lithium unacceptable as a routine antithyroid agent.

Potassium Perchlorate. Potassium perchlorate has not frequently been utilized for the treatment of hyperthyroidism in the past two decades, primarily because of early reports that aplastic anemia and nephrotic syndrome were not infrequent.[71a, 172] However, Wenzel and Lente[314] have studied potassium perchlorate and found that doses lower than 1000 mgm/day have fewer side effects than thionamides. Potassium perchlorate competes as a halogen with iodide for the thyroid trap and thus acts as an antithyroid drug. Wenzel and Lente[314] found that this agent was very effective, normalizing thyroid function as quickly as did the thionamide drugs and bringing about a decline in thyroid stimulating antibody activity as rapidly as did thionamides. Since there is no evidence that potassium perchlorate is directly immunosuppressive, this observation suggests that normalization of thyroid function, with a consequent positive effect on the immune system, may be the most important element in reducing thyroid stimulating antibody and bringing about remissions (see previous discussion). Clearly, this agent requires further study.

Iodides. The acute, transient inhibitory effect of iodide on thyroid hormone synthesis (Wolff-Chaikoff effect) depends on the intrathyroidal rather than the plasma iodide concentration and may be induced by the administration of approximately 2 mg of iodide to a normal subject.[265] However, the normal and hyperfunctioning thyroid soon escapes from the inhibitory effect of iodide on hormone synthesis by decreasing the active transfer of iodide into the thyroid.[307] A more important therapeutic effect of iodide is its ability to promptly inhibit thyroid hormone release, especially from the hyperfunctioning gland.[36, 221] Although iodides decrease hormone release and subsequently lower the plasma concentration of T_4 and T_3, thyroid hormone synthesis continues at an ac-

celerated rate, resulting in a gland rich in stored hormones.[72] Decreases in serum T_4 and T_3 concentrations occur for a few weeks but normal values are not always achieved.[72] Upon withdrawal of iodide, thyrotoxicosis will often become more severe, owing to the rapid release of stored hormones.[35] Iodides should not, therefore, be used for long in the treatment of thyrotoxicosis. The administration of 1 mg of iodide daily in addition to thionamide drug therapy may result in a more rapid fall in the serum hormone concentration during the early stage of treatment as compared with thionamide therapy alone.[152] Occasionally in some hyperthyroid patients large doses of iodide may aggravate the hyperthyroidism or indeed precipitate hyperthyroidism in persons previously euthyroid (Jod-Basedow's disease).[55] Patients with nontoxic goiter, typically in the presence of iodine deficiency, but now increasingly in its absence, may become thyrotoxic on iodine therapy.[288]

The two most commonly used iodine preparations are Lugol's solution, containing 5% elemental iodine and 10% potassium iodide, or a saturated solution of potassium iodide (SSKI) containing 1 gm of potassium iodide/ml. There is no documented advantage to using dosages in excess of 6 to 8 mg/day. Inorganic iodide, in the treatment of toxic diffuse and toxic nodular goiter, is reserved for the following: (1) neonatal thyrotoxicosis or thyroid storm, (2) preoperative preparation for thyroidectomy in association with thionamide, and (3) controlling serum thyroid hormone levels in the period after radioiodine therapy.[35, 192]

Finally, large quantities of iodide may result in toxic reactions including acneform rashes, salivary gland swellings, and gynecomastia.

Iodine-containing Radiologic Contrast Media and Drugs. Various radiologic contrast media that share a triiodo- and monoaminobenzene ring with a propionic acid chain (e.g., iopanoic acid) strongly inhibit the conversion of T_4 to T_3 peripherally. A similar "low T_3" syndrome has been described with amiodarone, a drug given in coronary artery disease. The effect of these compounds is not due to the iodine content but to competitive inhibition of conversion related to their structures, which resemble thyroid hormone analogues.[313] Sometimes, however, a combined effect is observed, with both iodide effects (e.g., Jod-Basedow's hyperthyroidism) and conversion inhibition effects. The result depends upon which element is most predominant. The potential of such agents as antithyroid drugs has yet to be fully explored; while preliminary evidence with iopanoic acid had suggested that this is of limited clinical value,[123, 247] another report indicated that sodium ipodate was effective and useful for long-term treatment of Graves' disease.[256a]

The Use of Antiadrenergic Agents in the Management of Hyperthyroidism

Although the adrenergic component of hyperthyroidism has been recognized for many years, antiadrenergic agents were not used in the treatment of hyperthyroidism until 1957. In that year, Canary and associates[43] first used oral or intramuscular reserpine to control many of the manifestations of hyperthyroidism in a small group of patients; reserpine is a *Rauwolfia* alkaloid that blocks the uptake and storage of norepinephrine and epinephrine, resulting in an overall depletion of tissue catecholamines. The agent appears to relieve the rapid heart beat, sweating, and heat intolerance within a few weeks after commencement of therapy. There is also some improvement in tremor, lid lag, stare, and convergence. Unfortunately, reserpine is rather slow in its onset of action, can actually aggravate some features of hyperthyroidism, and can lead to depression. It has thus gone out of common usage.[182]

Another agent that was studied in the 1960s was guanethidine, which acts at the terminal ends of the sympathetic nerve fibers to block the release of catecholamines. If used for any duration it also results in a depletion of stored tissue catecholamine. Levey[182] has summarized the effects of this agent in hyperthyroidism; it has been shown to cause limited decrease in the pulse rate, nervousness, fatigue, dyspnea, heat intolerance, and lid retraction. However, guanethidine is also slow to act, is not available in parenteral form, is associated with postural hypotension, and like other antiadrenergic agents can worsen congestive heart failure in patients with hyperthyroidism. In addition, patients treated with agents such as guanethidine and reserpine appear to be hypersensitive to the pressor effects of exogenously administered catecholamines and are thus more prone to anesthetic hazards.

Beta-Adrenergic Blockers. In recent years, the role of guanethidine and reserpine has been

usurped by the beta-adrenergic blocking drugs. There are several such drugs now commercially available of which D, L-propranolol was the first and still is the most commonly used drug. These agents block the beta-adrenergic receptors competitively, since they have chemical structures similar to the catecholamines. They thus compete for the available beta-adrenergic receptor sites in the various target tissues with the catecholamines.[182] Tissue catecholamine concentrations are therefore not depleted. Propranolol can be administered orally or intravenously.[60] The peak effect of this drug following oral administration occurs within 2 hours with a half-life of approximately 3 hours.[78] The peak effect with the intravenous administration of the agent is observed within minutes; consequently, these agents are advantageous in the initial management of severely and acutely ill patients with hyperthyroidism.[60, 182] The dosages of propranolol required to relieve those symptoms of hyperthyroidism related to adrenergic causes are variable. Dosages varying between 40 and 60 mg/24 hours, in divided doses, appear to produce sufficient blockade in most hyperthyroid patients.[182] In thyroid storm, intravenous doses of 1 to 2 mg have been given using careful electrocardiographic monitoring.[602] Generally, no further drug should be given for at least 4 hours.

The beta-adrenergic blockers will predictably and significantly reduce the cardiac rate although rarely to normal basal levels. The reason may be explained by the chronotropic effect of thyroid hormone on the heart independent of adrenergic stimulation of the heart. The drug also ameliorates nervousness, hyperactivity, sweating, and tremor. In consequence, these agents have proved to be valuable in the acute control of these symptoms, before other agents (e.g., thionamides) have had the time to induce positive clinical effects. In addition, these agents are sometimes helpful as preoperative therapy, in periods of diagnostic testing, in allergy to thionamides, in conjunction with ^{131}I therapy, and in treatment of thyroid storm.

The Effect of Beta-Adrenergic Blockade on Thyroid Function. There appears to be very little effect of these agents on the levels of serum thyroxine, the peripheral metabolism of thyroxine, or intrathyroidal iodine metabolism.[12] Moreover, the propranolol has no significant effect on oxygen consumption or on the urinary loss of calcium, phosphorus, or hydroxyproline.[99] Moreover, Ericksson and coworkers[77] have shown that propranolol alone will not prevent thyroid storm. It should be noted that propranolol does not reduce thyroid stimulating antibody,[60] although patients *who go into remission* after prolonged propranolol therapy do have a drop in thyroid stimulating antibody values. The serum thyroxine and radioactive iodine uptake values are unaltered by such therapy, although in the minority (about 20%) of patients who go into remission after propranolol therapy, these values fall to normal.[185, 196, 212, 228] (The possible mechanism by which remissions occur has been discussed.) There is some evidence, however, that the conversion of thyroxine to triiodothyronine is partially blocked.[291] This effect seems quite minimal in magnitude.

Contraindications. Propranolol is contraindicated in patients with congestive heart failure since catecholamine stimulation is important for the maintenance of cardiac output. If the cardiac failure in hyperthyroidism relates specifically to tachycardia, then beta-adrenergic blockers may actually ameliorate congestive heart failure. Propranolol is contraindicated in patients with evidence of bronchospasm and thus should not be prescribed in patients with asthma, chronic obstructive lung disease, and so forth. Other patients in whom contraindications exist are those with sinus bradycardia, those receiving monoamine oxidase inhibitors or tricyclic antidepressants, and those with spontaneous hypoglycemia. There is also some evidence that propranolol should not be prescribed to pregnant women, as it may cause prolonged gestation and labor. The drug is also occasionally associated with side effects such as nausea, vomiting, anxiety, insomnia, lightheadedness, bradycardia, and hematologic disturbances.[182]

Radioactive Iodine in the Treatment of Hyperthyroidism

Radioactive iodine has been used for the therapy of hyperthyroidism for over three decades. ^{130}I was the first isotope of iodine to be used for this purpose in 1941; since it had a very short half-life of 12 hours and was produced using cyclotrons, its therapeutic use was limited.[46] The availability of ^{131}I from nuclear reactors, commencing in 1946, has spread around most of the world, and huge numbers of patients have now been treated with this

isotope. More recently, a small number of patients have also been treated with ^{125}I, but as noted subsequently, there are disadvantages with this isotope, and it will likely never reach general favor.[38, 117, 118]

Treatment with ^{131}I. ^{131}I is primarily a beta emitter and only much less so is it a gamma emitter (Table 13–6).[323] The isotope, available as sodium iodide 131, is quickly absorbed after oral ingestion, briefly concentrated in the gastric mucosa, and then concentrated in the thyroid. While there is also uptake by the salivary glands, the thyroid gland is the only organ in which organification of the absorbed iodide takes place. Thus, the appearance of radioactive iodine in the gastric and salivary gland cells is transitory and the only prolonged effect takes place within the thyroid gland.

Table 13–6. The Photon and Electron Radiations Emitted by ^{131}I and ^{125}I*

Radiation	Energy (keV)	Mean Number per Distintegration (%)
^{131}I		
Beta electrons	191.7	90.4
	95.5	6.9
	70.1	1.6
Gamma rays	722.9	1.6
	637.0	6.9
	364.5	83.3
	284.3	4.8
	80.2	1.7
Internal conversion electrons	329.9	1.7
	45.6	2.9
X-rays	29.8	2.5
	29.5	1.3
Auger electrons	3.2	4.9
	0.9	11.7
^{125}I		
Gamma rays	35.5	6.8
Internal conversion electrons	34.7	8.0
	30.9	10.7
	3.7	74.6
X-rays	31.8	4.1
	31.0	19.9
	27.5	73.8
	27.2	37.8
	3.8	21.5
Auger electrons	30.2	0.9
	26.4	5.8
	22.7	13.7
	2.9	14.9
	0.8	35.9

*Reproduced from Wilson, G. M.: The treatment of thyrotoxicosis with radioiodine. In: The Thyroid, Physiology and Treatment of Disease, International Encyclopedia of Pharmacology and Therapeutics, Sect. 101, Hershman, J. M. and Bray, G. A. (eds.), Pergamon Press, New York, pp. 253–270, 1979, with permission.

The therapeutic effect depends on the beta emission and the range of 400 to 2000 μm exceeds the follicular diameter considerably.[323] Thus, both the nucleus and the cytoplasm of the thyroid cells are irradiated. ^{131}I within one follicle will irradiate adjacent follicles. However, structures outside the thyroid gland will not be damaged.

There have been several studies of the effects of ^{131}I irradiation on the thyroid gland both in humans and in experimental animals.[56, 57, 68, 323] These show evidence of cellular necrosis, breakdown of follicles, development of bizarre cell forms, nuclear pyknosis, and destruction of small vessels within the gland. Many abnormal cellular forms persisted for several years. Edema of the interstitial tissue and extensive fibrosis become evident.

Investigations in animals have indicated that these changes relate to the initial dosage. Radiation reduces the life span of the irradiated cells and prevents cellular division. The capacity of these cells to exercise their function and produce thyroid hormone persists for as long as they survive,[4] but at the termination of their life span, the radiation impairs their ability to divide and thus renew themselves.[117] This radiation effect appears to be important as a cause of late hypothyroidism many months or many years after ^{131}I therapy. However, as noted subsequently, it may not be the sole factor in bringing about late hypothyroidism.[190]

Practical Considerations. Radioactive iodine is a most convenient form of therapy, since it can be administered to an outpatient, without discomfort or ill effects. If sufficient radioactive iodine is administered, it will induce cure of the hyperthyroidism or indeed hypothyroidism in all patients with toxic goiter. As previously mentioned, the action of the isotope depends upon the destruction of thyroid tissue by internal radiation resulting from disintegration of the isotope. Aside from its efficacy and ease of administration, the vocal cords and organs other than the thyroid gland are not injured, and hypoparathyroidism following ^{131}I is exceedingly rare.[317] As the thyroid gland is the only tissue to retain iodine for any prolonged interval, it is an ideal organ for the use of therapeutic radioactive iodine. ^{131}I, with a half-life of 8 days,[317, 323] has been the isotope of choice since it became available, after a very brief experience with the short-lived ^{130}I.[46] However, when the atomic pile became available after World War II, ^{131}I became es-

tablished as an effective agent to control hyperthyroidism.

Possible Disadvantages of ^{131}I Therapy. Following the advent of ^{131}I therapy, the treatment was initially confined to those over 45 years of age.[323] This was done because of a concern about the possible subsequent induction of neoplastic thyroid disease, as well as a concern about radiation of the gonads with subsequent congenital abnormalities of newborn children.

There is no doubt that ionizing radiation is a cause of thyroid carcinoma both in experimental animals and in humans.[63, 195] However, the dosages that have brought about this high incidence of thyroid malignancies have been *low dose* irradiation (from 50 to 2000 rad). This observation occasioned great concern that the comparatively large dose radioactive iodine therapy for hyperthyroidism, given generally in doses from 6000 to 12,000 rad,[323] might induce thyroid carcinoma frequently in those so treated. However, Maxon and associates[195] have suggested that radioactive iodine as a source of thyroid neoplasia seems to be much less damaging on a rad/rad basis when compared with external radiation. In any event, this possibility, namely that radiation therapy from ^{131}I in the treatment of hyperthyroidism might lead to neoplasia of the thyroid, has been laid to rest. The Thyrotoxicosis Follow-up Project of the Radiation Health Section of the National Institutes of Health has shown no increased incidence of thyroid carcinoma in patients treated with ^{131}I as late as 20 years thereafter.[67] Indeed, there appeared to be a reduction in the expected number of such cases.[323] The reason for this finding appears to be that the large doses administered *reduced* the ability of the thyroid cells to undergo mitosis, thus decreasing the chances of neoplasia but increasing the chances of hypothyroidism. The major practical disadvantage of this treatment modality is the high incidence of hypothyroidism, which may occur early after the therapy or may appear gradually years or even decades later (see subsequent discussion).[190, 317, 323]

According to Werner,[317] 1 to 5% of patients treated with radioactive iodine develop the onset of exophthalmos or flare up in activity of eye changes, particularly with moderate to severe exophthalmos. This finding may be due to an effect of ^{131}I on intrathyroidal suppressor T lymphocytes, leading to an increase in autoantibody production (Fig. 13–12).[203, 229, 230]

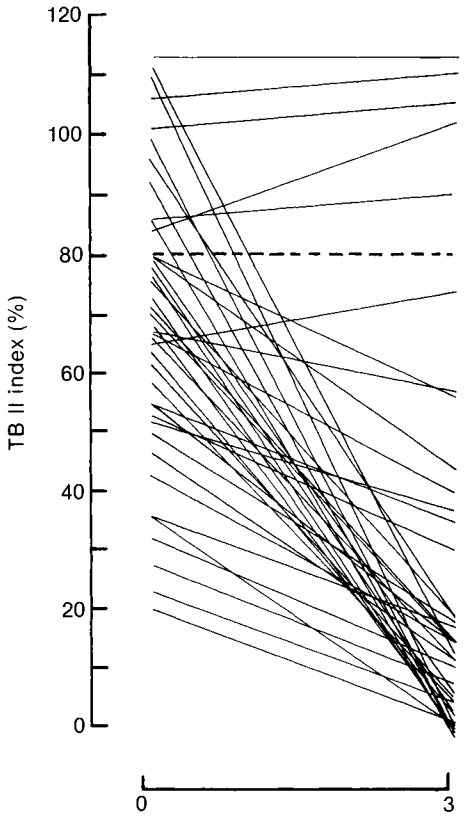

Figure 13–12. The effect of radioactive iodine therapy for Graves' disease on the titers of thyrotropin binding inhibitory immunoglobulin. Note that following the therapy, there is a rise in the titers, followed ultimately by a decline. (Results are expressed as in Figure 13–10.) (Redrawn from McGregor et al.: Effect of radioiodine on the thyrotrophin-binding inhibiting immunoglobulins in Graves' disease. Clin. Endocrinol. 11:437–444, 1979, with permission.)

Another concern has been expressed with respect to gonadal irradiation. However, there has been no increased incidence of mutations or congenital defects in children born to parents who have had radioactive iodine when compared with the general population. The dose to the gonads is clearly related to the blood dose, and in a patient who receives repeated treatments with ^{131}I for persistent hyperthyroidism, this dose may reach above 250 rad.[29] Robertson and Gorman[243] have calculated that the average dose of radiation to each ovary is approximately 0.12 rad/mCi, however. Thus, the conventional dose of radioactive iodine delivers approximately the same dose of radiation to the ovaries as does roentgenologic examination of the colon or kidneys. The dose of radioactive iodine usually

administered in hyperthyroidism would thus produce only a small amount of gonadal radiation and as a "genetically significant gonadal dose," this source does not contribute nearly as much to the whole of the North American population as does diagnostic radiology.[243, 294] Nevertheless, studies of peripheral leukocytes have shown persistent chromosomal damage after ^{131}I related to the dosage.[264] Calculations have suggested that the contribution of radioactive iodine for the treatment of hyperthyroidism to the genetically significant gonadal dose is only about 0.3% of the natural background.[243, 294, 323] Robertson and Gorman[243] have further estimated that the maximal increased risk for a mother who received ^{131}I therapy for Graves' disease of having a child with a harmful trait would be 0.003% or less, whereas the spontaneous risk of such abnormalities is 0.8%.

The increased incidence of leukemia predicted to follow the use of radioactive iodine in hyperthyroidism has not materialized.[249] There is in fact a slightly increased incidence of leukemia in patients with Graves' disease generally, no matter what form of treatment is prescribed and it is unrelated to the treatment itself. However, the incidence of leukemia in patients treated with radioactive iodine for hyperthyroidism and those treated surgically did not differ in a large follow-up study of 36,000 patients.[249]

Indications. For all of the aforementioned reasons, the age at which radioactive iodine has been prescribed for the treatment of hyperthyroidism has fallen considerably. While in Great Britain and Europe many clinicians do not employ this modality in patients under the age of 40, in North America the general rule is to treat patients over the age of 20.[294, 317, 323] Indeed in some clinics in the United States, even children are being so treated.[84, 124] In my view, ^{131}I is the ablative treatment of choice for Graves' disease after age 20. However, it is believed that all patients should receive antithyroid drug therapy first, with the expectation that those who will enter remission will obviously not require ^{131}I (see previous discussion). Since relapses may have more severe consequences in older patients, these considerations apply to those under age 50. After that age, the intention is to employ thyroid ablative therapy *without* permitting the patient to go into a remission after antithyroid drugs.

Practical Sequelae. Aside from the theoretic problems referred to previously, there are some practical sequelae that are of considerable importance. One is the increasing incidence of hypothyroidism in the years following therapy even despite initial induction of euthyroidism. Approximately 50% of patients are hypothyroid by 10 years and the incidence is continuing to climb. Dunn and Chapman[71] have reported that about 20% of patients are hypothyroid within the first year, with approximately 2.5% becoming hypothyroid each year thereafter. Follow-up must be continued not only in the early months following radioactive iodine therapy but also on an annual basis for life. Since the onset of hypothyroidism may be subtle, the patient must be exhorted to return for annual examinations to check the thyroid status. However, once patients have become hypothyroid and are taking thyroxine therapy, the major point of follow-up is to ensure that they continue to take their medication.

Radiation thyroiditis with tenderness in the neck is unusual after conventional doses for hyperthyroidism.[47] Occasionally with large doses, even severe painful thyroiditis has been observed, beginning 3 to 4 days after the administration of the agent.[47]

Treatment Considerations. Because the beta rays travel only about 2 mm, the ^{131}I within the thyroid gland will not damage surrounding structures, and therefore considerable radiation can be applied to the gland. The radiation destroys some cells, leaves others intact, and in some effectively reduces the synthesis of hormones. It has many of the following advantages: the treatment is usually definitive, it is convenient and can be generally administered without admitting the patient to hospital, it avoids the morbidity and complications of surgical treatment, and only rarely is ^{131}I administration followed by radiation thyroiditis.

The first clinical effects of radioactive iodine become evident no earlier than 1 month, with gradual improvement following treatment in most patients. The goiter usually disappears after one dose, and most patients are cured with a single dose; most of the remainder require only a second dose.[299]

Thus, it is important to define as closely as possible the factors that may determine the outcome of radioactive iodine treatment. The most important of these factors is the radiation dose received by the thyroid gland. Formulations for radiation therapy require the gather-

ing of certain data as well as certain assumptions for the calculation of the mean absorbed dose. These include (1) the thyroid weight, (2) the total thyroidal uptake of the radioactive iodine therapy dose, and (3) the duration of time in which the radioactive iodine will remain in the tissue (i.e., biologic half-life).

Other pertinent factors relate to the physical characteristics of the isotope used. In terms of ^{131}I, the half-life is 8 days and other physical characteristics are detailed in Table 13–6.[323]

Some aspects of thyroid physiology, however, cannot be ascertained in any precise fashion, e.g., the degree of activity of the thyroid gland and the degree of function of individual follicles within the gland. There is some variation in individual sensitivity to the radioactive iodine, and consequently one cannot predict with precision what the effect of a specific dose will be in any one individual. Since the major effects of ^{131}I on the thyroid gland are due to the beta rays, as noted, one can calculate, approximately, what the delivered radiation dose will be. Quimby and Feitelberg[234] have devised a formula as follows:

$$\text{dose (rad)} = \frac{90 \times \mu\text{Ci administered} \times \text{24-hour uptake}}{\text{gland weight (gm)} \times 100}$$

General experience suggests that a dose between 5000 and 10,000 rad is appropriate for the treatment of the diffusely enlarged thyroid gland encountered in Graves' disease. This dose would be equivalent to approximately 60 to 100 μCi per estimated gram of thyroid tissue. Once a dose of μCi/gm has been determined, the following simplified expression may be used:

$$\text{administered dose } (\mu\text{Ci}) = \frac{\mu\text{Ci/gm desired} \times \text{gland weight} \times 100}{\%\text{ uptake at 24 hours}}$$

With some experience the weight of the thyroid gland can be estimated. Such estimates are within 10 to 20% of actual gland size if the gland is 50 gm or less in size. This simplified system has been termed a "guesstimate." Thyroid scanning and ultrasonography may be of value in assisting with this estimate.

There is very considerable variation in turnover rate in the thyroid gland, making sophisticated dosage formulations of less value than heretofore expected. Most simply, 80 μCi per estimated gram of thyroid tissue may be administered when the 24-hour radioactive iodine uptake is approximately 50%. If the radioactive iodine uptake is considerably higher than this amount, the dosage is reduced somewhat. If the uptake is lower than 50%, the dosage is increased slightly. Generally, physicians have tended to overtreat patients with small goiters and thus increase the incidence of hypothyroidism. In comparison, large goiters are often undertreated. Although this "guesstimate" technique seems primitive, it seems to provide results that do not differ greatly from those obtained with more sophisticated formulations. Moreover, no great advantage seems to be gained by reducing the dosage because this procedure merely increases the number of patients who are hyperthyroid 3 months after the initial dosage; yet it does not prevent the late onset of hypothyroidism once the hyperthyroidism has been controlled, although it may delay that onset for several years (Fig. 13–13).[23]

Several variables have been shown to influence the outcome of radioactive iodine therapy. Men, for example, are less likely to have hypothyroidism than women, and blacks appear to be more resistant than others to ^{131}I.[23] Large thyroid glands appear to be more resistant than small thyroid glands, but there is a tendency to underestimate their weight.[299] Finally, patients who are most severely hyperthyroid also tend to be more resistant, and it has been suggested that this finding may be related to high levels of thyroid stimulating antibody.[210, 211]

It further should be emphasized that, in my view, it is always wise to have employed a course of antithyroid drugs prior to the administration of radioactive iodine (as previously discussed). The various reasons stated for this view will not be reiterated. Many clinicians, however, have been willing to prescribe radioactive iodine as primary therapy.[23, 24] All are agreed, however, if there is some other associated disorder, such as cardiac disease, severe diabetes mellitus, pulmonary disease, and so forth, or if there is very severe thyrotoxicosis, a course of antithyroid drug therapy must be given prior to the use of ^{131}I.[24, 294, 323] The prior use of antithyroid drugs in this manner generally prevents a sudden rise in circulating thyroid hormones, following the administration of the isotope owing to extensive rapid destruction of the thyroid gland. However, at the time of ^{131}I treatment in such patients, it is wise to

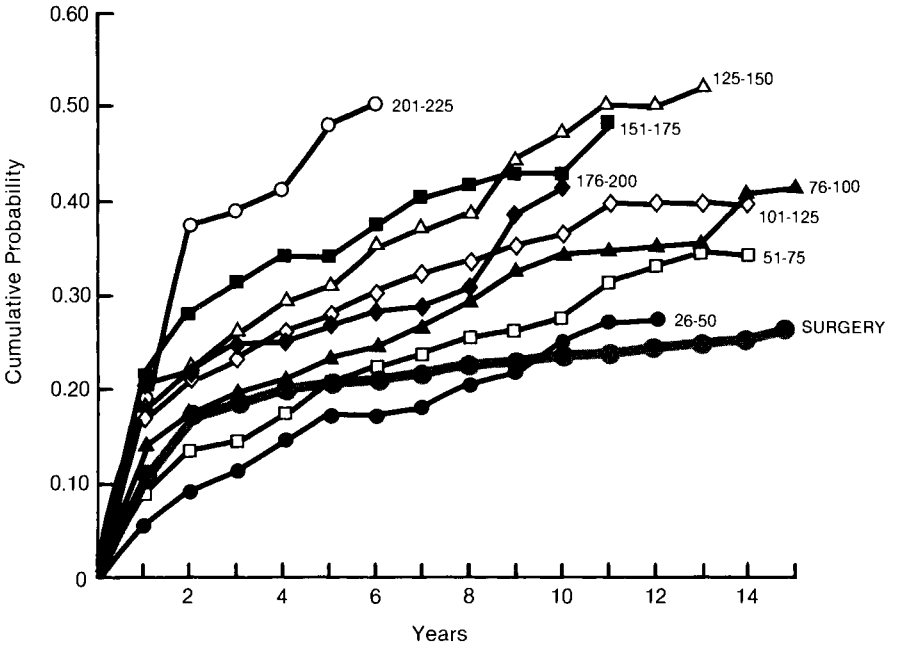

Figure 13–13. Experience of the Cooperative Thyroid Follow-Up Study initiated by the Bureau of Radiological Health of the United States Public Health Service. The 6000 patients included in this study had no previous treatment for hyperthyroidism and received a single dose of ^{131}I; the number of patients in each dose category averaged 750. The cumulative probability of becoming hypothyroid is plotted on the ordinate; on the abscissa is the interval in years from the time of administration of the ^{131}I. Radioiodine dose is indicated on the chart from the lowest of 26 to 50 μCi per estimated gram (μCi/gm) of thyroid to the highest of 201 to 225 μCi/gm; these assessments refer to μCi of ^{131}I delivered to the thyroid and not to the oral dose. The heavy line shows the appearance of hypothyroidism following surgical treatment in 5200 patients. After the third year, the probability of becoming hypothyroid, if less than 125 μCi/gm of gland had been given, was 2.3%/year; from the second year after surgery, the average probability of becoming hypothyroid was 0.7%. (Redrawn from Becker et al.)

administer a large dose of radioactive iodine sufficient to assure ablation of the thyroid gland with a single dose. Thus, for patients of this nature, doses in the range of 15 to 30 mCi may be given, depending on the size and condition of the thyroid.[323] In addition, those who have experienced severe recurrences of the disease after surgery should also be given such large doses of ^{131}I with the certainty of cure of the hyperthyroidism even though it is associated with a high incidence of subsequent hypothyroidism.[323]

^{125}I Therapy for Hyperthyroidism. The higher energetic beta-emission of ^{131}I as compared with ^{125}I combined with gamma photons of ^{131}I results in a rather uniform distribution of the radiation dose in the individual thyroid cell.[248] Since, as stated previously, the reproductive capacity of thyroid cells is more radiosensitive than the functional aspects, it seems unlikely that a decrease in the functional integrity can occur after administration of ^{131}I without ultimate cell death and eventual hypothyroidism.

However, ^{125}I is theoretically quite different from ^{131}I. Most of the radiation effect is probably due to the very low energy conversion electrons and auger electrons. The lower energy auger electrons (0.8 to 2.9 KeV) are particularly abundant. The range of these electrons in tissue is in the order of 0.4 μm to 20 μm only. Since the radioactive iodine is primarily in the colloid, the radiation dose distribution is markedly nonhomogeneous, affecting the cellular cytoplasm at the apex of the cell to a much greater degree than the nucleus.[323] Cell division might thus be preserved, and the relentless increasing incidence of hypothyroidism with the passage of time might be avoided.[117–119] However, even theoretically there was concern that damage to the secreting portion of the cell may be repairable if the nucleus remained intact, and thus only transient control of hyperthyroidism might be obtained.[117–119, 248, 249]

Several workers have put these considerations to the test. Greig and his colleagues[118] found that a mean dose of 38.2 mCi of ^{125}I

brought about euthyroidism within 8 weeks. There was a clear dose-response relationship; patients treated with larger amounts of ^{125}I showed a more prompt response but a higher incidence of hypothyroidism, while those treated with smaller dosages had the reverse situation. Long-term follow-up of these patients suggested that the incidence of hypothyroidism was increasing with time, although the experience is far too small to determine whether the incidence of hypothyroidism is comparable to that seen with ^{131}I. In any event, the theoretic considerations suggesting that there might be more favorable therapeutic properties of ^{125}I have not been confirmed in actuality. Rapid and permanent control of hyperthyroidism is thus associated with a high subsequent incidence of hypothyroidism. Thus, the place of ^{125}I in the treatment of this disorder cannot be established as yet. Moreover, it is not known whether there are late complications different from those seen with ^{131}I. There does not appear to be any convincing argument for studying this isotope further at this time with respect to its possible therapeutic role in hyperthyroidism.[47, 48]

Subtotal Thyroidectomy

Historically, thyroidectomy was the first effective treatment for hyperthyroidism. However, it did not become very feasible until Plummer[233] in 1923 demonstrated the value of iodine in the preparation of patients for surgery. Iodine was shown to reduce the vascularity of the gland and to produce a temporary involution.[113] It produced these effects by interfering acutely with the release of thyroid hormone and with the biosynthesis of thyroxine.[36, 72, 221, 265] Interference with the biosynthesis of thyroxine was prolonged in some patients and persisted almost indefinitely in at least a small number of patients when the iodide therapy was continued. In others, however, there was a problem with "iodine escape," i.e., an exacerbation of the hyperthyroidism by the excess amount of substrate available. In any event this form of preoperative preparation with iodide alone made it possible to operate in a patient who was no longer severely hyperthyroid, which reduced the incidence of complications considerably. Later the introduction of antithyroid drugs made it possible to have an even more effective preoperative preparation.[10, 117] It is clearly important to render the patient euthyroid before embarking on the surgical procedure. Since antithyroid drug therapy has been discussed extensively, it will not be further described at this point. The use of iodide therapy has likewise been discussed as an adjunct to antithyroid drug therapy just prior to the actual surgical procedure. Propranolol also has been found to be a useful agent for preparing patients for surgery, although it is generally held that unless individual circumstances dictate otherwise, it should not be used alone in preoperative preparation.[186, 267] With present advances in anesthetic techniques, surgical procedures, and postoperative care, mortality due to thyroidectomy is virtually nil.

Many medical centers in the world continue to provide subtotal thyroidectomy as the optimal treatment of a definitive nature for Graves' disease.[19, 25, 128, 252] In Toronto, most endocrinologists regard thyroidectomy as the treatment of choice only in selected cases, particularly children, adolescents who cannot be controlled with antithyroid drug therapy, or patients with toxic nodular goiters under the age of 50 (see subsequent discussion). Associated disorders must be treated accordingly; for example, a patient with cardiac disease should be digitalized, and arrhythmias must be corrected or controlled before surgery. Diabetes mellitus, if present, should also be properly controlled.

Although it is generally a safe procedure, subtotal thyroidectomy is attended by a variety of complications (Table 13–7).[128] From 3.6 to 42.8% of patients develop hypothyroidism postoperatively, either shortly after the procedure or many years later.[128] The higher in-

Table 13–7. Incidence of Complications Due to Thyroidectomy for Hyperthyroidism Reported from 13 Clinics*

Complication	Percent Incidence
Mortality	0 to 0.6
Hypothyroidism	3.6 to 42.8
Recurrence or persistence	0.6 to 17.9
Hypoparathyroidism	0 to 3.6
Tetany (transient)	0 to 8.0
Vocal cord paralysis	0 to 5.6
Vocal cord paresis	0.8 to 10.6
Wound problems and infections	3.7 to 15.5
Thyroid storn	0
Length of follow-up	
Minimum	0.5 to 5 years
Mean	2 to 10 years

*Adapted from Hershman, J. M.: Treatment of hyperthyroidism. Mod. Treat. 6:467–515, 1969 and Werner, S. C. and Ingbar, S. H.: The Thyroid, 4th ed. Harper and Row, New York, pp. 1–1047, 1978.

cidence was detected by follow-up studies many years after surgery, and so it appears that the thyroid remnant seems capable of continued secretion in many patients for a prolonged but ultimately limited period. The onset of hypothyroidism may be subtle. It often may go undetected for many years, while the patient's health gradually deteriorates. The cause of this late postoperative deterioration may be autoimmune destruction.[175, 213, 290] Consequently, it is clear that annual follow-up is essential—even in those patients who do exceedingly well. This is equally true no matter what type of treatment (antithyroid drugs, radioactive iodine, surgery) the patient has had. Patients who have had transient hypothyroidism after operation are particularly prone to develop permanent myxedema later. The presence of thyroid autoantibodies in moderate or high titers at the time of surgery may also be a harbinger of hypothyroidism.[175]

Postoperative hypoparathyroidism develops in about 1% of patients after subtotal thyroidectomy, and an occult form may occur in up to 10% of patients.[128] Patients with the overt form of hypoparathyroidism must be treated with lifelong calcium and vitamin D therapy. Untreated hypoparathyroidism is associated with a high incidence of cataracts, convulsions, and metastatic calcification. Vocal cord palsy resulting from trauma occurs in up to 5.6% of patients. At the very least, this complication alters the voice, particularly for singing, and usually results in chronic hoarseness. Paralysis of both cords produces spastic airway obstruction and may require tracheostomy.

The persistence or recurrence of hyperthyroidism has been observed in 0.6% to 17.9% of such patients and is more common in children.[128] A variety of other complications have been described, such as bleeding, scars, keloid formation, wound infections, and phlebitis. It should be emphasized, however, that in general subtotal thyroidectomy is an extremely effective form of therapy that controls the disease quickly and well in 90% of patients.

Special Considerations in Management of Hyperthyroidism

Pregnancy. Hyperthyroidism develops in pregnant women with the same frequency as would be expected in women of this age group. However, there seems to be some factor in the first trimester of pregnancy that can precipitate hyperthyroidism or aggravate preexisting hyperthyroidism.[6] Other women become pregnant during treatment of hyperthyroidism. Because some of the physiologic changes that take place during a normal pregnancy may simulate hyperthyroidism, the diagnosis should be carefully established before it is accepted or acted upon. The elevation in thyroxine-binding globulin that occurs in pregnancy produces an increase in serum thyroxine and a decrease in the T_3 resin uptake; in hyperthyroidism that develops during pregnancy, however, the serum thyroxine level is much more markedly elevated, whereas the T_3 resin uptake rises either into the normal range or into the high normal level.[41, 42]

Radioactive iodine is contraindicated in pregnancy because the fetal thyroid concentrates iodine after the 12th week of gestation.[49, 131] However, radioactive iodine has been prescribed inadvertently to some gravid women in the first trimester, without apparent injury to the fetus.[131] Surgery is to be avoided in the first trimester, because it results in a high incidence of spontaneous abortions but seems reasonably safe in the second trimester.[573]

Generally, the hyperthyroid state during pregnancy is treated with antithyroid drug therapy.[41, 42] In Toronto, antithyroid drugs are employed throughout the period of gestation. Many endocrinologists elsewhere also employ antithyroid drugs as the treatment of choice during pregnancy. In the early part of pregnancy, the usual doses of antithyroid drugs are employed as discussed. However, the drugs cross the placenta and if the dosage is excessive may produce goiter and hypothyroidism in the fetus.[41, 42] Such goiters may even interfere with vaginal delivery. While transient fetal hypothyroidism has not been clearly shown to have any lasting effects on the mental development of those children,[41, 42] it should nevertheless be avoided. Thus, particularly in the later part of the second trimester and throughout the third trimester, doses of antithyroid drugs must be reduced as much as possible; dosages of propylthiouracil below 200 mg/day are very unlikely to produce goiter in the newborn.[41, 42] In any event there is a reduction in thyroid stimulating antibody during the third trimester spontaneously, which makes it generally easy to reduce the dosage of antithyroid drugs to low levels without hyperthyroidism exacerbating.[297, 335] Indeed in some patients there is a transient "remission" during the last trimester, and some patients will continue to do well

throughout this interval, even without any medication.[335] It is safe however to continue propylthiouracil during the entire third trimester in modest dosages of 100 to 200 mg/day.

The dosage of propylthiouracil during pregnancy must be titrated to ensure that the mother does not become hypothyroid, because this state is associated with an increased incidence of spontaneous abortion.[41, 42] There is no point in adding thyroxine to the mother's treatment during this stage of pregnancy since T_4 virtually does not traverse the placenta.[41, 42] While of course thyroxine therapy would prevent maternal hypothyroidism, it is really better to reduce the dose of antithyroid drugs by titrating against response.

Occasionally, the amount of thyroid stimulating antibody, while it might decline in the last trimester, is still sufficiently high to cause hyperthyroidism to the fetus and neonate because of transplacental transfer.[297, 335] The fetus will have been treated with the maternal ingestion of propylthiouracil that crosses the placenta and maintains the fetus in a treated state throughout gestation. The baby thus may appear normal at birth because of this protective medication, only to become hyperthyroid several days thereafter, because there is no further propylthiouracil administration.[216]

Occasionally, the fetus becomes hyperthyroid during uterine life because the mother is on inadequate or no antithyroid drug therapy. Fetal hyperthyroidism is associated with such complications as failure to develop, craniosynostosis, and even death.[50, 134] If therefore the mother harbors large titers of thyroid stimulating antibody in the third trimester, she must be given propylthiouracil so as to treat the fetal hyperthyroidism during this interval. This is true even if the mother is no longer hyperthyroid at the time but still has high concentrations of TSAb in her circulation.[50, 297]

Following delivery, mothers who are still taking propylthiouracil may be permitted to nurse, since it has now been shown that there is only a negligible amount of this agent in mother's milk.[149, 184] However, methimazole given to lactating females is found in the milk in more significant amounts.[54]

Finally, it should be emphasized that after delivery, there may be a relapse of hyperthyroidism several weeks thereafter due to a rebound phenomenon.[6] Thus, patients may require more treatment in the postpartum state than that required during the last trimester of pregnancy.

Treatment of Neonatal Graves' Disease

The pathogenesis of neonatal hyperthyroidism has been discussed in the previous section. Recognition that a newborn infant has Graves' disease is very important, as this condition can be life-endangering. Indeed the disease has been responsible for deaths *in utero* or, failing that ultimate fate, abnormalities of development such as craniosynostosis.[41, 42, 50] Moreover, the condition will often be obscured at birth if the mother has been taking antithyroid drugs during her pregnancy.[216] Since the fetus has thus also been treated with transplacental antithyroid drug, it may be born appearing normal. After the effects of the antithyroid drug have been cleared, the disease will then express itself often 7 to 10 days post partum, by failure to thrive, vomiting, and tachycardia.

The laboratory-based diagnosis may also be somewhat difficult, in view of the rapidly changing thyroid function test results, which normally occur in the first few days of life. Generally speaking, however, the serum T_4 and T_3 will be unequivocally elevated in such cases, and TSAb can be demonstrated in the cord blood.[41, 42, 335]

Since the half-life of IgG is about 2 weeks, the spontaneous duration of this illness will depend on the initial burden of TSAb carried by the neonate. Generally by 12 weeks there is insufficient remaining TSAb to sustain the condition. Thus, treatment must be carried on for 8 to 12 weeks in most instances. Initial therapy consists of fluid replacement, antithyroid drug, and iodides, whereas, subsequently, antithyroid drug alone will be sufficient to sustain the clinical and biochemical improvement, until such time as TSAb declines and further treatment is no longer warranted.[41, 42, 134, 216, 297]

Thyroid Storm. Thyroid storm is a life-threatening condition that is characterized by the heightened signs and symptoms of hyperthyroidism and by hyperpyrexia.[137, 246] The elevation in body temperature may reach 106°F (41°C) and may be associated with marked restlessness, agitation, severe tachycardia, heart failure, profound prostration, nausea, vomiting, diarrhea, delirium, psychosis, jaundice, and subsequent dehydration. This disorder is now rare. Factors that precipitate this crisis include infection, trauma, surgery, and withdrawal from antithyroid drugs.[137, 246]

This condition is exceedingly dangerous and

even with therapy the mortality rate remains high. The condition is therefore a grave medical emergency, and it is important to commence therapy as quickly as possible. The therapeutic measures include the following:

1. Suppression of thyroid hormone formation and secretion
2. Antiadrenergic therapy
3. Administration of corticosteroids
4. Treatment of associated complications or coexisting disorders that may have precipitated the storm
5. Plasmapheresis and peritoneal dialysis to accelerate removal of thyroid hormones from peripheral tissues.[147, 246]

It is thus necessary to promptly initiate antithyroid drug therapy, either propylthiouracil or methimazole in large doses (800 to 1200 mg/day of propylthiouracil or 80 to 120 mg/day of methimazole).[147, 246] If patients are unable to take these drugs orally, the tablets can be crushed into suspension and instilled by gastric tube. The mechanism of action of these drugs has been discussed. Since some weeks of treatment with these agents alone would be necessary before levels of thyroid hormone approach normal, it is important to add iodide treatment so as to block thyroid hormone release. Dosages of 0.5 to 1 gm of sodium iodide may be given intravenously, or oral preparations of iodine may also be provided. The oral preparations include Lugol's solution, 10 drops every 8 hours, or a saturated solution of potassium iodide, 5 drops every 8 hours.[246] It should be emphasized that the antithyroid drugs should be initiated at least a few hours *before* the iodide therapy is begun. If iodide is administered first it would provide the substrate permitting the synthesis and storage of a large amount of thyroid hormone in the thyroid gland, and this would then prolong the duration of hyperthyroidism thereafter.

Wartofsky and associates[304] have shown that the serum thyroxine level declines rapidly with this regimen. It may be noted that lithium salts have an action qualitatively and quantitatively similar to that of iodine.[251, 252] As previously discussed, since the combination of lithium and propylthiouracil has no clear advantage over iodide and propythiouracil, and since lithium carbonate in the doses required may have significant toxicity, which may be difficult to detect in patients suffering from thyroid storm, lithium has not achieved any popularity in this context.[100, 276]

There are beneficial effects to be anticipated from antiadrenergic therapy in thyroid storm. The inotropic and chronotropic responses to adrenergic stimulation and to catecholamines are actually not altered in the hyperthyroid heart, and the tachycardia and enhanced myocardial contractility in hyperthyroidism appear to result from additive effects of thyroid hormones and catecholamines.[181] Nevertheless, because of the adrenergic component, the results of blocking adrenergic effects will prove useful in the treatment of hyperthyroidism and, in particular, of thyroid storm.[137, 246]

While reserpine, a tissue depletor of catecholamines, and guanethidine, a depletor of catecholamines and an inhibitor of neuronal release of neurotransmitters, have been employed with benefit,[66, 197] beta-adrenergic blocking agents have been most frequently used.[60, 95] Doses of propranolol on the order of 2 to 5 mg intravenously have been administered in order to provide prompt control of tachyarrhythmias.[60, 95] Appropriate oral dosage of 20 to 80 mg every 4 hours is equally effective.[240] As mentioned, precautions must be observed in the use of these agents in thyroid storm, since congestive heart failure and bronchospasm may be aggravated. In insulin-dependent diabetics, these agents may impair hepatic glycogenolysis and thus induce hypoglycemia. These agents must be considered adjunctive, not to be employed alone.[246]

The use of corticosteroids is also recommended in the therapy of thyroid storm, generally by the intravenous route.[197] Rosenberg[246] has pointed out that there is no convincing evidence of adrenocortical insufficiency in thyroid storm, and the benefits derived from corticosteroid therapy may be via their antipyretic action and their effect of stabilizing blood pressure.

A variety of general supportive measures may also be necessary.[137, 197, 246] It is important to treat the underlying associated disorders, which may have helped to precipitate the thyroid storm. The use of intravenous electrolyte and water replacement is obviously essential. Hyperpyrexia can be treated with alcohol sponge baths or cooling blankets. Mild sedation may also have to be employed.

Finally, plasmapheresis[9] and peritoneal dialysis[127] have been used in this situation. This approach should be considered in a patient who does not respond to the more conventional procedures described, particularly when characterized by impaired consciousness or ex-

tremely high circulating hormone concentrations.

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14
Other Forms of Hyperthyroidism

ROBERT VOLPÉ

The previous chapter is devoted to one form of hyperthyroidism, namely, that of Graves' disease. Although Graves' disease is by far the most common form of hyperthyroidism, other types of hyperthyroidism must be considered and together make up about 20% of the total of all cases. This chapter describes the various other forms of hyperthyroidism that may be encountered.

HYPERTHYROIDISM DUE TO AUTONOMOUS FUNCTIONING THYROID NODULES

Hyperthyroidism due to autonomous functioning thyroid nodules was first described by Plummer[34] in 1913, who characterized two types of thyrotoxicosis as follows: (1) exophthalmic goiter (Graves' disease) and (2) toxic adenomatous goiter. The latter was associated with either single or multiple nodules with variable histologic patterns, whereas in the former the hyperthyroidism was associated with diffuse hyperplasia of the thyroid gland. Plummer recognized that hyperthyroidism was often an end stage of a slowly growing autonomous functioning follicle, occurring in the life history of sporadic or endemic goiter.

The pathogenesis of toxic solitary adenomas or toxic multinodular goiters is not fully understood. It would appear that a true thyroid adenoma might develop, which if functional could enlarge to the size that would produce hyperthyroidism. More often, however, toxic nodular goiter seems to evolve from a euthyroid nontoxic nodular goiter over a long period of time. To understand how this may develop, it is essential to understand the pathophysiology of euthyroid nontoxic nodular goiter. Studer and Ramelli[43] in 1982 showed evidence that in nodular goiter there are autonomous follicular cells probably present from birth, which slowly replicate over time until there is a sufficient mass of such cells to produce and secrete excess thyroid hormone into the circulation.

In practice, autonomy of follicular function is most readily recognized when it appears as the solitary or dominant nodule in an otherwise normal thyroid gland by palpation.[29] The finding of such a small area in the thyroid gland, which ultimately functions more actively than the rest of the tissue, may be incidental for many years in a clinically euthyroid person.[29, 53] (This is discussed further.) In any event, such

nodules will prove to be solid on ultrasonographic examination; on scintiscanning, the nodule or nodules will tend to pick up more of the isotope than the surrounding, resting thyroid parenchyma (Fig. 14–1).[29, 53]

Clinical Picture

As previously mentioned, patients with autonomous thyroid nodules may have no clinical features whatever to suggest hyperthyroidism. Conversely, they may occasionally have quite severe manifestations of hyperthyroidism as described in the preceding chapter. Most frequently, however, the patients with this form of hyperthyroidism have a mild form of the disease, often with a prolonged and subtle history.[29, 53]

Since the majority of patients with toxic solitary or multinodular goiter are in the older age group and since the lesion is a slowly evolving one, it follows that the hyperthyroidism is often "masked." Patients with this disorder may lose weight only very slowly over years without an obvious cause.[18] They may deny nervousness, tremulousness, excessive sweating, or excessive appetite. Quite frequently, a tachyarrhythmia (e.g., rapid atrial fibrillation) will alert the suspicious physician to investigate the thyroid status. Such patients have been aware of a goiter for decades and may often state that they have never been bothered by its presence. Moreover, there may have been no observed enlargement of the goiter over a long period of observation. However, long-standing goiter with no obvious change over many years may be thought by the patient to have enlarged in the months or years prior to presentation.[18, 53] When associated with overt hyperthyroidism, the nodule or nodules are generally at least several centimeters in diameter.[21]

As noted, the clinical presentation is subtle, and most patients are in the older age group. There is no exophthalmos associated with this condition. Moreover, the disorder does not respond as readily to the usual measures of management as does Graves' disease.[15, 29]

Prevalence, Age, and Sex Incidence

In southern Ontario, the incidence of overt hyperthyroidism due to toxic nodular goiter has greatly declined and is currently in the order of about 5% of all patients with hyperthyroidism.[54] However, in Great Britain, the incidence of Plummer's disease is approximately 15% of all patients with hyperthyroidism. It would appear that the incidence of hyperthyroidism due to toxic nodular goiter has declined, since in New York between 1924 and 1944, about one third of the patients operated upon for hyperthyroidism were shown to have toxic nodular goiter.[53]

Generally, toxic nodular goiter is a disorder of the older age group.[53] Most patients with

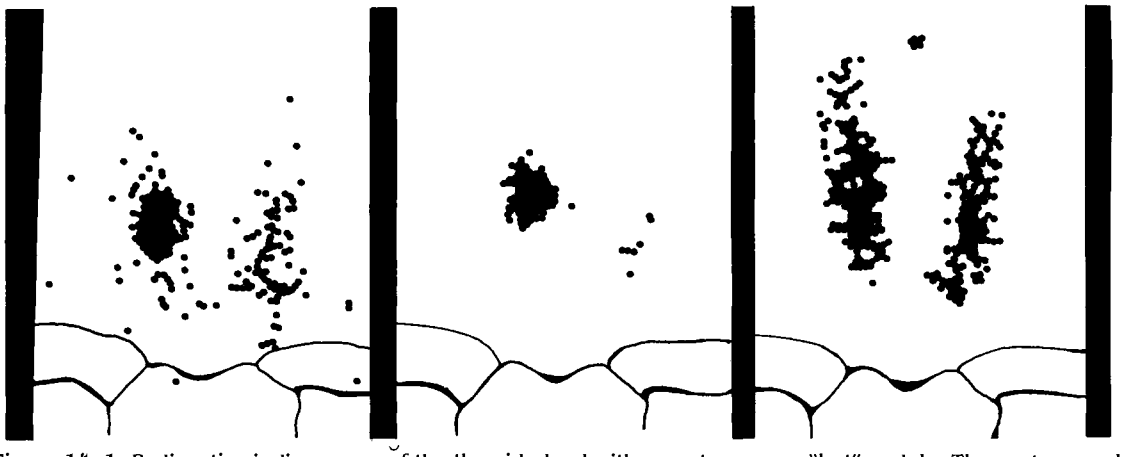

Figure 14–1. Radioactive iodine scans of the thyroid gland with an autonomous "hot" nodule. The center panel shows the thyroid scan after the patient was given 100 μgm of triiodothyronine for 10 days. The nodule proved to be nonsuppressible. The right panel shows the scan after the patient was given 10 units of bovine thyroid-stimulating hormone (TSH) intramuscularly daily for 3 days. The left thyroid-stimulating hormone lobe, which had been in a resting state due to suppression by the autonomous nodule, was thus stimulated by the TSH and then concentrated the radioactive iodine.

this condition are seen after the fourth or fifth decade of life. This is particularly true for patients with toxic multinodular goiters, but even solitary hyperfunctioning adenomas are more common in the older age group; however, solitary hyperfunctioning adenomas have been described in children, probably representing true adenomas. These young patients with such cases are very uncommon.[46, 55]

Diagnosis

In patients with nodular goiters, even those who may seem clinically euthyroid, it is important to pay close attention to thyroid function tests (see the next discussion). As previously mentioned, even when hyperthyroidism is detectable by clinical means, it may be very subtle and only very slowly developing. Older patients with gradual weight loss and tachyarrhythmias should always be suspected of suffering from hyperthyroidism.[18, 53] The disease, of course, is not a diagnostic problem when overt hyperthyroidism is evident. The combination of hyperthyroidism with an obviously multinodular goiter or even a solitary nodule should provide appropriate direction for suitable thyroid investigations.

Thyroid Testing

Measurements of thyroid hormone in the circulation are, of course, important. In some cases, both the serum thyroxine and triiodothyronine values will actually be within the normal range. Nevertheless, the thyroid-stimulating hormone (TSH) value will be low if measured by some of the new and sensitive assays now available.[30] If older radioimmunoassays are employed for TSH, the response to TRH will be shown to be flat. In the circumstance in which the serum thyroxine and total serum triiodothyronine values are normal, but either the TSH is low or the TSH response to TRH is flat, it is evident that there is indeed "chemical" hyperthyroidism, whereby sufficient thyroid hormone is being produced by the autonomous thyroid tissue to be able to suppress the TSH-secreting cells of the anterior pituitary.[36] This minimal increment of thyroid hormone secretion may not be sufficient to elevate the levels of thyroid hormones above the normal range nor may it be sufficient to cause clinical manifestations of hyperthyroidism.

As the toxic nodular goiter evolves, however, the levels of thyroid hormone in the blood will slowly rise until they are clearly above the normal range. At times the serum T_4 may be normal or only slightly elevated, but the serum T_3 will be definitely elevated (T_3 thyrotoxicosis).[29] Finally, both serum T_4 and T_3 concentrations will become unequivocally elevated; although, unlike Graves' disease, the serum T_3 concentration will usually be elevated only in proportion to the serum T_4 concentration.[18]

Thyroid scanning will be variable, depending on whether the entire thyroid glandular secretion rate is isofunctional or even minimally hyperfunctional (see Fig. 14–1).[18, 29] While the gland is still isofunctional, the autonomous nodules may appear "warm," but since there is not yet suppression of TSH the remaining normal thyroid parenchyma will not be suppressed.[6] Both with solitary hyperfunctioning adenomas and multinodular toxic goiters, as chemical hyperthyroidism develops, there will be sufficient suppression of TSH to suppress those areas within the thyroid gland that are normally dependent on TSH. Thus, the autonomous nodules will begin to be contrasted by their activity from the suppressed areas within the thyroid gland. Moreover, in many multinodular goiters, there will also be areas of degeneration that will additionally appear as hypofunctional. It is sometimes of interest to administer bovine TSH to a patient with areas of suppression within the thyroid gland so as to demonstrate that the paranodular tissue is indeed resting and will again pick up radioactive iodine or technetium in response to the administration of TSH.[29]

The occurrence of carcinoma is rare within toxic nodular goiters. Warm or hot nodules due to thyroid carcinoma are very rare indeed.[14, 28, 29, 45] However, a solitary "cold" nodule in an otherwise "hot" gland should be considered suspicious as possibly malignant and should be investigated as a "cold" nodule.[17]

Ultrasonographic examinations will prove to be useful in order to show that the toxic solitary or multinodular goiter is due to solid nodules.[53]

Treatment

The treatment of toxic nodular goiter may prove to be difficult. Surgery may be considered mandatory when there is evidence of compression by large multinodular goiters on

the trachea and other vital organs. Thus, larger multinodular goiters should be removed surgically unless there are contraindications in terms of general health.[29]

The treatment of toxic multinodular goiter, whether solitary hyperfunctional thyroid adenoma or multinodular goiter with hyperthyroidism, with radioactive iodine involves considerations that are quite different from those that pertain to the treatment of hyperthyroidism due to Graves' disease. Radioactive iodine will control hyperthyroidism in large autonomous multinodular goiters, but the following factors must be considered.[15, 29] In patients with complicating problems of congestive heart failure, cardiac arrhythmias, dehydration, and protein loss, corrective measures should precede the administration of ^{131}I. These measures particularly include antithyroid drug therapy to be maintained until the patient is virtually euthyroid. The dose of ^{131}I required is relatively large, since 50 mCi produced permanent remission in no more than half of the patients observed by Miller and colleagues.[29] Even with doses of 75 to 100 mCi, control of the disease may not be predictable, and the hyperthyroid state may persist for several months. The hazard of these large doses in the induction of thyroid carcinoma is yet to be determined but is very remote, as most patients so treated are already in the older age group.

There is almost always considerable residual goiter after such treatments in these patients. The possibility of recurrence in subsequent years exists, but if life expectancy is reasonably short, this may not pose a problem. Moreover, the incidence of hypothyroidism is low, since the non-nodular resting thyroid parenchyma does not pick up ^{131}I.

The treatment of solitary hyperfunctioning thyroid adenomas with radioactive iodine is much simpler and much more successful.[15] Such treatment involves minimal inconvenience, usually does not require hospitalization, and has a very low rate of postradioactive iodine myxedema. However, even when precautions are taken to minimize extranodular ^{131}I uptake, the nonfunctioning portion of the thyroid does receive some gamma radiation, mainly from radioactive iodine trapped in the hyperfunctioning nodule but also, to a minor extent, from ^{131}I located in distant body sites or circulating in the blood. It has been observed that much larger doses are necessary to treat patients with toxic adenomas as opposed to those with Graves' disease. Since 83% of patients are cured with doses of 30,000 rad/adenoma, Gorman and Robertson[15] have calculated the doses of ^{131}I needed to deliver this amount of radiation for various sizes of adenoma.

Gorman and Robertson also have pointed out that as the nodule size of an adenoma increases from 2 to 6 cm, the amount of radioactive iodine administered to the patient to deliver the same dose (30,000 rad to the nodule center, assuming a 30% uptake) increases from 5.6 to 135 mCi. Concurrently, the suppressed thyroid tissue receives a radiation dose as high as 2300 rad. Despite these potentially carcinogenic doses, few patients with radioiodine-induced thyroid tumors have been reported, possibly because most patients with solitary hyperfunctioning thyroid adenomas are in the older age group. Moreover, ^{131}I radiation may not be as carcinogenic as external radiation.[27] Following treatment, as with multinodular toxic goiter, some residual goiter tissue remains. The suppressed thyroid tissue does not concentrate ^{131}I, and after the nodule is ablated the suppressed tissue returns to normal function. Thus, hypothyroidism is uncommon.

Although surgical treatment offers prompt control of hyperthyroidism and the certainty that the nodule will disappear, there are surgical risks that cannot be ignored, particularly in elderly patients with other diseases. (See Surgical Treatment of Graves' Disease, Chapter 13.) The main advantages of radioactive iodine are the absence of surgical risk and the lesser expense. Disadvantages include failure of the nodule to disappear in about 20% of cases, inability to examine the tissue histologically, and presence of low-dose radiation to the suppressed thyroid tissue and to the whole body.

For these reasons, Miller[29] and Gorman and Robertson[15] have recommended surgery for patients less than 40 years of age and for those who may have large nodules and no increased surgical risk.

HYPERTHYROIDISM SECONDARY TO EXCESSIVE HUMAN CHORIONIC GONADOTROPIN

In 1940, Smilie and Clements[40] first described hyperthyroidism associated with trophoblastic disease (uterine chorioepithelioma). In 1955, Tisne and colleagues[47] reported hyperthyroidism secondary to a benign hydatidiform mole.

There were later sporadic reports of hyperthyroidism associated with benign and malignant trophoblastic disease, but it was not until 1963 that Odell and coworkers[32] studied 93 women with choriocarcinoma systematically and found evidence of hyperthyroidism in seven. After demonstrating thyroid stimulating activity in the serum of two patients and in the tumors of two other patients, these investigators suggested that the choriocarcinoma itself was secreting an abnormal thyroid stimulator.

In 1978, Higgins and Hershman[20] reviewed the literature on this subject. They noted that while many of the patients with hydatidiform mole or choriocarcinoma had increased thyroid function, often there was little clinical evidence of hyperthyroidism.

In comparison, some patients had not only overt clinical evidence of thyrotoxicosis but even severe features of this disorder. Higgins and Hershman noted that hydatidiform moles occur more commonly in the Orient than in North America and Western Europe and that increased thyroid function occurs in about 50% of patients with this condition. The mole is usually made evident by bleeding, nausea, vomiting, and pre-eclampsia; in those who have been found to be clinically hyperthyroid, the uterus has always been large for the length of the pregnancy. The diagnosis of hydatidiform mole may be made by the observation of molar tissue being passed *per vagina*; ultrasonographic scans, amniography, and human chorionic gonadotropin (hCG) assays help to settle the diagnosis. Curry and coworkers[8] have reported that repeated hCG assays that fail to show a decline from high levels after 3 months of pregnancy are suggestive of hydatidiform mole.

Higgins and Hershman have further reviewed the thyroid function of 20 patients with hydatidiform moles, of whom 12 were euthyroid, two were mildly hyperthyroid, and six were severely hyperthyroid. In these last six patients, all had goiters; two had very rapid supraventricular tachycardia and pulmonary edema. Moreover, the progression to even more severe hyperthyroidism in these patients was quite rapid.

The nature of the thyroid stimulator, while clearly related to hCG, is not entirely settled.[2] Patients with this disorder tend to have extremely high levels of hCG. Although a thyroid stimulator resembling pituitary TSH is found in human placenta, this does not appear to be increased in hydatidiform moles or choriocarcinomas and thus hCG itself has been implicated in causing the hyperthyroidism.[20]

Bioassays of highly purified hCG have been found to have intrinsic thyroid stimulating activity, although only 1/4000 that of pituitary TSH.[39] There is evidence that hCG binds to the TSH receptor on the follicular cell and will displace TSH from a thyroid cell membrane. Serum hCG concentrations generally exceed 300 IU/ml in patients who are hyperthyroid. Nevertheless, Amir and colleagues[2] have challenged the thesis that hCG is the cause of the hyperthyroidism per se; when they utilized highly purified preparations of hCG they could not demonstrate the aforementioned actions.

Management of this condition involves removing the offending tumor as quickly as possible. While one should attempt to control the thyrotoxic state with antithyroid drugs, this should not cause delay in removing or treating the tumor.[20] In the case of hydatidiform mole, the uterus should be emptied as soon as possible (Fig. 14–2), whereas in the case of choriocarcinoma, treatment should be directed against the tumor at the same time that antithyroid drug therapy is also utilized.

IODIDE-INDUCED HYPERTHYROIDISM

The discovery in 1820 of the use of iodine for the treatment of endemic goiter is generally attributed to Coindet.[22] However, in 1821,

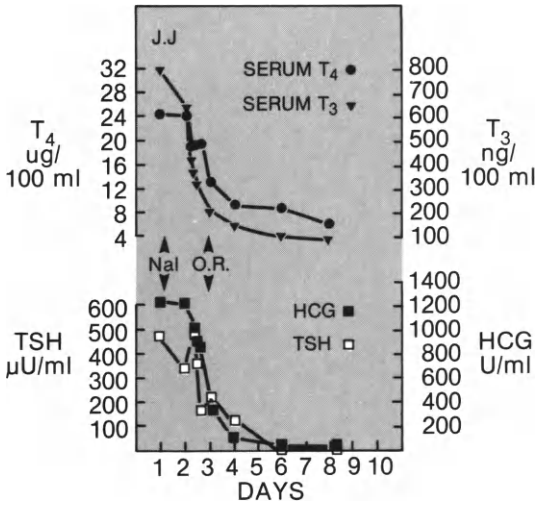

Figure 14–2. The effect of 1 gm of sodium iodide (NaI) intravenously and surgical removal of the molar tissue (O.R.) on the circulating levels of serum thyroxine (T_4), serum triiodothyronine (T_3), human chorionic gonadotrophin (HCG), and TSH in a hyperthyroid patient are depicted. (From Higgins, H. P., et al. Ann. Int. Med. 83:307, 1975, with permission.)

Coindet also reported that patients who took iodide suffered "annoying symptoms of tremor, tachycardia, rapid loss of weight and strength, despite increased appetite, and insomnia."[22, 23] Greer[16] in 1973 recognized that Coindet's report was almost certainly that of hyperthyroidism induced by iodine. Breuer[3] in 1900 coined the term "Jod-Basedow syndrome" to describe the condition of iodine-induced thyrotoxicosis.

It is since the era of prophylactic iodization programs that the incidence of hyperthyroidism has increased markedly.[23] Following the introduction of iodized bread in Tasmania, there was a sharp increase in the reported incidence of hyperthyroidism.[42] The use of iodophor disinfectants in the dairy industry with elevation in the iodine content of milk also brought about local increases in the incidence of hyperthyroidism.[7, 42]

In 1975, Vagenakis and Braverman[49] reported that hyperthyroidism developed in four of eight patients with multinodular goiters who were given a saturated solution of potassium iodide in a dosage of approximately 180 mg of iodine daily, after 6 to 18 weeks of treatment. After the iodine was discontinued, the hyperthyroidism still persisted for several weeks.

Thus, Jod-Basedow's syndrome has now been reported in patients with pre-existent goiters living in nonendemic regions of the world.[12] Indeed, there are several compounds containing organic iodides that have now been reported to produce the same disorder. These compounds include contrast dyes, iodochlorhydroxyquin, and now and most spectacularly amiodarone, a drug used for treatment of cardiac arrhythmias.[12] Most patients who have this condition have had pre-existent multinodular goiters, although the disorder has also occurred in patients who had previous Graves' disease and in one patient who had an autonomous thyroid nodule.[7, 12]

It is important to emphasize that some of the organic iodide compounds mentioned will cause an elevation of the serum thyroxine level, accompanied by a fall in the total serum triiodothyronine and a slight rise in TSH levels. This so affected group of patients does not have clinical hyperthyroidism. It is essential to document true iodine-induced hyperthyroidism by means of the demonstration of an elevated serum thyroxine and total serum triiodothyronine with suppressed TSH values. One cannot accept an elevation of serum thyroxine level alone as evidence for the diagnosis of Jod-Basedow's syndrome.[7]

Mechanism

The basic mechanism responsible for the development of Jod-Basedow's disease is unknown. The importance of iodide to intrathyroidal metabolism is discussed elsewhere in this text. Usually, excessive or pharmacologic amounts of iodide given to normal subjects cause a slight but temporary impairment of hormone synthesis. This is termed the "Wolff-Chaikoff" effect and is a phenomenon of substrate inhibition of hormonal synthesis.[12] It seems evident that this autoregulatory mechanism has somehow failed in patients who are vulnerable to Jod-Basedow's disease, so that the increased iodide acts not to inhibit hormone synthesis but as substrate for further intrathyroidal hormone production. The basic mechanism whereby this autoregulatory effect becomes abnormal in vulnerable patients is not yet clarified. The fact that this condition is more common in endemic goiter regions, associated with previous iodine deficiency, suggests that prolonged iodine deficiency will cause some defect in autoregulation of iodine metabolism.

Nevertheless, the few reports that suggest that iodide-induced hyperthyroidism may occasionally occur in a patient with a previously normal thyroid gland cannot readily be accepted. It is more likely that the thyroid gland even in such a patient has some intrinsic abnormality of iodine metabolism, although the patient may have been clinically normal prior to the onset of Jod-Basedow's syndrome.

Diagnosis

As previously mentioned, this syndrome is generally diagnosed by the appropriate history of iodine intake; the usual presence of a previous goiter, which often is multinodular; the finding of symptoms and signs of hyperthyroidism clinically; and the demonstration of elevated levels of serum thyroxine and total serum triiodothyronine, accompanied by a suppressed TSH determination. It is of interest that there is one report of a case of iodide-induced thyrotoxicosis with elevation of only triiodothyronine concentration.[1] The radioactive iodine uptake is also suppressed by virtue of saturation of the thyroid gland with iodine. Since the serum thyroid hormones are elevated

and the radioactive iodine uptake is reduced, the differential diagnosis has to include silent thyroiditis and factitious thyroxine administration.

In silent thyroiditis, there is, of course, no history of iodine administration. There may be thyroid autoantibodies, and the amount of iodine present in the urine would be compatible only with the increased rate of degradation of thyroid hormone. In the case of factitious thyroxine administration, the serum thyroglobulin level would be very low. Under these circumstances, this finding can virtually be caused only by thyroid hormone administration in an otherwise normal person.[26] In the case of all other types of hyperthyroidism, including Jod-Basedow's syndrome and thyroiditis, serum thyroglobulin is elevated or is at the upper end of the normal scale.[4, 33]

Clinical Course

Iodide-induced hyperthyroidism is usually mild and will ultimately remit spontaneously. Occasionally, it is severe and responds only very slowly to antithyroid drug therapy. This finding is presumably due to the large amount of stored hormone and the increased circulating levels of iodide, which slow the response to treatment. The use of beta-adrenergic blocking agents may be helpful under these circumstances. Radioactive iodine cannot be employed as therapy because the gland is already saturated.

Although iodide must be withdrawn to effect the remission, Vagenakis and Braverman have reported that Jod-Basedow's syndrome may be exacerbated initially following withdrawal of the iodide, possibly because of removal of some degree of inhibition of hormone secretion by the high levels of iodide.

THYROIDITIS

Many types of thyroiditis may be associated with hyperthyroidism. These forms include subacute thyroiditis, silent thyroiditis, postpartum thyroiditis, and even Hashimoto's thyroiditis. (Since these disorders are discussed elsewhere in this text, there will be no further discussion of this category in this chapter.)

TSH-INDUCED HYPERTHYROIDISM

Hyperthyroidism per se causes an undetectable or low serum thyrotropin (TSH) concentration. The obvious reason for this finding is that hyperthyroidism when not caused by excess TSH itself will exercise normal feedback control via TRH and the anterior pituitary itself and will inhibit TSH production. Rarely, hyperthyroidism is caused by increased pituitary TSH secretion, with consequent thyroid hormone secretion excess. In all of the cases reported thus far, the increased TSH secretion has been of anterior pituitary origin itself, and there has been no report of TSH production from ectopic sources.[7, 48]

Causes of TSH-induced Hyperthyroidism

Production of TSH by the thyrotrophs is normally regulated by a direct inhibitory action of the thyroid hormones on the thyrotrophs themselves.[48] These cells are very sensitive to these hormones so that the slightest increment will cause a decrease in TSH production, whereas the slightest decrement in thyroid hormone concentrations will cause a definite increase in TSH secretion. At the cellular level, most of the inhibitory effect appears to be due to T_3 acting via nuclear receptors. It is now well known that conversion of T_4 to T_3 occurs within the thyrotrophs; T_3 produced within the pituitary appears to account for a greater proportion of thyroid hormone action in these cells than does conversion of T_4 to T_3 in other tissues.

Hypothalamic thyrotropin-releasing hormone (TRH) also is an important regulator of TSH secretion. The action of TRH appears to be readily inhibited by small increases in circulating thyroid hormone concentration.[41] There is, however, little evidence that the secretion of TRH in turn is regulated by thyroid hormones, so that the prime effect of these hormones seems to be directly on the thyrotrophs.[25] The physiologic regulation of TSH secretion also depends on various other factors, such as glucocorticoids, somatostatin, and dopamine.

As previously mentioned, TSH will rise significantly with the most minimal decrement of thyroid hormone concentration, and thus the most common cause of TSH hypersecretion is primary hypothyroidism. In this situation, hyperplasia and hypertrophy of the thyrotrophs are present, and the pituitary may enlarge even so as to become clinically evident.[9] When thyroid hormone therapy is provided, TSH

declines and all of the aforementioned changes subside.

Potential causes of TSH-induced hyperthyroidism have been listed by Utiger.[48] Most patients with this syndrome can be shown to have pituitary adenomas, but in others tumors could not be detected. Utiger has concluded that TSH-induced pituitary hyperthyroidism in all patients, whether they have a pituitary tumor or not, results from resistance to T_4 and T_3 within the thyrotrophs. It is important to emphasize, however, the sequence may rather be that the tumors themselves *cause*, but do not necessarily themselves result from, resistance to T_4 and T_3. In any event, this resistance causes a reduction in the intracellular signal, which inhibits the secretion normally generated by T_4 and T_3. Increased TSH secretion thus persists despite the presence of increased levels of thyroid hormone in the blood. There appear to be other cases in which pituitary tumor cannot be demonstrated, and these have been termed as "pituitary resistance to thyroid hormone."[13]

Some investigators consider that there is a fundamental difference between these entities, and indeed most of the patients with definite pituitary tumors have had an increase in serum concentrations of TSH alpha subunit, even when serum TSH was only modestly increased. The patients without demonstrable tumors have tended not to have increases in the alpha subunit (Table 14–1).[7, 24, 48] Since these patients are clinically hyperthyroid, it is clear that there is no resistance to thyroid hormone action elsewhere in other tissues. It is not as clear whether, in fact, there is any fundamental difference between these two forms as is so often proposed.

However, thyrotropin resistance to thyroid hormones, whether in the pituitary itself or generalized throughout all tissues, could result from several abnormalities. These include impaired cellular entry of T_4, T_3, or both; reduced intracellular metabolism of T_4 to T_3; reduced thyroid hormone receptor numbers of affinity; and postreceptor abnormalities or accelerated intracellular conversion of T_4, T_3, or both to biologically inactive metabolites.[48] Some or all of these abnormalities might ensue from tumor growth. I suspect that at least in some instances very small and thus occult tumors of the thyrotrophs have been thrust into the category of "pituitary resistance to thyroid hormones," whereas obvious tumors are so classified. It could also be that when tumor tissue is exceedingly minute in amount, it may be reflected by qualitatively different laboratory values when compared with those of the much larger or macroadenomas.

There has been no definite proof that any of the cases of TSH-induced hyperthyroidism are due to excessive production of thyrotropin-releasing hormone (TRH), with one possible exception.[19] Indeed, pituitary TRH receptors are "down regulated" by TRH. Moreover, the actions of TRH are readily inhibited by T_4 and T_3.[31]

Composition of TSH

TSH is a glycoprotein hormone that is composed of two dissimilar noncovalently linked subunits, an alpha and a beta. The alpha subunit is common to TSH, luteinizing hor-

Table 14–1. Characteristic Patterns of Thyroid-stimulating Hormone (TSH) Secretion in Patients with TSH-induced Hyperthyroidism*

	Pituitary Tumor	No Pituitary Tumor
Serum TSH		
Basal, μU/ml	1.7 to 525	2.7 to 260
Response to thyrotropin-releasing hormone (TRH)	No change	Increase
Response to T_4 or T_3	No change	Decrease
Response to glucocorticoid	Decrease	Decrease
Response to dopaminergic agonist	Variable	Variable
Response to somatostatin	Variable	—
Serum alpha-subunit		
Basal	Increase	Normal
Response to TRH	No change	Increase
Response to T_4 or T_3	No change	Decrease
Response to glucocorticoid	No change	Decrease

*Reproduced from Utiger, R.: TSH-induced hyperthyroidism. In: Delange, F., Fisher, D. A., and Malvaux, P., (eds.), Pediatric Thyroidology (Pediatric and Adolescent Endocrinology, Vol. 14). Basel, S. Karger, 1985, with permission.

mone (LH), follicle-stimulating hormone (FSH), and hCG. The beta subunit of each glycoprotein hormone is unique and thus determines the immunologic and biologic specificity of each hormone.[35] Both subunits are synthesized separately in the thyrotrophs and later linked to form TSH. However, small amounts of the subunits are secreted into the circulation in normal individuals.

Serum TSH concentrations in patients with TSH-induced hyperthyroidism are either elevated or in the high normal range.[7, 24, 48] Even if a normal result is obtained, that would be inappropriately elevated in the face of high levels of circulating thyroid hormones. In the various reported cases, TSH concentrations have varied widely from normal values up to extremely high values. In comparison, patients are usually only moderately hyperthyroid with elevations of thyroid hormones no higher than two to three times normal. This finding suggests that in those patients with extremely high levels of TSH, the hormone may have relatively reduced biologic activity. The observation that there are usually substantial increases in serum alpha subunit concentrations found in some patients with TSH-induced hyperthyroidism indicates the disorder in intracellular TSH production that occurs in some of these patients. In one thyrotrophic tumor in culture, there was a discordant rate and pattern of alpha subunit and TSH secretion.[11]

Clinical Manifestations

The youngest patient with this disorder so far reported was 4 years of age. This child, however, did not have an overt pituitary tumor. The youngest person with a pituitary tumor was 16 years of age.[38] Most patients have been adults, with equal frequency between males and females. While generally not familial, there is one instance in which several members of the same family have been affected.[37]

The patients have suffered only mild hyperthyroidism with no evidence of ophthalmopathy or dermopathy. The relative mildness of the hyperthyroidism has been remarked upon frequently and, as mentioned, seems remarkable in the light of some of the extremely high values of TSH that have been reported.[7, 48]

Quite often, the initial manifestation of the disorder was that of a pituitary mass rather than hyperthyroidism itself. Many of the patients had received antithyroid drug therapy or ablative treatment for the hyperthyroidism, and in these circumstances some patients required multiple doses of radioactive iodine or repeat thyroidectomies. The goiters are generally small, and following ablative therapy recurrent goiters are common.[48]

The pituitary tumors may produce TSH alone or may produce excessive amounts of growth hormone or prolactin. Thus, acromegaly and galactorrhea may be seen in these patients.[5, 7, 48, 52] Visual field involvement has been observed secondary to the compression of the optic tracts by the tumor, and enlargement of the sella turcica is frequently observed with roentgen examination.

Laboratory Findings

Laboratory studies, of course, show increases in serum thyroxine and triiodothyronine concentrations, which are often proportionate, unlike the finding in Graves' disease, which is a disproportionate increase in serum triiodothyronine concentration. Although the TSH will be normal or elevated, the values in general are higher in those patients with overt pituitary tumors. The values become much higher following various forms of treatment, whether antithyroid drug therapy, radioactive iodine, or thyroidectomy, and patients with serum TSH concentrations greater than 100 mU/L usually have had such treatment. The alpha subunits are commonly increased proportionate to the total amount of TSH (see Table 14–1).[7, 24, 48]

The response of TSH to TRH has been studied[5, 7, 13, 24, 28, 51, 52] Generally, with overt tumors, TSH concentrations change little in response to TRH but rise in response to antithyroid drug therapy in about half of the patients so treated. The serum alpha subunits also change little following TRH stimulation. Glucocorticoid administration will cause a paradoxical rise in total TSH in many of the patients with overt tumors so far studied but not in the alpha subunit. Conversely, TSH secretion in those without overt tumors is remarkably different from those with such tumors. In the "nontumorous" group, TSH concentrations generally increased with TRH administration and following antithyroid drug therapy. In comparison, administration of excess thyroid hormones would cause some drop in the TSH values. Glucocorticoids cause serum TSH levels to decline in this group of patients and the alpha subunit proportions are generally normal.[13, 51]

These differences in response between those patients with overt pituitary tumors and those without such tumors have been heralded as representing fundamental differences and thus two diseases. The evidence so far would support such a view, since the nontumorous patients have not yet developed pituitary tumors despite follow-up of several years.[48] However, another view is that both disorders do represent "tumors." The tumors obviously have a doubling time such that they become overt over a matter of years, whereas the "nontumorous" group may represent those with an extremely slow rate of doubling, in which even a lifetime may pass with little change. Indeed, this question has yet to be answered.

Diagnosis

The question certainly must be asked, Should serum TSH be determined in all hyperthyroid patients? It is obvious that TSH-induced hyperthyroidism is very rare, or conversely the cause of hyperthyroidism in the vast majority of patients is reasonably obvious. However, the newer and very sensitive TSH assays may simplify the making of the diagnosis.[30] With the new immunoradiometric techniques, patients with hyperthyroidism of all other causes will be shown to have less than 0.3 mU/L of TSH in the sera. It thus becomes possible, for the first time, to rely on TSH determinations even when they are not elevated. I thus favor the carrying out of TSH determinations in all patients with hyperthyroidism. In those with an inappropriately high level of TSH, measurements of serum alpha subunit concentrations shoud also be carried out.

Treatment

If a tumor can be demonstrated, transsphenoidal resection of the adenoma should be undertaken. This treatment has resulted in long-term cures in several instances, although it is not yet possible to ensure that a permanent cure has been achieved.[7, 48] In some patients with very large tumors, multiple operations and postoperative radiation therapy might be considered, since TSH-secreting tumors may be ameliorated by such therapy.[6]

The treatment of patients who do not have pituitary tumors is not entirely satisfactory. Amelioration of the hyperthyroidism with antithyroid drugs or with radioactive iodine therapy certainly must be offered. However, it is noteworthy that such therapy does result in increasing serum TSH concentrations and could conceivably result in the development of a pituitary tumor, although that consequence has not yet been realized. The use of adjunctive symptomatic measures, such as beta-adrenergic blockers, might be useful in this respect.[7, 48]

THYROID CARCINOMA

Actual hyperthyroidism secondary to carcinoma of the thyroid is rare, despite the fact that thyroid function persists in most cases of follicular carcinoma of the thyroid. In the majority of about 30 cases now reported, the hyperthyroidism has been made evident after complete thyroidectomy.[7] Probably this is only because the widespread metastases did not appear until somewhat later, although it is possible that basal levels of TSH were elevated after removal of the thyroid itself; these may have caused further stimulation of the metastatic thyroid neoplastic tissue. In any event, there have been other examples in which the hyperthyroidism was documented prior to thyroidectomy. The evidence suggests that the tumor metastases almost invariably function in a suboptimal fashion when compared with normal tissue. The reason that hyperthyroidism develops is because of the large mass of tumor tissue; although each unit of tissue is producing less thyroid hormone than a comparable unit of normal tissue, the function of the neoplastic tissue is autonomous. The net result of the functioning large mass of tissue is the production of excess amounts of thyroid hormone. TSH is suppressed, as would be expected in any type of hyperthyroidism other than that due to TSH itself.

There has been at least one case of hyperthyroidism in which thyroid-stimulating antibody (TSAb) was present, owing to Graves' disease, and was capable of stimulating the metastases to produce hyperthyroidism.[17]

A few unusual features should be mentioned. Abnormal thyroglobulin has been found in one follicular carcinoma of the thyroid, which, while immunologically identical to normal thyroglobulin, was quite different in its physical characteristics and amino acid composition.[10] In addition, there has been one case of T_3 thyrotoxicosis.[44] There is at least one example of a lymphoma involving the thyroid that caused rapid destruction of thyroidal tis-

sue, with a clinical picture analogous to that of subacute thyroiditis.[38]

There are only very few examples in which follicular carcinomas of the thyroid are themselves hyperplastic, although it is obvious that carcinoma of the thyroid can coexist in a person who has hyperthyroidism as a result of Graves' disease or other benign processes.[14, 28, 45] Miller[29] has stated that in all reports in which "hot" thyroid carcinomas have been reported, further careful investigation has failed to validate those claims.

HYPERTHYROIDISM DUE TO EXOGENOUS THYROID HORMONE EXCESS (FACTITIOUS THYROTOXICOSIS)

The syndrome of factitious hyperthyroidism must be at least briefly considered.[7] The patients who suffer this disorder, of course, usually have major personality disorders, often want to be extremely thin, and may be hysterical, rigid, perfectionistic, and immature in their behavior. Indeed, one patient actually submitted herself to total thyroidectomy and two courses of radioactive iodine treatment.[7]

The diagnosis should be suspected in the presence of long-standing elevations of thyroid function test results and low radioactive iodine uptake values, in the absence of thyroid enlargement. The differential diagnosis includes painless thyroiditis; Jod-Basedow's syndrome; and the rare examples of functioning thyroid carcinoma with thyroid either removed or destroyed, and ovarian teratoma (struma ovarii).

A determination of the serum thyroglobulin concentration is extremely useful in differentiating among these disorders. In the case of factitious thyrotoxicosis, the serum thyroglobulin level will be markedly reduced, whereas in all of the others mentioned the thyroglobulin level will be either high or well above normal.[26]

A very unusual variant of factitious hyperthyroidism has been described, owing to the inadvertent ingestion of foods containing large amounts of thyroid tissue. A recent "epidemic" due to the ingestion of hamburger laden with such thyroid tissue has been reported.[18a]

EXTREMELY RARE FORMS OF HYPERTHYROIDISM

Mention should be made of hyperthyroidism due to hyperactivity of thyroid tissue in struma ovarii. This extremely rare entity should be considered in instances of hyperthyroidism in which the "neck uptake" is very low, but the serum thyroglobulin is not suppressed.[7, 26] A pelvic scan with ^{131}I would be useful when this condition is being seriously considered, but false positive results have been reported.[7]

Hyperthyroidism has also been reported in association with McClune-Albright syndrome.[55] This condition is characterized by the polyostotic fibrous dysplasia of bone, cutaneous pigmentation, sexual precocity, and a variety of other endocrine disturbances, including thyroxicosis. The hyperthyroidism is of mild severity and appears to be related to autonomous nodules; TSH is suppressed, and there are no demonstrable abnormal thyroid stimulators. The cause of the syndrome is unknown and that is also true for the component of hyperthyroidism. The treatment is the same as that for other cases of toxic nodular goiter.

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15
Hypothyroidism

ROBERT VOLPÉ

Hypothyroidism may be defined as the result of inadequate production or delivery of thyroid hormone to its site or sites of cellular action.[93] Generally, hypothyroidism is due to an inadequate output of thyroid hormone and may occur at any time of life, even *in utero*. The inadequate production may be caused by disorders of the thyroid gland itself, by anterior pituitary insufficiency, or uncommonly by hypothalamic abnormalities.[54] Resistance to the action of thyroid hormones is a very rare cause of hypothyroidism.[69]

Virtually every tissue in the body is affected to a greater or lesser extent by thyroid hormone deficiency. The clinical picture will depend upon a variety of factors, such as the rate of onset of the deficiency and its severity.[38] The term myxedema, often used as a synonym for hypothyroidism, has another connotation, namely, the presence of mucinous edema that can be recognized clinically. Because many patients with hypothyroidism do not show such marked clinical changes, the term myxedema should be applied to only those with severe disease and specific tissue alterations.[38]

Table 15–1 will list the causes of hypothyroidism. The great majority of patients who present with hypothyroidism suffer from primary thyroid disease. Pituitary hypothyroidism is uncommon and should be suspected if there is other clinical evidence of primary pituitary disease, such as acromegaly, Cushing's syndrome, visual field defect, history of pituitary surgery or irradiation, or other more unusual causes of hypopituitarism.[54] A rare cause of hypopituitarism with secondary hypothyroid-

Table 15–1. Causes of Hypothyroidism

1. Marked reduction in thyroid parenchymal cells
   a. Thyroid agenesis or hypogenesis
   b. Thyroiditis (almost always secondary to autoimmune thyroiditis; rarely, after subacute thyroiditis; very rarely, after Riedel's struma)
   c. Radiation (radioactive iodine or external)
   d. Thyroidectomy
   e. Cancer
   f. Infiltrations or degenerations of the thyroid gland
2. Types due to normal or hyperplastic thyroid gland
   a. Enzymatic defects (includes Pendred's syndrome)
   b. Iodine deficiency
   c. Goitrogens
3. Decrease in stimulation of thyroid gland (reduction of thyrotropin due to pituitary or hypothalamic disease)
4. Peripheral resistance to thyroid hormone effects (Refetoff's syndrome)

ism, namely, lymphoid hypophysitis, can also be associated with primary hypothyroidism due to Hashimoto's thyroiditis.[49] It most commonly occurs in the postpartum period and may be confused with Sheehan's syndrome (postpartum pituitary necrosis), another cause of postpartum pituitary insufficiency.

Primary hypothyroidism occurs most frequently in women, with a sex ratio of females:males of 10:1 in symptomatic patients. The disease can occur at any age, although the most common age of presentation is between 30 and 65 years. Permanent forms of hypothyroidism are categorized in Table 15–1; transient forms are listed in Table 15–2.

The clinical syndrome of hypothyroidism was first described by Gull in 1874.[37] The term myxedema was coined by Ord in 1888,[66] and as mentioned, myxedema and hypothyroidism were considered synonymous terms for several years. However, it has long been clear that hypothyroidism varies in biochemical and clinical severity, and this is discussed subsequently.

EPIDEMIOLOGY

A study by Tunbridge[89] indicated that overt hypothyroidism, accompanied by low levels of thyroxine and raised thyroid-stimulating hormone (TSH) values, occurred in a survey in northeastern England in 14 of 1000 females and in less than 1 of 1000 males. This finding is similar to previous survey results reported by Gordin and associates.[35] In these surveys, the overall prevalence of both treated and untreated cases of overt hypothyroidism was in the order of 0.6 to 0.8%. These studies, however, were done in areas without iodine deficiency; it should be emphasized that the frequency of hypothyroidism in areas of iodine deficiency is clearly much higher. Ibbertson[45] has shown that the serum TSH level may be elevated in up to 50% of persons who live in areas of severe iodine deficiency. In these same regions cretinism may occur in 1 to 10% of the population. The presence of minor degrees of hypothyroidism (both mild and subclinical forms) will always be more common than the overt forms of the disease. The term will also embrace patients with "compensated" hypothyroidism, i.e., normal circulating thyroid hormone levels with high TSH values, as well as those with equivocal or slightly low thyroid hormone concentrations and elevated TSH levels.[26] Up to 3% of the population can be placed in this category; it is much more common in females and has a much higher incidence with advanced age.[35, 89]

It should be emphasized that the progression of compensated hypothyroidism to overt hypothyroidism is by no means a certainty. Indeed, Tunbridge[89] suggests that only about 2%/year of patients with both thyroid autoantibodies and raised TSH levels will develop overt hypothyroidism. The incidence of development of overt hypothyroidism in patients with only thyroid autoantibodies on the one hand and raised TSH levels on the other, is much more uncommon. Indeed, thyroid autoantibodies and TSH levels can fluctuate spontaneously, and many patients with histologic evidence of autoimmune thyroiditis never develop clinical evidence of thyroid disease during their lifetimes.[18, 99]

Hypothyroidism may occur at any time of life and has different characteristics and consequences at different stages of life. The term cretinism relates to hypothyroidism that commences in fetal or neonatal life, and this is discussed next.

Table 15–2. Transient Hypothyroidism Circumstances*

1. Drug administration (antithyroid drugs, iodine, lithium, other goitrogens)
2. Subacute thyroiditis
3. Silent thyroiditis
4. Postpartum thyroiditis
5. Autoimmune thyroiditis
6. After discontinuing long-term thyroid hormone therapy
7. Postoperatively in Graves' disease
8. Following radioactive iodine treatment
9. Hypothyroidism recovering, then changing into hyperthyroid Graves' disease
10. Neonatal transient hypothyroidism

*Reproduced from Lamberg, B. A.: Etiology of hypothyroidism. Clin. Endocrinol. Metab. 8:3–19, 1979, with permission.

CRETINISM

Etiology

Cretinism results from an inadequate output of thyroid hormone during uterine and neonatal life.[45, 47, 60] It may be endemic (related to severe iodine deficiency) or due to enzymatic defects within the thyroid gland. A goiter may be associated with these forms of cretinism (goitrous cretinism).[45, 60] The more uncommon sporadic cretinism is usually not associated with goiter, and the genetic aspects of this

disorder remain to be clarified.[21] Indeed, many such patients have no demonstrable thyroid tissue (athyreotic cretinism due to thyroid aplasia) or may have thyroid hypoplasia.[54] Thus, the causes of cretinism include developmental abnormalities of the thyroid gland, genetic enzyme defects, iodine deficiency, and excessive maternal intake of goitrogens.[54] When the thyroid is hypoplastic, it may often be found in unusual sites, for example, lingual thyroid.[54]

While autoimmune thyroiditis rarely makes its appearance within the first years of life, there are at least two reports suggesting that cretinism may have been conceivably due to autoimmunity.[11, 85] The passive transfer of TSH-blocking antibodies may also be a cause of transient neonatal hypothyroidism.[59] However, contrary to some recent reports,[91] such antibodies, including "thyroid growth inhibiting" antibodies are very unlikely to result in permanent hypothyroidism. Very rarely, resistance to thyroid hormone at the cellular level may also result in cretinism.[70]

Clinical Aspects

Thyroid hormone is essential during embryonic life for the growth and development of many tissues, especially brain tissue. The influence of thyroid hormone on brain metabolism has been investigated extensively.[47] There is evidence of depressed RNA and protein synthesis, decreased activity of specific central nervous system enzymes, reduced neuronal cell population, decimated synaptic interactions, and deficit of myelin,[36] which may be irreparable, following severe fetal hypothyroidism.

In humans, the critical period during which thyroid hormones influence brain development encompasses the last months of fetal development and the first postnatal year. This period is associated with rapid myelinization, proliferation of dendritic and axonal processes and glial cells, and continuing division of neuroblasts.[74] Mental development in children with congenital hypothyroidism depends largely on the duration of postnatal thyroid deficiency; mental retardation, unlike growth retardation, cannot "catch up" later in life, even under optimal replacement therapy with thyroid hormones.[47] Children treated before the age of 3 months show a much higher percentage of IQs above 90 than those treated after the age of 3 months.[52]

Thus, the outcome of mental development in the individual child depends on the duration and severity of thyroid deficiency during fetal and neonatal life. The infant completely deprived of thyroid hormone during fetal life will be retarded, no matter how early thyroxine is initiated following birth. The retardation can be ameliorated to a greater or lesser degree if thyroid hormone is begun immediately after birth.[75–77] Indeed, if the fetal thyroid has produced even a modicum of thyroid hormone during gestation, thyroxine therapy begun even some weeks after birth may result in ultimate normal mental and physical development. However, whether "normal" mentation attained under these circumstances represents the optimal that that particular person might have attained had he not suffered intrauterine hypothyroidism is uncertain; rather, studies suggest that subtle mental and motor system abnormalities may persist.[75–77] All other clinical manifestations are readily and completely reversible when thyroxine therapy is utilized in this fashion.

Clinical Diagnosis

Clinical recognition of hypothyroidism during the earliest period of life may be difficult. The full-blown picture develops slowly over weeks or months.[74] At birth, most infants with hypothyroidism and even some with athyreosis appear normal.[74] However, there may be some nonspecific signs or symptoms or abnormalities of behavior present during the newborn period that are attributable to hypothyroidism, at least in retrospect. Thus, such elements as prolonged jaundice, feeding problems, somnolence, and constipation may be present quite early in life.[47]

Later, the characteristic features of cretinism can readily be recognized.[21, 47] The cretin is dwarfed and has short limbs (Fig. 15–1). The nose is broad, the eyelids are puffy and wrinkled, the tongue is thick, the voice is husky, and the forehead is short. The teeth appear late. The fontanelles also close late. The hands and feet are short, broad, and puffy. The skin is coarse and dry; the hair is sparse and dry. The abdomen usually protrudes, and umbilical hernias are common. Mental retardation may be severe. Cretins are slow to crawl, sit, or stand; their movements are slow. The physician should suspect cretinism in infants who show marked retardation of development, sluggishness, dwarfism, delayed bone development, bradycardia, and often hypothermia (see Fig. 15–1).

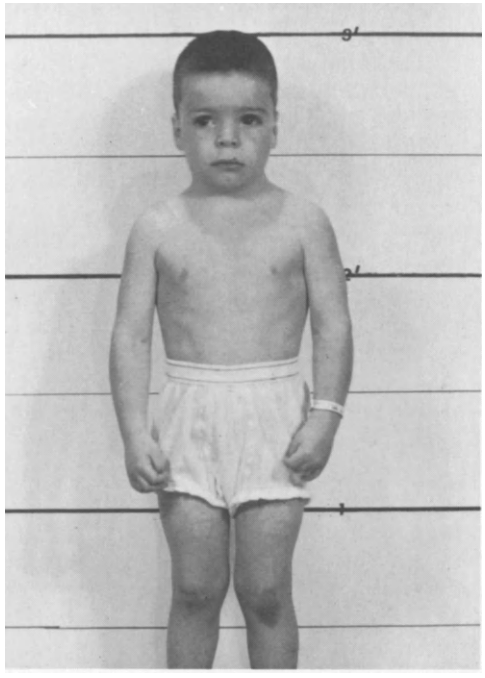

Figure 15–1. Five-year-old child with severe cretinism. Note the marked shortness of stature, depressed bridge of the nose, and short fingers. This child was also very markedly mentally retarded.

Diagnosis

In the newborn period, as in later life, low thyroxine (T_4) and high thyrotropin (thyroid-stimulating hormone, TSH) values constitute the characteristic finding of primary hypothyroidism.[53] However, the physiologic changes in thyroid function occurring during the normal neonatal period have to be taken into consideration when laboratory tests are performed.[31] Thus, TSH concentrations in cord blood are normally significantly higher than in maternal blood. Ater birth, there is a further sharp increase, reaching peak values within the first 2 hours of life that are many times higher than basal TSH levels. There is, thereafter, a rapid decline in TSH concentrations, reaching adult levels by the second day of life.

Serum triiodothyronine concentrations are, in contrast, three times lower in cord blood than in maternal blood.[69] Thyroxine, in comparison, shows no significant difference from the level in maternal blood.[31] Both thyroid hormones rise within the first 24 hours and then remain slightly above adult levels for several days. Reverse T_3 (3,3′5′-triiodothyronine, rT_3) will be found to be elevated during the first 4 days of life, decreasing to adult levels thereafter.[31]

It should also be emphasized that the mean level of serum thyroxine in prematurely born infants are significantly lower than in term infants and this too has to be kept in mind.[31]

Neonatal Screening for Congenital Hypothyroidism

Screening programs are now functioning on a large scale in North America, Europe, and Japan.[22, 47] Hormone determinations may be done either in cord blood or in dried blood spotted on filter paper. While cord blood permits a relatively large quantity of plasma and allows the determination of a whole spectrum of thyroid hormones by conventional radioimmunoassay methods, nevertheless, there are distinct disadvantages to the use of cord blood for this purpose. First, the shipment of plasma samples is not convenient for large scale programs. Second, there is a considerable overlap between normal and pathologic ranges directly at birth.[47] Therefore, most medical centers are now employing filter paper blood spots, usually taken by heel pad stabbing 5 days after delivery. These filter paper spots can be readily handled logistically in large numbers, and new automated techniques have been made available for determination of thyroxine, TSH, or both.

The disadvantage of T_4 screening is the high rate of retesting of specimens by TSH (and T_4) determinations, and a recall rate of more than 1%. Thus, many centers have gone directly to TSH determination as a single screening procedure. Values above 25 mU/L are considered abnormally high. When such values are obtained, the diagnosis of primary hypothyroidism is achieved by a second test of TSH and T_4 in dried blood before thyroxine therapy is instituted.

Because of its low recall rate, TSH screening seems most suitable for a mass screening program. Of course, it is limited to the discovery of primary hypothyroidism on the order of one infant in every 3000 to 5000 live births.[22, 47] This program does not permit the detection of secondary hypothyroidism which, however, is quite rare at this age, and such infants may not be so crucially affected by early diagnosis and treatment. These screening programs are clearly cost effective and are recommended for all countries that have the organization to mount them.[22]

In the absence of screening procedures, serum thyroxine and TSH determinations car-

ried out at the time of examination will establish the diagnosis. However, the importance of screening procedures for early diagnosis and treatment cannot be overemphasized.[77] Roentgenograms will reveal abnormalities of osseous development, such as delayed ossification, delayed closure of fontanelles, delayed appearance of epiphyseal centers, delayed bone age, and retarded growth of the base of the skull. Ossification centers often have an unusual stippled appearance because several small subcenters unite to form an irregular center.[21, 47]

Cretinism may occasionally be confused with mongolism.[7] However, children with Down's syndrome, while retarded, are active, and have hyperextensive joints and fine features. They display no evidence of retarded ossification; although their growth is below the average, they are usually not dwarfs.[7] Of course, test results of thyroid function are usually normal, although there is an increased incidence of autoimmune thyroid disease in patients with Down's syndrome.[28]

Treatment

While therapy for all forms of hypothyroidism is discussed at the end of this chapter, a few points in relation to the management of cretinism will be made at this juncture. Treatment should be initiated as soon as possible, even if the diagnosis has not yet been substantiated definitively by hormone determinations in plasma. In the TSH screening programs, substitution therapy with thyroxine commences immediately after blood sampling at the recall examination, which is usually within the second week of life. The initial dosage in a newborn is usually 0.025 mg of thyroxine daily, to be increased after 3 weeks to 0.050 mg daily.[47] By the age of 3 or 4 years, the dosage can be increased gradually to an adult lifetime maintenance dosage of approximately 0.1 to 0.15 mg/day. Infants are evaluated at 1- to 3-month intervals, particularly to ensure that the TSH values have returned to and then remain at normal levels.

As previously mentioned, it should be noted that transient neonatal hypothyroidism, if not recognized as a transient disturbance, may lead to lifelong, unnecessary thyroxine therapy. Transient hypothyroidism may be caused by iodine deficiency; by placental passage of thyroid suppressive factors or goitrogens, such as antithyroid drugs or iodine; and by maternal thyroid autoantibodies. Radiocontrast iodine–containing materials or topical application of iodine-containing antiseptic agents may also cause transient neonatal hypothyroidism. In any event, only 1 to 2% of infants whose condition is diagnosed by newborn screening have transient hypothyroidism.[16]

Because these patients will be identified in the screening programs and started on thyroxine therapy as if they were permanently hypothyroid, it is wise to recheck thyroid function at a later date. This should not occur before the age of 1 year and, preferably, about 3 years, and should take the form of temporary withdrawal of thyroid hormone therapy.[16] To do this with the least disturbance, the thyroxine should be replaced by triiodothyronine therapy for 3 weeks.[47] The triiodothyronine can then be withdrawn for 1 week, and a TSH determination carried out. If the TSH determination is then above 20 mU/L the patient may be safely considered to be truly permanently hypothyroid and reinstituted on lifelong thyroxine therapy.

Of course, this section would not be complete without at least a brief comment about the prevention of iodine deficiency in the world, a common cause of goitrous cretinism in many regions. This prevention is accomplished by the provision of iodine to the mother and the neonate. This simple statement, however, begs the question of the logistics of iodine administration to people in iodine-deficient areas—a problem that has yet to be solved in several parts of the world.[17]

CHILDHOOD AND ADULT HYPOTHYROIDISM

Unlike cretinism, hypoplasia of the thyroid gland is a less common cause of hypothyroidism, beginning later in childhood.[32, 54] Acquired primary hypothyroidism is more common and may be caused by milder genetic enzymatic defects, thyroiditis, or medical or surgical intervention (subtotal thyroidectomy, radioactive idoine therapy). In addition goitrogens, iodine deficiency, and malignant destruction of the thyroid gland may cause hypothyroidism. A very rare cause of familial hypothyroidism should again at least be mentioned, namely, cellular resistance to or lack of recognition of thyroid hormones (Refetoff's syndrome).[69, 70] This condition is characterized by minimal or occasionally severe clinical hypothyroidism, accompanied by *high* serum levels of T_4, T_3, and TSH.

Iodide-induced myxedema is of considerable interest; it results from the prolonged ingestion of moderate or large doses of iodide usually in the form of an expectorant mixture.[3, 97] Certain proprietary drugs used in the treatment of asthma contain large amounts of iodine. It appears to be that only persons who are especially responsive to the inhibitory effects of iodide develop goiters or hypothyroidism under such prolonged therapy. Some of these patients have coexisting thyroid abnormalities, such as Hashimoto's thyroiditis, which predisposes them to this excessive response to iodide.[92] In any event, the iodide acts directly on the thyroid gland, preventing the organic binding of iodide that changes it to organic iodine.[97] Thus, the further synthesis of thyroid hormone is reduced, resulting in increased pituitary TSH secretion. The thyroid gland hypertrophies (iodide goiter). It may thus overcome the iodide-produced block and result in a euthyroid goiter, but if the block is severe, the individual will become hypothyroid. The process, of course, can be reversed by discontinuing the iodide.[3, 97]

It is curious that both iodide excess and iodine deficiency can result in hypothyroidism. In many regions of the world, iodine deficiency remains the commonest cause of hypothyroidism. This subject has been touched upon as a cause of cretinism and is not discussed further herein.

The most common cause of spontaneous hypothyroidism in adolescence or adulthood is autoimmune thyroiditis associated with either goiter or atrophy of the thyroid gland. Mild forms of this disorder are quite common. This disorder has been considered in depth in Chapter 11, and the etiology is not discussed in this section. In addition, in postpartum thyroiditis, a variant of autoimmune thyroiditis, there is commonly a transient phase of hypothyroidism (see Postpartum Thyroiditis, Chapter 11). Autoimmune thyroiditis may also be associated with a state of chronic "compensated" or subclinical hypothyroidism,[27] which is discussed further subsequently. Indeed, there may be variations over time in the clinical disorder, varying from overt hypothyroidism to less marked hypothyroidism to disappearance of the disorder completely.[95] Moreover, mild forms of this disorder may similarly vary over time clinically, biochemically, and serologically.[19, 92] However, the vast majority of patients who are found to be hypothyroid from this disorder will remain hypothyroid for life and accordingly should be appropriately treated (see later discussion).[44]

Another form of hypothyroidism that should be emphasized is post-therapy hypothyroidism occurring after the surgical or medical treatment of Graves' disease or other thyroid disorders. Patients with Graves' disease, whether treated with radioactive iodine or surgery, often become hypothyroid weeks, months, or many years later (see Graves' Disease, Chapter 13). Indeed, many patients treated with antithyroid drugs for Graves' disease may become spontaneously hypothyroid years after such therapy. In these situations, the therapy does not induce the hypothyroidism, rather the glandular deficiency results from concomitant spontaneous autoimmune thyroiditis with ultimate destruction of the thyroid gland.[86, 92, 95]

It is also obvious that hypothyroidism may result from secondary causes, namely pituitary or hypothalamic disease. Under this circumstance, the clinical features are milder and are often associated with the other clinical features of hypothyroidism. Other more uncommon causes of hypothyroidism are listed in Table 15–1.

Clinical Features (Figures 15–2 and 15–3)

The identification of patients with overt hypothyroidism rarely causes problems.[38] The recognition of mild degrees of thyroid insufficiency may, however, be difficult since individuals with mild hypothyroidism frequently have minor or nonspecific symptoms. Such complaints as lethargy, fatigue, and facial puffiness should certainly raise the suspicion of thyroid deficiency, particularly in persons who are at risk by virtue of their family history of autoimmune disease.[43] In addition, patients with Down's syndrome or Turner's syndrome, who manifest an increased incidence of autoimmine thyroid disease,[28, 41] should be screened for this possibility. It should be kept in mind, however, that many patients with complaints of lethargy, lassitude, fatigue, and even puffiness of the face may have only functional disturbances and no evidence biochemically of thyroid disease.

The subtle and prolonged evolution of this disorder is another obstacle to quick diagnosis. It may take years for the condition to become overt.[1, 86] Spontaneous severe primary myxedema in adults is usually due to atrophic autoimmune thyroiditis, whereas goitrous

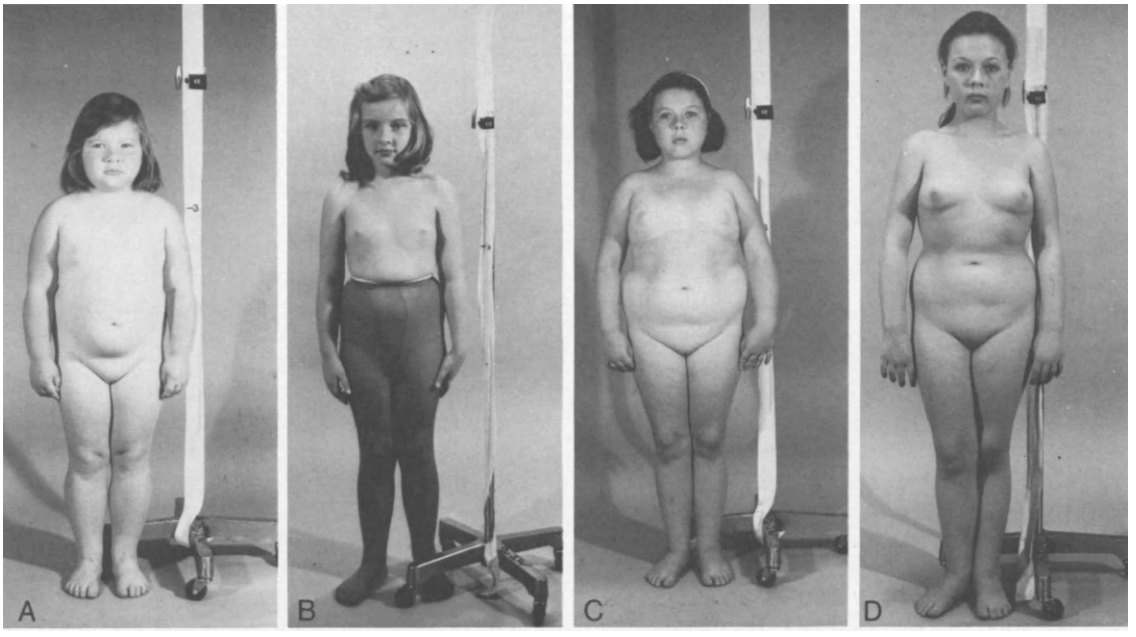

Figure 15–2. *A,* A 9-year-old girl with juvenile myxedema secondary to autoimmune thyroiditis. Note the puffy face, protuberance of the abdomen, and shortness of stature. *B,* The same child after 8 months of thyroxine therapy. *C,* Sister of girl in *A* and *B,* age 13. This photograph was taken when this girl was also myxedematous, likewise a result of autoimmune thyroiditis. *D,* The same patient 8 months after thyroxine therapy had been instituted. Note the sharp gain in height and physical development.

Hashimoto's thyroiditis does not frequently reach this severe form.[1]

Thyroid hormone deficiency causes a wide variety of disturbances; it decreases the metabolic activity of virtually all body cells and allows mucinous interstitial fluid to accumulate.[38] Thus, in overt hypothyroidism, patients will complain of dryness of hair and skin, fatigue, mild weight gain, cold intolerance, puffiness of face and body, decreased sweating, muscular aches and pains, constipation, bloating, hoarseness, dyspnea, menorrhagia, deafness, difficulty in concentration, and lapses of memory.[86] These symptoms may have developed subtly; the associated lethargy and apathy often ensures that the patients do not bother to seek medical advice. Under such circumstances, they may decline into a vegetative existence and might even die in coma after years of myxedema. The sequence is particularly important in the elderly because these manifestations may be confused with senility or may act synergistically with minimal brain dysfunction to create a severely ill patient.

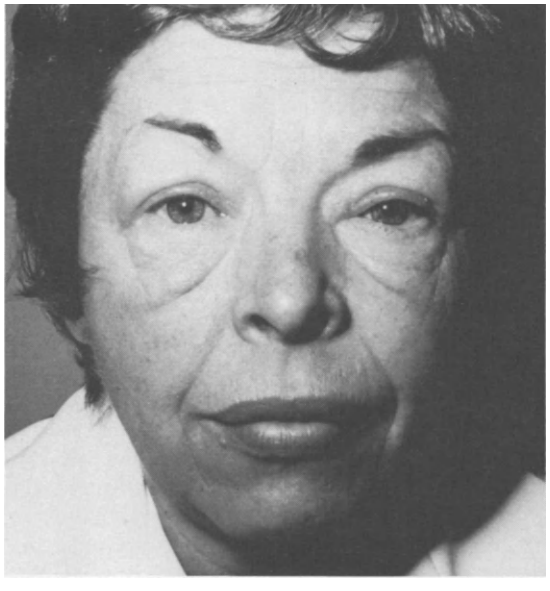

Figure 15–3. An adult with severe myxedema secondary to autoimmune thyroiditis. Note the marked periorbital edema.

The skin is characteristically dry and flaky with hyperkeratosis over the flexures. The dryness under these circumstances is at least partly due to the decreased sebum excretion rate.[34] The skin feels cool, and there may be a faint yellow color to the skin, particularly on the palms, soles, and creases. The color results from hypercarotenemia due to reduced conversion of carotene to vitamin A.[24] The coarsened features and subcutaneous swelling noted

in severe hypothyroidism are due to the presence of mucinous interstitial fluid (a mixture of polysaccharides, hygroscopic hyaluronic acid, and chondroitin sulfate).[33] Thus, the eyelids are puffy, as are the hands and feet (Fig. 15–3). The skin is thickened, coarse, and cold. The heart may be enlarged, the myocardium is pale and flabby, and the swollen myocardial fibers may be separated by an accumulation of mucinous material. Similar material may accumulate on the serous surfaces. Congestive heart failure may occur. Hearing loss and poor memory may become evident. Depressive disorders, agitated states, or paranoia may be features of severe hypothyroidism. Vertigo may appear, and rarely true cerebellar ataxia may be demonstrable. Speech is characteristically slow, and the voice is hoarse. The tongue is often thickened. Carpal tunnel syndrome may be seen.[38, 86]

Muscle involvement may be reflected by general aches, pains, and stiffness. The relaxation phase of the tendon jerk is prolonged, while the contraction phase may remain quite brisk. This is a primary muscle abnormality and can be measured as the Achilles tendon reflex time. It is a feature of advanced hypothyroidism and may not be detected in very mild forms of the disorder. In addition, the patient may manifest pseudomyotonia, which reflects a slowness of the response of the muscle belly when it is directly struck by a sharp reflex hammer. This, too, is seen only in severe myxedema.[38, 86]

Bloating and constipation are common, and ascites occasionally develops. Vital capacity and maximal ventilatory capacity may be reduced, and pleural effusions may be demonstrable.[38, 86] When hypothyroidism develops in children or adolescents, the growth rate is markedly slowed, and developmental distortions may occur (see Fig. 15–2).[32] In young males, there may be enlargement of the genitalia, while in females there may be retardation of the menarche and secondary sex characteristic development or precocious puberty and galactorrhea (see Fig. 15–2).[32] Long-standing primary hypothyroidism will also result in enlargement of the anterior pituitary gland due to the greater number of thyrotrophs. This finding may be evident in roentgenograms, particularly when patients are young.[87]

Diagnosis

Investigation of patients with suspected hypothyroidism falls into two categories.[9] The first is to determine the status of thyroid function so as to confirm or rule out a diagnosis of hypothyroidism. The second is to determine the etiology of the hypothyroidism.

Tests of Thyroid Function

Direct techniques for measuring circulating thyroid hormone concentrations are widely available and are discussed elsewhere in this text. Serum thyroxine (T_4) is now generally measured by radioimmunoassay. This procedure measures total serum T_4 concentration, and thus alterations in the capacity of thyroxine binding proteins may lead to total hormone concentrations at variance with the clinical assessment of the patient. Serum T_4 concentration can be corrected for changes in thyroxine-binding globulin (TBG) by carrying out an indirect measure of thyroxine binding, such as the T_3 resin uptake. For the combination of these two measurements, a free thyroxine index (FTI) can readily be calculated. In addition, the free thyroxine (T_4) can now be directly measured, although there are theoretic problems with the interpretation of most of the results of many of the commercial procedures.

The serum total triiodothyronine (T_3) concentrations can also be measured directly by radioimmunoassay. It is of interest that as hypothyroidism develops, the T_4 will fall below normal values long before the T_3. Nevertheless, it is worthwhile testing for T_3, since it does correlate fairly well with clinical status; when the T_3 is still normal or only slightly subnormal, the patient will not be nearly as severely hypothyroid clinically as when the T_3 finally falls to significantly low levels. However, the serum total triiodothyronine determination should be interpreted with some caution and understanding, since in the euthyroid "sick" syndrome (a condition that does not equate with true hypothyroidism), the serum total triiodothyronine may be very low indeed. However, as noted subsequently, the TSH concentration is not elevated in this curious nonthyroidal syndrome, whereas this finding is the first and most sensitive indicator of primary hypothyroidism.

The radioactive iodine uptake will not be useful in the diagnosis of hypothyroidism. While in severe destruction of the thyroid gland the uptake values for this test are low, there are many types of hypothyroidism not associated with such low uptake values. Thus,

the results obtained may be misleading and this test should not be performed in the investigation of suspected hypothyroidism.

As previously mentioned, the TSH determination is exceedingly important and is measured by radioimmunoassay. Indeed, newer extremely sensitive techniques using immunoradiometric assay (IRMA) and other technical innovations have made the TSH procedure extremely valuable. A rise in TSH will precede any other abnormality of thyroid function as the first evidence of primary hypothyroidism. Indeed, the state of "compensated" or subclinical hypothyroidism should be emphasized here.[27] This is a state in which the serum thyroid hormone value is normal, yet the TSH is elevated. This finding is due to a disturbance in thyroid function, whereby normal thyroid hormone output is maintained only by virtue of increased stimulation of the thyroid gland by increased TSH production. Patients so affected may have either no symptoms or extremely mild symptoms suggestive of hypothyroidism.[71] This state of compensated hypothyroidism may give rise to overt hypothyroidism in some patients,[25, 95] while in many others there is no further progression of thyroid deficiency.[25, 55, 95]

In past years, assessment of the TSH response to thyrotropin-releasing hormone (TRH) was performed and showed an exaggerated response in patients with primary hypothyroidism. This is of little current value, particularly because of the sensitive TSH procedures available. However, in patients with secondary hypothyroidism, the TRH test may be useful to determine the nature of the pituitary or hypothalamic disease. In the case of pituitary insufficiency, the TSH values will be low and will not respond to TRH. In hypothalamic disease, the basal TSH may be normal and there is usually a sluggish response to TRH.

Other Associated Biochemical and Hematologic Abnormalities

Hyperlipidemia (Fredrickson's types II or IV) is common.[56] Dilutional hyponatremia,[53] increases in serum aspartate transaminase, lactate dehydrogenase, creatine phosphokinase, and serum magnesium levels may be seen.[2] Hypoplastic anemia is observed in moderately severe and severe cases.[43] The serum prolactin level is often modestly elevated.[32, 87]

Other Laboratory Procedures

The electrocardiogram may show bradycardia and low voltage. The measurement of the ankle tendon reflex duration may be slowed. Both of these responses, however, are nonspecific.[27]

The Possibility of Screening Procedures for Adult Hypothyroidism

Since mild or subclinical (compensated) hypothyroidism is quite common in the elderly, particularly elderly females, it has been suggested that screening procedures should be carried out in institutions where the elderly live or even in the elderly in the general population.[90] However, such screening procedures have not become popular.[23] The reason is that the yield of overt hypothyroidism from such studies has been rather small and does not appear to justify the cost of doing tests on every institutionalized elderly person, let alone those in the general population. However, a high index of suspicion should be maintained when reviewing the status of an elderly person who shows evidence of mental or physical deterioration or both.

INVESTIGATION INTO THE ETIOLOGY OF HYPOTHYROIDISM

The various causes of hypothyroidism are listed in Table 15–1. It should be emphasized that the most common spontaneous cause of hypothyroidism in the Western world is autoimmune thyroiditis. This condition is most commonly associated with a firm diffuse goiter, although the gland, conversely, may be atrophied. Thyroid autoantibodies should be routinely performed, but biopsy is usually not indicated unless there are grounds for considering the possibility of thyroid malignancy (see Autoimmune Thyroiditis, Chapter 11).

The other causes of hypothyroidism are generally self evident. Dietary iodine deficiency will generally be an endemic problem. The hypothyroidism following subacute thyroiditis or silent thyroiditis is usually self evident and often transient. The same is true for postpartum thyroiditis, although such an affected patient should be carefully monitored and may ultimately remain permanently hypothyroid. Riedel's struma is very rare but may occasionally lead to thyroid failure. Other goitrous causes of hypothyroidism as listed in Table 15–1 can be appropriately investigated.

MANAGEMENT OF HYPOTHYROIDISM

The treatment of hypothyroidism has its beginnings in the 19th century. After the Gull commission first accurately described the nature of hypothyroidism,[37] it was not long before Murray in 1891[62] began to treat patients with myxedema with injections of sheep thyroid extract and reported success with this treatment. This treatment was later followed by desiccated thyroid treatment, a preparation that has been used extensively throughout the world until the present generation.[80] It now seems evident, however, that synthetic sodium levothyroxine is superior to other preparations for long-term replacement therapy.[15, 80] The use of thyroid replacement therapy has been greatly facilitated by the ability to precisely measure thyroid function. (The sensitive radioimmunoassays for thyroxine (T_4), triiodothyronine (T_3), free thyroid hormones, thyroxine-binding globulin, and thyrotropin (TSH) have been discussed elsewhere in this text.) The response of TSH to thyrotropin-releasing hormone (TRH) has been advocated for "fine tuning" of thyroid hormone replacement[42] although in my view this procedure is unnecessary.[94] Drug toxicity from the thyroid hormones relates only to overdosage and otherwise is virtually unknown.

Therapeutic Use of Thyroid Hormone Preparations (Table 15–3)

Sodium Levothyroxine (Thyroxine, T_4)

Sodium levothyroxine is now considered the drug of choice by most endocrinologists for the long-term management of hypothyroidism.[15, 42, 80] While it is clear that T_3 (not T_4) is the active thyroid principle within peripheral cells,[8, 12, 13, 64, 65, 78] T_3 is freely available to the tissues in patients who receive thyroxine, since T_3 production is predominantly via peripheral conversion of T_4 to T_3.[6, 48, 72, 79, 82, 84] Indeed, it has been suggested that T_4 is primarily a prohormone.[13] It has been calculated that when normal persons are on no medication, approximately 70 to 75% of T_3 comes from the peripheral monodeiodination of T_4 to T_3, whereas the remaining 25 to 30% comes directly from the thyroid.[6, 12, 13, 48, 64, 65, 72, 78, 79, 82, 84]

When patients are taking thyroxine, either for hypothyroidism or for goiter suppression, virtually no T_3 comes directly from the thyroid, and the entire source of T_3 under these circumstances is from the exogenous thyroxine.[29, 98] Daily administration of thyroxine results in constant concentrations of T_4 and T_3 in the peripheral blood throughout the day.[83] In general, when complete physiologic replacement doses of thyroxine are provided to hypothyroid patients, the T_4 value tends to be high normal or above normal, but the important point is that the total serum triiodothyronine determination will be well within normal.[29, 98] The reason for the constancy in blood levels relates to the polonged half-time of T_4 (1 week), associated with a relatively constant rate of conversation of T_4 to T_3.[48, 72, 79, 84] Because of this prolonged half-time of T_4, initiation of thryoxine therapy will not result in rapid clearing of hypothyroid manifestations.[15, 80] In comparison, it allows for some variation in compliance without obvious ill effects. The absorption of levothyroxine in the gastrointestinal tract is about 50 to 60% and is somewhat variable from patient to patient.[39] Diseases of the gastrointestinal tract,[88] including malabsorption syndrome, removal of large portions of the small bowel, cholestyramine administration,[63] and ingestion of soybean formula[68] may interfere with the absorption of thyroxine. Preparations of thyroxine contain no significant quantity of either active or inactive analogues of thyroid hormone and are not contaminated with nonhormonal iodide.[80] Thyroxine is of synthetic origin and therefore no thyroid gland proteins or other proteins are present in this formulation.

Initiation of Treatment. The conventional goal of treatment of hypothyroidism is obviously the reestablishment of a eumetabolic state. However, a number of factors must be considered in the selection of a starting dose of thyroxine, as well as of a time before which the dose should not be increased. These factors include the age of the patient, the presence of associated disorders, and the severity and duration of the hypothyroidism itself.[15] In the neonate, as previously mentioned, it is important to initiate thyroid hormone replacement as soon as hypothyroidism is suspected, since mental retardation may be prevented or markedly ameliorated by rapid restoration of the euthyroid state.[21, 47, 52, 53, 74–77] With the advent of neonatal screening programs, it has now become possible to shorten the time between diagnosis and commencement of such therapy.[20] In children, the dosage of thyroxine may be calculated by weight,[30] as noted in Table

Table 15–3. Available Preparations, Usual Maintenance Dosage, and Thyroid Hormone Levels During Maintenance Therapy*

Preparation	Composition	Tablet Strengths	Maintenance Dosage	Serum Hormone Levels	
				T_4	T_3
Levothyroxine sodium	T_4	0.025, 0.05, 0.1, 0.15, 0.3, and 0.5 mg†	0.1 to 0.2 mg daily or 1 to 2 mg weekly	Normal or somewhat elevated	Normal
Liothyronine (levotriiodothyronine) sodium	T_3	5, 25, and 50 μgm‡	25 μgm two or three times daily	Low	Elevated
Thyroid USP (desiccated thyroid)	25 to 35 μgm T_4 and 8 to 14 μgm T_3§ per 60 mg (1 grain)	15, 30, 60, 90, 120, 180, 240, and 300 mg	90 to 180 mg daily	Low or low normal	Usually elevated
Thyroglobulin	20 to 35 μgm T_4 and 11 to 21 μgm T_3 per 60 mg (1 grain)	15, 30, 60, 90, 180, and 300 mg	90 to 180 mg daily	Low normal	Usually elevated
Liotrix	Euthyroid: 60 μgm T_4 and 15 μgm T_3 Thyrolar: 50 μgm T_4 and 12.5 μgm T_3	½, 1, 2, and 3 "grain equivalents"	1.5 to 3.0 "grain equivalents"	Low normal	Often elevated

*Modified from Cobb, W. E. and Jackson, I. W. D.: Drug therapy reviews: management of hypothyroidism. Am. J. Hosp. Pharm. 35:51–58, 1978, with permission.
†Parenteral preparation: lyophilized powder, 0.5 mg, with mannitol, 10 mg, and normal saline 5 ml, as diluent.
‡Parenteral preparation: powder (for solution) 114 μgm/ml; not commercially available, but Smith Kline will supply kit upon request for use in myxedema.
§Range of T_4:T_3 ratio is 2 to 3:1 for pig glands and 3 to 4.5:1 for beef or sheep glands.

15–4. Other children and young adults may be initiated on the appropriate maintenance dosage rather than on a very low initial dosage, particularly if the hypothyroidism is mild and of short duration.[15, 80]

For adults, this maintenance dosage is in the order of 0.1 to 0.15 mg/day.[15, 80] If compliance is a problem, a weekly single dose of 2.0 mg may be perscribed.[5]

In older patients, particularly those with other overt associated diseases, such as cardiovascular disease, the dosage amount initially should be determined cautiously, with daily doses on the order of 0.025 mg.[50] In such patients, there is no necessity to increase the dose rapidly, as subsequently noted. The same considerations should obtain with elderly patients, even if there is no obvious cardiovascular disease, as it has to be assumed that such disease could exist in an occult form. In any event, even with full maintenance dosage schedules, it takes several weeks for clinical and biochemical normality to be reached.

Table 15–4. Recommended Replacement Dosage of Sodium Levothyroxine in Childhood*

Age (years)	Dose (μgm/kg/day)
0–1	9
1–5	6
6–10	4
11–20	3

*From Bernstein, R. S. and Robbins, J.: Intermittent therapy with L-thyroxine. N. Engl. J. Med. 281:1444–1448, 1969, with permission.

Adjustment of the Dosage. The half-time for thyroxine is approximately 1 week, as mentioned previously. Thus, steady-state equilibrium for thyroxine will be virtually complete after approximately 1 month. For that reason in older patients or in those with other associated disorders, one should allow about 1 month between dosage adjustments. In older patients, increments of dosage increases should be quite small on each occasion, since there is no necessity for rendering these patients euthyroid quickly.

In patients with associated coronary artery disease, as mentioned, the initial dosage should be on the order of 0.025 mg daily, with a monthly incremental increase of the same

amount.[85, 86] If symptoms of the associated cardiovascular disease, e.g., angina pectoris, become more evident as the dosage is slowly increased, one may have to be content with a maintenance dosage far below what otherwise would be considered optimal. In contrast, if antiangina or other cardiac medications are being employed successfully, the dosage of thyroxine can be cautiously increased.[48, 86]

Maintenance Dosage. Optimally, the objective would be to reproduce exactly the euthyroid status of that particular person. Since there is extremely sensitive regulation of the hypothalamic-pituitary-thyroid axis, it seems evident that for every individual there is an ideal thyroid hormone concentration.[4, 61] However, this ideal concentration is very difficult to achieve with precision by exogenous thyroid therapy, and it does not seem essential to achieve this ideal concentration exactly.[62, 76]

While it would be useful to have some clinically valuable index of the effect of thyroid hormone on peripheral tissues, such an index does not exist. Thus, the general rule is to use the clinical status of the patient on the one hand,[44] and the thyroid hormone and TSH levels on the other, to determine the adequacy of thyroxine replacement.[29, 98]

One means of establishing an optimal maintenance dosage of thyroxine is to ensure that the TSH levels have been brought down to within the normal range.[9, 15, 29, 38, 42, 84, 98] By such means it is certainly possible to determine when sufficient thyroxine therapy has been prescribed. The average dosage that causes the TSH to fall within normal limits varies between 0.1 and 0.2 mg per day. One could use the TSH response to TRH to achieve "fine tuning," but this is time-consuming and unnecessary for the purpose of achieving a clinically euthyroid state.[94]

The serum thyroxine or, for that matter, the free thyroxine cannot be employed to determine the precision of the replacement dosage. When patients are receiving physiologically optimal replacement dosages, the serum thyroxine and free thyroxine levels are either high normal or above normal in many, if not most, instances.[29, 98] However, what is more useful is an estimation of the serum total triiodothyronine radioimmune assay (T_3 RIA) for reasons already explained.[29, 98] If this value and the TSH determination are both within normal limits, the dosage can be stated to be optimal. Of course, it is necessary to calculate the effects of thyroxine-binding proteins on these values for proper interpretation of results.

I clearly prefer to use synthetic levothyroxine for maintenance therapy for virtually all hypothyroid patients. With the exception of those patients who have some other associated disorder, it is truly remarkable how severely myxedematous patients will respond so readily to a regimen as described. With the return of well-being, it is particularly rewarding to often note the return of a euthyroid state and normal mentation in elderly patients who may have previously been considered senile. It is almost invariably necessary to maintain hypothyroid patients on thyroid hormone replacement for life. This regimen should then be considered the dogmatic rule, accompanied by suitable exhortations to the patient and appropriate follow-up for the purpose of ensuring compliance.

In patients who have severe cardiovascular disease, it is often impossible to reach such optimal levels. Daily dosages as low as 0.05 to 0.075 mg per day may be as maximal as such patients can reach without causing aggravation of the vascular disease.[46, 50]

Other Preparations

Dessicated Thyroid. Desiccated thyroid is manufactured simply by drying the thyroid glands of various animals, such as cattle, pigs, or sheep.[15] The power so obtained contains a mixture of thyroid hormones, tyrosines, and iodinated proteins, and the standardization of the tablets has continued to be based on total iodine content rather than on the amount of hormone in the preparation. Thus, variable potency has been a problem, although some pharmaceutical companies have made considerable efforts to establish uniformity of potency in their products.[58] While in most instances 60 mg of desiccated thyroid is roughly equivalent to 0.1 mg of sodium thyroxine, this may vary considerably from lot to lot and from preparation to preparation.[57, 58] This variation depends considerably on the different T_4/T_3 ratios found in the thyroid glands of different animals.[81]

Because there is a considerable amount of T_3 in desiccated thyroid, the levels of T_3 will be higher in the blood shortly after taking the medication, falling to lower levels later in the day.[83] The serum T_4 concentration will generally remain at low normal or even below normal when patients take desiccated thyroid.[15]

Thus, serum concentrations of the thyroid hormones cannot be used to accurately assess efficacy of treatment with this medication.

For all of the aforementioned reasons, most endocrinologists no longer employ desiccated thyroid as treatment for hypothyroidism and, instead, generally use synthetic L-thyroxine.[9, 15, 46]

1-Triiodothyronine. Triiodothyronine is very rapidly absorbed when ingested and is three to four times as potent as thyroxine in restoring a hypothyroid patient to a euthyroid state.[15, 80] Since it has a half-life of approximately 1 day,[8, 12] euthyroidism could be achieved within days rather than weeks, as would be required with L-thyroxine. Because of its almost complete absorption[40] and rapid biologic activity, however, in patients with coronary artery disease it might prove to be dangerous[61]; in any event, bringing a patient rapidly to a euthyroid state is often not of any great value. Moreover, when a patient takes triiodothyronine, the serum levels of this hormone vary widely during the day, although this does not seem to cause any particular side effects.[83] Generally speaking, however, it seems L-thyroxine is more physiologic, which consequently deiodinates constantly providing stable levels of both T_4 and T_3 within the circulation. Incidentally, when a patient is taking triiodothyroinine, it is necessary to provide the tablets in divided dosages during the day, so as to minimize the wide fluctuations in serum T_3 that are observed with this medication.[15] Moreover, the serum thyroxine is depressed to very low levels on such a regimen and, thus, cannot be used as any index of treatment.

Thyroglobulin. Thyroglobulin (Proloid) is an extract of animal thyroid, which is brought to a greater level of purification than desiccated thyroid. It is standardized by bioassay and is thus more constant as a preparation than is desiccated thyroid. It is also more expensive and really has no advantages other than its relative uniformity when compared with desiccated thyroid.[15, 80]

Liotrix. Liotrix consists of a combination of synthetic T_4 and T_3 in a ratio of 4:1. This ratio was thought to mimic the proportions of T_4 and T_3 found in human thyroid glands,[81] but this ratio proved to be incorrect. The actual ratio in the human thyroid gland is approximately 20:1.[14] This product has most of the disadvantages of desiccated thyroid, primarily because of the rather high amount of T_3 contained in the preparation and has no advantages over desiccated thyroid, other than its standardization.

HYPOTHYROIDISM IN INFANCY AND CHILDHOOD

Hypothyroidism in infancy and childhood is best treated by using sodium levothyroxine.[30] The drug is given orally in a single daily dose. The optimal maintenance dose is that which corrects the total serum triiodothyronine and TSH determinations as previously noted. Recommended replacement of T_4 for hypothyroidism in infancy and childhood is age- and weight-related for dosage, as noted in Table 15–4.

In the neonate, it is important to commence therapy as quickly as possible, particularly in a patient with severe hypothyroidism so as to minimize or obviate mental retardation. However, excessive dosage in infancy can result in accelerated bone maturation and premature craniosynostosis.[67]

While it has been suggested that patients with congenital hypothyroidism appear to have alterations in the pituitary threshold for TSH secretion, such that with normal levels of serum thyroxine the serum TSH concentrations and the serum TSH responses to TRH are increased, this may not represent a true disturbance in the pituitary threshold. The same may be noted in adults and relates to the fact that T_3, not T_4, is the active thyroid principle. Since normally 70 to 75% of T_3 comes from peripheral conversion of T_4 to T_3, while the remaining 25 to 30% comes directly from the thyroid, the thyroid's contribution to T_3 is no longer provided in instances of hypothyroidism. Thus, when one is relying solely on exogenous T_4 for the replacement of T_3, it is necessary to provide sufficient exogenous thyroxine to ensure that there is an adequate supply of T_3. Consequently, when there is an optimal replacement dosage of exogenous thyroxine, the serum T_4 level will tend to be high normal or above normal; this level ensures that the serum T_3 will be in the middle of the normal range and that the TSH will then likewise be normal, as has been discussed previously.

With hypothyroidism acquired in older children, there is no concern about developmental risks or mental retardation. However, the

same considerations regarding optimal dosage of thyroxine apply (see Maintenance Dosage.)

HYPOTHYROIDISM FOLLOWING TREATMENT OF HYPERTHYROIDISM

Radioactive iodine or surgical treatment of hyperthyroidism accounts for a large number of cases of hypothyroidism in adult life. Quite often after such therapy, patients may appear clinically euthyroid and have only "compensated" hypothyroidism.[25] This state is characterized by normal levels of serum thyroxine and total serum triiodothyronine but by high levels of TSH.[50] In patients with compensated hypothyroidism, occurring after ablative treatment for hyperthyroidism, it should be assumed that overt hypothyroidism will ultimately follow.[49] Moreover, even transient hypothyroidism with subsequent recovery following such treatment will usually herald the ultimate development of permanent hypothyroidism (see Graves' Disease, Chapter 13). For these reasons, I am generally quick to prescribe lifelong thyroxine therapy for such individuals so as to obviate the need for close medical supervision thereafter. While generally the same conditions regarding optimal dosage prevail as previously discussed, replacement dosage of thyroid hormone for postablative hypothyroidism can sometimes be lower than anticipated because of continued nonsuppressible function in the thyroid remnant.[98] One, therefore, should not use a standard dosage in such patient but should ensure that the tests results of thyroid function, particularly the T_3 RIA and TSH determinations, are well within normal limits. It should thus be emphasized that when "standard" doses on the order of 0.1 to 0.2 mg of thyroxine are given to patients with nonsuppressible thyroid function, the total serum T_3 value may be elevated. Thus, a high serum T_3 value may be used as an index of nonsuppressibility in patients taking such "standard" dosages of thyroxine.

AUTOIMMUNE THYROIDITIS AND OTHER SPONTANEOUS FORMS OF HYPOTHYROIDISM

The treatment of hypothyroidism due to autoimmune thyroiditis or other spontaneous forms of hypothyroidism does not differ in principle from that already discussed. Generally speaking, in adults, dosages of 0.1 to 0.2 mg per day will suffice in most patients with these disorders. The achievement of optimal maintenance dosage should be individualized as previously described. Compensated hypothyroidism in this special group deserves special mention. Treatment actually may not be essential when patients are still in this phase. If one could predict which patients were going to develop overt hypothyroidism then it would be useful to start them selectively on thyroxine therapy. Since, however, overt hypothyroidism is a surreptitious disease, accompanied by patient disinterest and apathy, I prefer to start all such patients with compensated hypothyroidism on thyroxine at the time of initial recognition. Since the therapy itself is innocuous, whereas the consequences of overt hypothyroidism can be severe, this approach seems reasonable. Moreover, Ridgway and associates[71] have reported that in compensated hypothyroidism therapy does effect some subtle improvements in well-being, although this view has been disputed.[4]

PITUITARY AND HYPOTHALAMIC HYPOTHYROIDISM

Hypothyroidism secondary to pituitary or hypothalamic disease usually occurs in the context of panhypopituitarism or at least multiple anterior pituitary polypeptide hormone deficiencies and, rarely, occurs as a single unitropic hormone deficiency. When hypothyroidism occurs with a low TSH concentration and with a poor or flat response of TSH to TRH, it may be assumed that the abnormality lies in the hypothalamic or pituitary region. It is important to distinguish this form of hypothyroidism from primary hypothyroidism because of treatment considerations.

If one commences therapy with thyroxine alone in a patient with pituitary hypothyroidism, an occult pituitary adrenocorticotropic hormone (ACTH) deficiency state might be unmasked by such therapy and result in adrenal crisis. It is important that this possibility be precisely assessed, and corticosteroid initiated prior to the commencement of thyroid hormone supplementation.[94] Clearly, TSH determinations cannot be employed here to determine adequacy of thyroxine replacement, so that in this context, the serum total triiodothyronine determination is of greatest importance.

MYXEDEMA COMA

Myxedema coma, a rare problem, is a reflection of the most extreme expression of the most severe degree of hypothyroidism and frequently represents an end stage of that disorder. It is a medical emergency; even with vigorous therapy, the mortality rate may still be as high as 50%.[10, 73, 96] Two of Ord's 12 fatal cases of myxedema reported in 1888 apparently died in coma.[66]

Clinical Aspects

While the condition is based on severe hypothyroidism there are many other elements that are of importance as precipitating factors.[96] Often, the patients are elderly and precipitating events include respiratory infections, congestive heart failure, and cerebrovascular accidents. Indeed, pulmonary infections may also be secondary to the coma, since patients hypoventilate as a result of this disorder. Often, myxedema coma occurs during the winter suggesting that external cold may be an important precipitating factor. Drugs, such as sedatives, tranquilizers, narcotics, or antidepressants, which may depress the respiratory center, may also be important precipitating factors.[16, 73, 96]

Clearly, coma or marked stupor are important features of myxedema coma and hypothermia is equally important. The disorder usually comes to the physician's attention in an Emergency Department in a hospital where a previously undiagnosed patient is found to have severe hypothyroidism and coma simultaneously. Often, as mentioned, the illness has been complicated by infection or other systemic disease. On occasion a history of thyroid disease, including hyperthyroidism may be elicited, and it is not rare to learn that a patient has had medical or surgical thyroid ablative therapy in the distant past. In some instances, the patient has taken thyroid hormone replacement in the past only to discontinue it for one reason or another. Myxedema coma is almost always related to primary hypothyroidism, although a few patients with this condition will be found to have pituitary or hypothalamic disease as the basis for hypothyroidism.

There are other important clinical features relating to this disease. These include depression of respiration due to a reduced ventilatory response to hypoxia and hyperapnea. The resulting reduction in ventilation leads to CO_2 narcosis and further deepens the coma.[96]

The cardiac status is typical of severe hypothyroidism with enlargement of the cardiac silhouette, slowed cardiac rate, and generally the presence of pericardial effusion. The electrocardiogram will often show low voltage, prolonged QT intervals, and flattened or inverted T waves. An echocardiogram will show decreased action of the heart and pericardial effusion.

Decreased intestinal motility and even paralytic ileus may be seen. Increased body water with decreased serum sodium and plasma osmolarity with increased urine sodium and osmolarity are typical of this condition and are apparently secondary to impaired diuresis due to reduced delivery of water to the distal nephron. The hyponatremia may be responsible for seizures occasionally, and cerebellar signs may be secondary to the severe hypothyroidism.[10, 73, 96]

Hypoglycemia is also common, secondary to severe hypothyroidism. This hypoglycemia is rarely severe in itself but may contribute as still another factor to the stupor or coma.

Treatment of Myxedema Coma

The objectives of treatment are to replace the extrathyroidal hormone pool and to control the life-threatening complications of the condition. Treatment should be initiated immediately when the diagnosis is established on clinical grounds, because undue delay can occur if the physician waits for laboratory confirmation. It is clear that parenteral replacement is required initially.[10, 73, 96] The general consensus is that a large dose of thyroxine (about 500 μgm) should be administered intravenously. Some workers have suggested that small doses of triiodothyronine (12.5 μgm via nasogastric tube every 6 to 12 hours) should be provided on the grounds that an earlier onset of action would be to the patient's advantage.[10] An intravenous triiodothyronine preparation is not readily available commercially, although triiodothyronine could be given by that route in doses of 10 to 50 μgm every 6 to 12 hours.[73] It also has been suggested that T_4 and T_3 should be administered simultaneously because there might be an impairment of peripheral conversion of T_4 to T_3.

The case for the very large doses of thyroxine generally recommended has never been well established. It is clear, however, that unless the physician acts promptly, the mortality rate is exceedingly high. The parenteral

thyroxine or triiodothyronine should be discontinued as soon as the patient has recovered sufficiently to take therapy orally. Oral thyroxine should then be increased gradually as previously discussed. Adequate ventilation and oxygenation should be maintained throughout to treat the hypercapnia and hypoxia commonly seen in this disorder. Hypothermia generally does not require any particular therapy. Pressor amines should not be used because there is a significant risk of precipitating cardiac arrhythmias. Corticosteroid administration is generally recommended on the assumption that the patient is cortisol deficient. Infection, when present, should be treated rigorously and appropriately. Glucose infusions are generally useful, because many patients are hypoglycemic. However, fluid intake should be restricted because the patient often has what appears to be inappropriate production of excessive antidiuretic hormone, but probably the hyponatremia is due instead to reduced delivery of water to the distal nephron of the kidneys.[96] Although the serum sodium is low under these circumstances, this condition does not reflect a fall in total body sodium. Thus, hypertonic saline is not indicated.

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16
Thyroid Diseases in Pregnancy

GERARD N. BURROW

THYROID FUNCTION DURING PREGNANCY

Hormonal changes and metabolic demands during pregnancy result in complex changes in thyroid function. Furthermore, pregnancy outcome may be profoundly altered by abnormal thyroid function. Changes that occur in the various parameters of thyroid function are mainly due to increased thyroxine-binding globulin (TBG), which is induced by increased estrogen production during pregnancy. Although the normal pregnant woman is considered to be euthyroid, there is also an increase in the basal metabolic rate, radioactive iodine thyroid uptake, and thyroid gland size.

Thyroid disease is much more common in women than in men and is not rare during pregnancy. Clinically, the diagnosis of thyroid dysfunction in the pregnant woman can be difficult, and thyroid function tests may offer little help. Once a diagnostic decision has been reached, therapy is complicated by the presence of the fetus. Pharmacologic therapy that is beneficial to the mother may be harmful to the fetus. An understanding of the normal physiologic processes of the thyroid gland during pregnancy will be very helpful in understanding pathologic processes during gestation.

GOITER

The histologic picture of the thyroid gland during pregnancy is that of the active formation and secretion of thyroid hormone. Characteristically, the gland has large follicles with abundant, well-stained colloid, and frequent vacuolization. Papillary infolding indicative of follicular hyperplasia may be seen, and this impression is reinforced by the finding of columnar follicular cells.[213]

The prevalence of goiter during pregnancy varies with the geographic area studied. In a study done in Scotland, 70% of pregnant women were diagnosed as having goiter in contrast with 38% of nonpregnant women.[40] Goiter was considered to be present if the glands were both palpable and visible. Previous pregnancies did not appear to affect this incidence, since goiters were found in 39% of nulliparous women and 35% of nonpregnant parous women. The investigators repeated the study in Iceland under the same experimental conditions but noted no increase in goiter during pregnancy.[41] Goiter was found in 19%

of nonpregnant and 23% of pregnant Icelandic women. A multiple observer design, blind study done in the United States on 49 pairs of pregnant and nonpregnant women also failed to show any increase in goiter during pregnancy.[131]

Increased Iodine Excretion During Pregnancy. The differences between these studies have been attributed to differences in the dietary iodine content, which is low in Scotland but high in Iceland and the United States. An early and sustained rise in the renal clearance of iodine has been considered to be the major factor in the decreased plasma inorganic iodine concentration in pregnancy.[1] The increased glomerular filtration rate during pregnancy results in an increased renal loss of iodine, beginning early in pregnancy. The thyroid gland compensates by enlarging and increasing the plasma clearance of iodine to produce sufficient thyroid hormone to maintain the euthyroid state.

Whether goiter ensues depends on the ability of the thyroid gland to compensate, which in turn depends on the concentration of the plasma inorganic iodide. Iodine deficiency goiter is unlikely to occur at a plasma iodine concentration above 0.08 μgm/100 ml.[2] In most Europeans, the plasma inorganic iodine concentration ranges from 0.10 to 0.15 μgm/100 ml and during pregnancy may fall below 0.08 μgm/100 ml.[125] In residents of North America and Iceland, the plasma inorganic iodine is about 0.30 μgm/100 ml. Even if this value is halved during pregnancy, it remains above 0.08 μgm/100 ml. An iodine balance study done in the United States revealed no difference between pregnant and nonpregnant women.[54] The pregnant women in North America also have increased renal clearance of iodine, but ample dietary intake prevents excess iodine loss. Iodized salt should be sufficient to supply an adequate intake of 250 μgm of iodine needed during pregnancy, and the iodine in most prenatal vitamin supplements ensures an adequate intake. In areas of marginal iodine intake, e.g., those of 50 μgm per day, supplementary iodine (160 μgm per day) given to pregnant women reduced neonatal goiter from 33 to 7%.[233] An excessive iodine intake because of unusual dietary practices, e.g., greater than 2000 μgm daily, may cause difficulties for both mother and child and is subsequently discussed.

RADIOIODINE THYROID UPTAKE

The decreased plasma inorganic iodine concentration during pregnancy results in a smaller iodine pool and an increased thyroid clearance of iodine. Since the thyroid radioiodine uptake depends on the size of the iodine pool in addition to thyroid-stimulating activity, the thyroid radioiodine uptake is elevated in pregnancy.

Specific problems occur with the use of radioisotopes in the pregnant woman, because possible radiation effects on the fetus must be considered. Whether radiation effects depend on a threshold dose is not clear, and all radiation to the fetus should be regarded as harmful.[210]

However, when pregnant women have been studied, the radioactive iodine thyroid uptake has been increased. Three of five women had elevated radioactive iodine thyroid uptake at 12 weeks of pregnancy.[93] Urinary excretion of administered radioactive iodine is an indirect measure of thyroid uptake. In 22 women in the third trimester, the mean urinary excretion of radioiodine was in the range between normal and thyrotoxic values.[169] However, with this method, maternal thyroid uptake cannot be distinguished from fetal uptake. In one study, the short-lived isotope ^{132}I was administered to 25 pregnant women and the 2-hour thyroid uptake measured at 12, 24, and 36 weeks of gestation as well as 1 and 6 weeks post partum.[93] The thyroid uptakes during pregnancy and at least 1 week post partum were significantly elevated compared with both nonpregnant values and those at 6 weeks post partum.

Two pregnant patients also had a triiodothyronine suppression test with this isotope, and thyroid uptake was suppressed to the same extent as in four nonpregnant women. The same worker also compared the effect of a single injection of TSH in three pregnant and three nonpregnant women with a similar response found in both groups. The uptake doubled 22 hours after the administration of TSH and returned to normal on the third day.

BASAL METABOLIC RATE

Thyroid function was originally monitored by the basal metabolic rate (BMR), and studies indicated that the BMR was elevated in pregnant women.[161] The BMR began to increase during the fourth month of pregnancy and

continued to rise slowly until the eighth month. There was a 15 to 20% increase, the largest occurring in patients who had the lowest BMR when they were not pregnant. Under scrupulously basal conditions, it was demonstrated that the uterus and its contents could account for 70 to 80% of the rise in oxygen consumption values above nonpregnant values. Increased maternal work accounted for the rest.[27]

Although clinical tests continue to be introduced for the appraisal of thyroid function, a true BMR is still a good indicator of overall thyroid function. However, even in experienced hands the BMR correlates with the final clinical appraisal in only about half of the patients. The major reason is the difficulty in separating basal from total metabolism, which includes increases in oxygen consumption from digestive and muscular activity. Only the true basal metabolism is a measure of thyroid activity. Most errors in the interpretation of the BMR are due to a failure to recognize this distinction.

THYROXINE-BINDING GLOBULIN

The other major change in thyroid function during pregnancy is a rise in TBG concentrations to about twice normal values. The increased estrogens in pregnancy induce TBG and cause a fall in thyroxine-binding prealbumin capacity.[80,180] About 85% of thyroid hormone is transported in serum-bound TBG, and 15% by thyroxine-binding prealbumin (TBPA). The maximum binding capacity of these proteins can be determined by the addition of saturating concentrations of thyroxine. TBG has a normal binding capacity that ranges from 19 to 30 μgm/100 ml of thyroxine and increases to 40 to 60 μgm/100 ml of thyroxine during pregnancy. TBG concentration can also be measured directly by radioimmunoassay and has a normal range of 12 to 30 mg/L which increases to 30 to 50 mg/L during pregnancy.[21] The maximum binding capacity for TBPA has been determined to be 219 and 393 μgm thyroxine/100 ml.[172] Although TBPA has a greater binding capacity for thyroxine, TBG has a greater affinity and actually binds more thyroxine *in vivo*. Thyroxine binds more tightly to TBG than does triiodothyronine. This difference in affinity of TBG for thyroxine and triiodothyronine is the basis for the resin triiodothyronine uptake. The role of the increased TBG in pregnancy has also been studied by examining pregnant patients with partial or total TBG deficiency.[167, 180] Although there was no increase or only a minimal increase in TBG during pregnancy, no significant changes in thyroid function occurred. Serum thyroxine does not increase unless there is an increase in TBG. Conversely, there must be adequate amounts of thyroid hormone produced to maintain normal thyroid function in the presence of increased binding. In one study, the administration of estrogen to euthyroid patients resulted in an increase in the serum protein-bound iodine concentration; however, no increase was noted in hypothyroid patients who received inadequate thyroid hormone replacement even though estrogens resulted in an increase in thyroxine-binding capacity.[59]

Although hazardous, the temptation to speculate on the reason for estrogen induction of TBG is overwhelming. A number of hepatic proteins are induced during pregnancy, including cortisol-binding globulin; ceruloplasmin; and the blood clotting factors I, VII, and IX. Perhaps the increased estrogens during pregnancy switch on a genome that results in the rise in certain clotting factors and incidentally in a rise in TBG. The hypothesis has been suggested that maternal thyroxine plays a vital role in early fetal neurogenesis, and the greater TBG ensures an adequate supply of thyroxine throughout fetal life.[56]

THYROXINE PRODUCTION DURING PREGNANCY

After free thyroid hormones enter the cell, they exert their effect, perhaps by binding to nuclear receptors and initiating new protein synthesis. Thyroid hormone is subsequently degraded, and this degradation can be measured with radioisotope-labelled thyroxine. In the steady state, thyroxine degradation is a measure of thyroid hormone production. Serum thyroxine has an approximate volume of distribution of 10 L. With a normal serum thyroxine of 8 μgm/100 ml, the entire thyroidal pool of thyroxine is approximately 800 μgm T_4. Thyroxine disappears from the serum of a euthyroid nonpregnant adult with a half-life of 6 to 8 days, which results in a fractional turnover of about 10% per day. Therefore, about 10% of the extrathyroidal pool of T_4, or about 80 μgm, turns over per day. In a steady state, 80 μgm of T_4 is produced daily.

One study showed a decrease in the fractional rate of thyroxine turnover when TBG capacity was increased by estrogen administra-

tion.[48] These investigators suggested that thyroxine binding by TBG exerted a rate-limiting effect upon the peripheral metabolism of thyroid hormone. However, the absolute rate of thyroid hormone disposal was unchanged, because the total serum thyroxine concentration increased. In another study, net thyroxine turnover and presumably thyroid hormone requirements were unchanged in normal human pregnancy.[42] Net thyroxine turnover was 90 μgm per day in the nonpregnant women and 97 μgm per day in pregnant women. The two values were identical when expressed as the daily turnover per square meter of body surface. These findings are for one period in pregnancy, but because of necessary restrictions on the use of radioisotopes in pregnant women, further data will not be available. Increased thyroxine turnover has been reported during pregnancy in monkeys.[215]

Most of the studies of thyroid hormone turnover in pregnancy have concerned thyroxine, because until recently triiodothyronine was considered to be relatively unimportant in thyroid hormone economy. However, triiodothyronine is now known to be the major thyroid hormone. The weaker binding affinity of TBG for triiodothyronine leads to a greater volume of distribution and a more rapid turnover. Triiodothyronine has been estimated to have an extrathyroidal distribution space of approximately 40 L with a fractional turnover rate of 70% per day. The T_3 extrathyroidal pool approximates 45 μgm. Therefore, T_3 turnover would approximate 33 μgm per day. Since T_3 is three or four times as potent as T_4 on a weight basis, the metabolic effects of triiodothyronine would at least equal those of thyroxine.

LABORATORY TESTS OF THYROID FUNCTION

The increase in TBG results in changes in the laboratory parameters of thyroid function. These changes are important to understand because diagnostic and therapeutic decisions are based on the interpretation of these laboratory tests. Before the radioimmunoassay for TBG was developed, determination of thyroxine-binding capacity was technically difficult. Therefore, an indirect measure of thyroxine-binding capacity was, and still is, obtained with the resin T_3 uptake (RT_3U) test.[224] With the increase in TBG during pregnancy, there is a marked increase in the number of thyroxine-binding sites. Even though more thyroid hormone is bound, the number of unsaturated binding sites still exceeds normal nonpregnant levels. As a consequence, the RT_3U tends to be in the hypothyroid range during pregnancy. The exact values depend on the particular test used. The combination of an elevated serum thyroxine value and an RT_3U in the hypothyroid range is characteristic of pregnancy. However, any condition that increases thyroid-binding protein capacity may result in similar changes.

Serum T_4 and T_3. Serum thyroxine and triiodothyronine concentrations are now determined directly by radioimmunoassay. These determinations measure the total thyroxine or triiodothyronine content in the serum and therefore are elevated during pregnancy because of the increased thyroid hormone binding. The serum T_4 and T_3 concentrations are significantly elevated early in pregnancy and remain elevated throughout gestation (Fig. 16–1).[95, 173] The values return to normal shortly after delivery.[240] Although triiodothyronine may play a dominant role in thyroid hormone economy, changes in the serum thyroxine concentration are for the most part mimicked by changes in serum triiodothyronine concentration. Therefore, a serum thyroxine determination usually will suffice as an indicator of thyroid function during pregnancy. Since the total thyroxine concentration is being determined, this should be accompanied by some measure of thyroxine-binding capacity, such as RT_3U.

Free Thyroid Hormone. Although the bound thyroxine and triiodothyronine are increased, the free or unbound thyroid hormones have been found within the normal range. Although there is disagreement,[110] two studies have reported elevated values for both free T_4 and

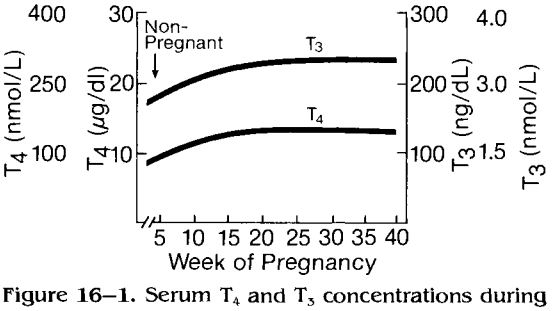

Figure 16–1. Serum T_4 and T_3 concentrations during normal pregnancy.

free T_3.[95, 240] In the larger study the elevated levels were not above the normal range of the nonpregnant female controls, and this finding was borne out by a third study.[173] One group of investigators have reported a significant increase in the free T_4 concentration during the first trimester of pregnancy with a return to control levels by the third trimester.[90] During the first 5 weeks of pregnancy, the mean serum T_4 concentration was 50% higher than in nonpregnant women. These findings would be consistent with a thyrotropic effect of elevated concentrations of human chorionic gonadotropin (hCG) present during the first trimester. Urinary thyroid hormone levels correlate closely with circulating free thyroid hormone concentrations.[31] The elevation in free thyroid hormone concentration could result from changes in peripheral metabolism or increased secretion of thyroid hormone. As mentioned previously, there are no data clearly relating the slightly elevated free thyroid hormone concentrations with altered metabolic effects of thyroid hormones.

The absolute concentration of free T_4 and T_3 is determined with tracer amounts of ^{125}I-T_4 and equilibrium dialysis of the patient's serum. A percentage of dialyzable fraction is found and, when multiplied by the serum thyroxine determination, yields the free thyroxine concentration. The free thyroxine concentration is the only direct method of estimating thyroid function that compensates for changes in the TBG capacity, but the procedure is technically difficult. Another method of determining the free thyroxine concentration, utilizing the kinetics of binding to ligands such as glass beads, has been developed.[236] The determination is relatively easy to perform and will probably supplant the more cumbersome and technically difficult equilibrium dialysis method. However, in some of the procedures the values are not accurate in the presence of large amounts of thyroid-binding protein, similar to those present during pregnancy.[236]

To compensate for the effect of increased thyroxine binding on the serum thyroxine, a derived value, the free thyroxine index has been obtained. Derived from the determination of the serum thyroxine and the RT_3U, the free thyroxine index gives an indirect approximation of the absolute free thyroid hormone concentration. However, similar to some of the nonequilibrium dialysis methods, the test may not provide a true index of free T_4 concentration.[21, 206] This may be due to the failure of the RT_3U to determine TBG capacity accurately. Whatever the cause, the free thyroxine index is not directly proportional to the free thyroxine concentration, and the two tests should not be used interchangeably in the pregnant woman.

HYPOTHALAMIC-PITUITARY-THYROID AXIS

Thyroid hormone secretion is dependent on thyroid-stimulating hormone (TSH), and TSH secretion in turn is dependent on the circulating thyroid hormone concentration. Thyroid function is, therefore, an example of the classic negative feedback mechanism. The level of thyroid hormone—particularly pituitary triiodothyronine, which shuts off TSH—is determined by thyrotropin-releasing hormone, a tripeptide, L-pyroglutamyl-L-histidyl-L-prolinamide. This hypothalamic releasing hormone determines the set point at which circulating thyroid hormone suppresses TSH secretion of the pituitary.

There are conflicting reports about the responsiveness of the hypothalamic-pituitary-thyroid axis during pregnancy. Two pregnant women given 80 μgm of T_3 for 1 week had depression of the thyroidal uptake[162] similar to that in nonpregnant controls.[177] The increase in ^{131}I uptake with TSH was also similar in pregnant and in nonpregnant women. Pregnant women in the second and third trimester were suppressed with 75 to 125 μgm T_3 daily for 7 days. Thyroid uptakes were suppressed to the same extent as in nonpregnant women.[232] Serum protein-bound iodine values fell more than 1.0 μgm/100 ml at all time periods studied, but more triiodothyronine was needed to lower the serum protein-bound iodide (PBI) concentration in the later months of pregnancy. Some patients did not respond regardless of the dose of triiodothyronine. One group found that only one of five pregnant women who received 150 to 200 μgm T_3 daily had serum PBI determinations consistent with complete suppression.[182] The ^{131}I uptake appears to be normally responsive to thyroid hormone suppression during pregnancy, but the serum PBI concentration is not. Part of this apparent lack of responsiveness may be due to the increase in TBG during pregnancy.

We administered thyrotropin-releasing hormone (TRH) to patients in different stages of pregnancy who were to undergo therapeutic abortions.[26] Women between 16 and 20 weeks

of pregnancy had increased TSH responses to TRH compared with women between 6 and 12 weeks of pregnancy. This increase appeared to be due to estrogens, since nonpregnant women on oral contraceptive steroids also had a greater response to TRH. Other workers, however, have not found an increased TSH response to TRH in pregnant women.[115, 221, 241] The failure to find an increase in the TSH response to TRH might be due to iodine deficiency,[125] although the mechanism is not clear. TRH crosses the placenta and stimulates the fetal pituitary in animal studies.[43, 122] TRH activity was found in the human placenta, and lower levels of thyrotropin-releasing hormone degrading activity were found in both cord and maternal sera,[201] compared with sera from euthyroid nonpregnant adults.[165] These data all suggest that TRH may play a role in the modulation of thyroid function during pregnancy. However, fetal pituitary TSH secretion appears to be controlled independently of both the maternal and the fetal hypothalamus.

Thyroid-Stimulating Activity. TSH concentrations during pregnancy have been reported to be within normal limits or slightly higher during the early trimesters.[140, 184] These conflicting results are attributable in part to lack of sensitivity of particular assays. Further work suggests that serum TSH concentrations are not increased during pregnancy and in fact are decreased during the early weeks of gestation.[95, 240]

Three thyroid stimulators have been reported in normal pregnancy as follows: (1) normal pituitary TSH (hTSH), (2) chorionic TSH (hCT), and (3) chorionic gonadotropin (hCG). In patients with hydatidiform mole and choriocarcinoma, markedly elevated levels of hCG may result in hypothyroidism.[102, 115]

Material with thyrotropic activity has been extracted from some human placentas (hCT).[100] Bioassayable thyrotropin in the sera of pregnant women, as well as hCT activity in the placenta, has been found.[98] With improved assays, the levels of hCT have been found to be quite low. Only one third of the samples in one report contained more than 0.25 μU/ml of activity.[95] With improved techniques it has also been impossible to recover significant hCT activity from the placenta.[94] The hCT does not appear to play a significant role in the modulation of thyroid function during pregnancy.

In comparison, hCG has an activity in the TSH bioassay of 0.2 μU TSH/U of hCG.[95] Based on this activity, hCG concentrations in early pregnancy would be equivalent to 3 to 10 μU TSH/ml. The concentrations of TSH activity are high enough to stimulate thyroid function mildly. The slight decrease in serum TSH concentrations might be explained by the increase in hCG.[95, 240] However, recent studies have suggested that pure hCG may not have intrinsic thyrotropic activity.[7]

FETAL THYROID FUNCTION

The fetal thyroid gland must reach a certain stage of development before thyroid hormone is produced. Any thyroid hormone necessary for fetal development before that stage is reached must be supplied by the maternal thyroid gland.

Ontogenesis of Thyroid Function

No organic iodine is present in the fetal thyroid gland before 10 weeks of gestation.[202] By 11 to 12 weeks of gestation, the fetal thyroid attains maturity with the ability to produce iodotyrosines and iodothyronines. When radioactive iodine was administered to women immediately prior to termination of pregnancy, the fetus was found to concentrate iodine at about 12 to 14 weeks.[33, 61, 90] Serum TSH is detectable in fetal serum as early as 10 weeks[86] but it remains relatively low until 20 weeks of gestation, when it increases over the next 10 weeks up to 15 μU/ml and then falls until it is about 7 μU/ml.[171] Fetal serum T_4 concentrations progressively increase in response to the TSH rise after midgestation, increasing from 2 to 3 μg/100 ml at 10 weeks to 5 to 10 μg/100 ml at 30 weeks. A similar progressive increment in serum-free thyroxine also occurs.[68] These data suggest that fetal pituitary control of thyroid function must exist as early as 12 weeks of gestation and 1 month of postnatal life. The ability of anencephalic fetuses to synthesize iodotyrosines has been taken as evidence that TSH is not necessary. However, careful studies suggest that in these fetuses pituitary tissue is usually present but the hypothalamus is absent; in fact, it was demonstrated that anencephalic fetuses had a hyperresponse of TSH to TRH.[98]

Requirements for Fetal Thyroid Hormone. Whether the fetus requires its own thyroid hormone or whether the hormone can be supplied by the mother is unresolved. Fetal access

to maternal thyroid hormone is suggested by athyreotic infants who are normal or only mildly retarded at birth. However, maternal thyroid hormone probably cannot completely replace fetal thyroid production, and most athyreotic infants do show a lack of thyroid hormone. The role of maternal thyroid hormone in early fetal development remains unknown, but it does not appear to be necessary during the later part of pregnancy.[208]

Amniotic Fluid

The increased frequency of amniocentesis in pregnant women has led to an interest in thyroid hormone concentrations in amniotic fluid. TSH has been difficult to detect in amniotic fluid.[36] During the first half of pregnancy, amniotic fluid thyroxine concentrations increase progressively, reaching peak concentrations at 25 to 30 weeks (Fig. 16–2).[9, 121]

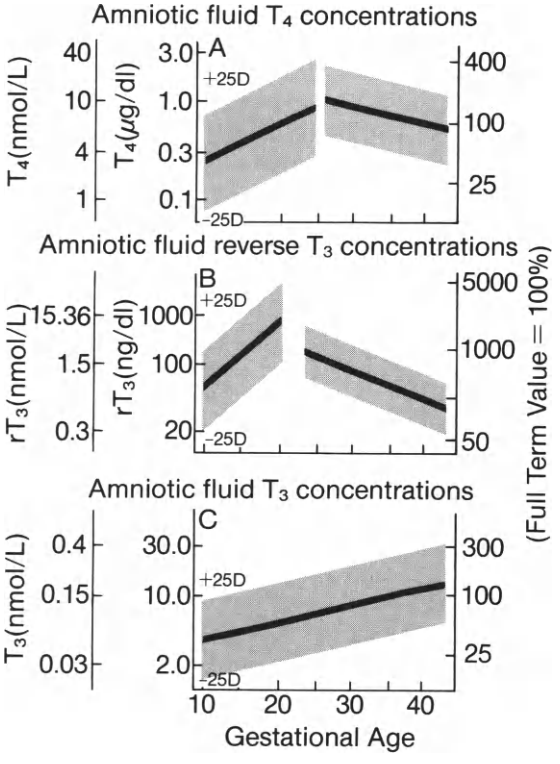

Figure 16–2. Amniotic fluid T_4, T_3, and reverse T_3 concentrations during pregnancy. *A*, Amniotic fluid T_4 concentrations. The vertical axis on the left is a logarithmic scale of standard concentrations and the one on the right is the percentage of the term mean value ± 2 SD calculated from the regression lines, which are also plotted. *B*, Amniotic fluid reverse T_3 concentrations plotted in the same manner. (Reproduced from Klein, A. H., Murphy, B. E. P., Artal, R., et al.: Am. J. Obstet. Gynecol. 136:626, 1980, with permission.)

Amniotic fluid triiodothyronine concentrations are low during early pregnancy and rise slowly. During the last half of pregnancy, amniotic fluid thyroxine concentrations decrease while amniotic fluid thyroxine continues to increase. Most of the triiodothyronines (3,5,3′-triiodothyronine) are formed from monodeiodination of the outer ring. Deiodination of the inner ring produces 3,3′,5′-triiodothyronine or reverse T_3 (rT_3), which has minimal biologic activity. In the fetus, thyroxine is predominantly metabolized to rT_3, perhaps because of immaturity of the enzyme systems.[36] Reverse T_3 concentrations are markedly increased in the amniotic fluid, reaching peak levels at 17 to 20 weeks. This pattern of change in amniotic fluid thyroid hormones is compatible with an elevation in 5′-iodothyronine monodeiodinase activity in the fetal compartment. The placenta appears to be an important site for fetal thyroid hormone metabolism. In addition to the deiodination of T_4, T_3 is also deiodinated in the placenta to form mono- and di-iodothyronine.[30]

Whether amniotic fluid thyroid hormone concentrations reflect the fetal compartment is of particular interest. The suggestion has been made that amniotic fluid iodothyronine concentrations can be used for the prenatal diagnosis of fetal thyroid abnormalities.[36, 105] A normal amniotic fluid rT_3 concentration was found in a fetus whose mother had inadvertently received a therapeutic dose of ^{131}I at 10 to 11 weeks of gestation. The fetus was treated with thyroxine injected into the amniotic fluid and was euthyroid at birth.[132] Furthermore, the amniotic fluid rT_3 concentration rose after the T_4 injection. However, amniotic fluid concentrations have been found to be high in a hypothyroid infant and low in a normal infant.[129] TRH also has been found in amniotic fluid.[156]

NEONATAL THYROID FUNCTION

Serum T_3 values in cord serum are low, and T_3 concentrations are elevated (Table 16–1). Immediately following birth, there is a sharp rise in the serum TSH concentration caused by increased pituitary secretion of TSH.[71] TRH degrading activity is absent in the newborn but appears after 3 days.[8] This acute surge in TSH may be mediated by TRH secretion, which has been reported to be elevated in newborns.[135] Neonatal serum TSH concentration increases minutes after birth from a mean of 7.5 μU/ml

Table 16–1. Thyroid Function Tests in Material and Cord Blood at Term

Test	Maternal	Cord
Serum thyroxine μgm/100 ml	10–16	6–13
Free thyroxine ngm/100 ml	2.5–3.5	1.5–3.0
Serum triiodothyronine ngm/100 ml	150–250	40–60
Reverse triiodothyronine ngm/100 ml	35–65	80–360
Resin T_3 uptake	22	25–35
Thyroxine-binding globulin mgm/L	30–50	12–30
Serum TSH μU/ml	0–6	0–20

to a peak of 30 μU/ml within 3 hours (Fig. 16–3).[216] In response to TSH stimulation, there is a sharp rise in total and free serum thyroxine concentrations. Serum triiodothyronine also increases dramatically, but this rise is at least in part TSH independent.[194] Neonatal tissues rapidly acquire a greater capacity to monodeiodinate T_4 to T_3, which contributes importantly to the early rise in serum T_3 concentrations and the fall in serum T_3 concentrations. The capacity of neonatal tissues to monodeiodinate T_4 to T_3 is reflected in the progressively changing serum T_3/T_4, rT_3/T_4 ratios between 30 weeks of gestation and the first postnatal month.[67]

Neonatal radioactive iodine thyroid uptake is elevated as early as 10 hours post partum. Thyroid uptake reaches a peak by the second day and drops to adult normal limits by the 5th day post partum.[70] The plasma inorganic iodine and iodine pool are increased, as is the absolute amount of iodide taken up by the thyroid gland.[178] The factors responsible for this stimulation of iodide transport are unknown but probably involve more than stimulation of the hypothalamic-pituitary-thyroid axis.

PLACENTAL TRANSFER

Any effect of maternal thyroid hormone on the fetus must depend on placental transfer. In fact, there are a number of agents that may affect fetal thyroid function depending upon whether or not they are transferred across the placental barrier (Table 16–2).

Transfer of Thyroid Hormone

Pregnant women at term were administered ^{131}I-T_3 or T_4, and the ratio of radioactivity in maternal and fetal sera was determined at the time of delivery.[89] Both hormones were transferred slowly, but ^{131}I-T_3 appeared to be transferred at a somewhat faster rate. Similar observations were made of women who had received the isotopes between the 11th and 26th weeks of pregnancy prior to therapeutic abortions. In another study, 13 pregnant women were given infusions of 500 to 8000 μgm of L-thyroxine at term, and the serum PBI and RT_3U were determined in maternal and cord blood.[69] Neonatal serum butanol-extractable iodine (BEI) values increased progressively with greater amounts of maternal hormone and diffusion time, but the values did not approach those of maternal sera. The placenta appeared to be relatively impermeable to thyroxine, at least at term, and persisted despite progressive saturation of maternal TBG and a presumed increase in maternal free thyroxine concentrations.

Triiodothyronine was administered to preg-

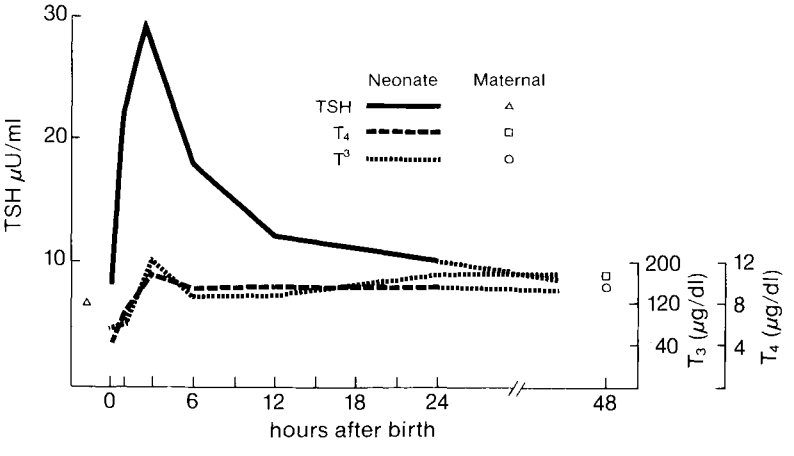

Figure 16–3. Serum (TSH), Thyroid-stimulating hormone T_4, and T_3 concentrations during the first 48 hours in the neonate. (Modified from Stubbe, P., Gatz, J., et al.: Horm. Metab. Res. 18:58, 1978, with permission.)

Table 16–2. Placental Transfer and Fetal Thyroid Function

Placental transfer without difficulty
Iodides
Thioamides
Thyroid-stimulating immunoglobulins
Thyrotropin-releasing hormones
No transfer
Thyrotropin
Minimal transfer
Triiodothyronine
Thyroxine

nant women for 4 to 6 weeks before delivery and serum thyroxine was determined in maternal and cord sera.[182] Transfer of triiodothyronine across the placenta would be expected to decrease fetal TSH level with a resulting decline in serum T_4 values. In five of eight infants born to mothers who had received 300 μgm T_3 daily, which is approximately three times the normal dose, serum T_4 values were low. In the other three children, however, values were comparable with control values. Little decrease in cord blood T_4 was noted in infants whose mothers received 150 to 200 μgm T_3 daily. The need to administer large doses of triiodothyronine in order to suppress cord blood T_4 again appeared to reflect placental impermeability. In a similar study, the serum T_3 concentration was determined directly in maternal and cord blood.[53] Cord serum T_3 concentrations were elevated in those infants who had decreases in serum T_4, but there was further evidence of minimal placental transfer.

Available evidence suggests that triiodothyronine and thyroxine cross the placenta but do so with difficulty. Placental transfer of thyroid hormone may also change with duration of pregnancy and aging of the placenta. Triiodothyronine appears to cross more easily than thyroxine; however, fetal serum triiodothyronine concentrations are normally low. The available evidence also suggests that thyroid hormone transfer across the placenta is such that administration of physiologic amounts of thyroid hormone to the mother is not helpful in elevating the fetal serum thyroid hormone concentration.

Animal studies have indicated that it may be possible to modify the structure of the thyroid hormone molecule to increase placental transfer. A nonhalogenated thyroid hormone analogue, dimethyl-isopropyl thyronine (DIMIT), was found by us to be 20 times as effective as thyroxine in preventing fetal rat goiter without inducing maternal thyrotoxicosis.[37] Placental transfer depends on molecular weight, protein binding, and lipid solubility,[9] and DIMIT is smaller, binds less tightly, and is more lipid soluble than thyroxine. Whether DIMIT has any role in the treatment of hypothyroidism *in utero* is not clear. The data raise the possibility that the thyroid hormone molecule can be altered to improve transfer across the placenta.

MATERNAL HYPOTHYROIDISM

Hypothyroidism is relatively uncommon in the pregnant patient.[55] This low incidence of hypothyroidism during pregnancy, coupled with the widely held belief that fertility and thyroid function are closely related, is probably responsible for statements that hypothyroid women are infertile.[179]

"The relationship of the thyroid gland to the sex organs is the most ancient and classical relation of the function of the glands of internal secretion. Known to the ancients and a subject of daily gossip, it was passed down through the ages."[134]

In one study, 7 of 10 myxedematous women were anovulatory.[83] Because of this reported association with infertility as well as the absence of definitive therapy, thyroid hormone has been administered to euthyroid infertile women. Desiccated thyroid hormone or placebo was given to 339 euthyroid women with either infertility or menstrual disorders; 75 infertile women eventually received thyroid hormone and 50 received placebo. The investigators concluded that thyroid hormone appeared to have no definite benefit.[27] We have treated 20 women who had no identifiable causes of infertility with a combination of thyroxine and triiodothyronine or placebo in a randomized double-blind study. Two of six women who received hormone became pregnant, while 6 of the 14 women who had received placebo also became pregnant. Controlled studies offer no support for the use of thyroid hormone for infertility in euthyroid women.

Pregnancy Outcome in Hypothyroidism

Animal data suggest that mild or moderate hypothyroidism has a minimal effect on fertility, although hypothyroid animals do have difficulty maintaining pregnancy.[113] This was confirmed in a study of 244 pregnant hypothy-

roid women in whom the rate of stillbirths was double that in controls.[168] Other workers studied a group of children whose mothers had proven or suspected thyroid disorders during pregnancy.[88] The outcome of six of seven pregnancies in women with clinically suspected hypothyroidism and low BEI values was poor. In addition to a spontaneous abortion and a stillbirth, one child was born with congenital defects and three children had undifferentiated developmental retardation at 8 months of age. Only two of the mothers had received even inadequate thyroid hormone replacement during pregnancy. Although there was no firm evidence that these women were actually hypothyroid, an attenuated rise in the serum BEI concentration was associated with a poor pregnancy outcome.

One group monitored thyroid function in women during pregnancy and subsequently obtained developmental data on the children born to these mothers.[142, 144] About 4% of pregnancies were classified as "hypothyroxinemia" based on two low thyroid hormone values relative to normal pregnancy values or one low value relative to clinical hypothyroidism, previous reproductive failure, or thyroidectomy. Of the 135 pregnancies in which the diagnosis of hypothyroxinemia was made, 81% of infants examined 8 months post partum whose mothers had received adequate thyroid hormone replacement were classified as normal. In comparison, only 46% of infants whose mothers had received inadequate replacement were classified as normal. In a 7-year follow-up study, the progeny of inadequately treated "hypothyroxinemic" women had lower psychologic scores.[143] There was no compelling clinical evidence that these patients were actually hypothyroid, and perhaps their socioeconomic situation might have played a role in the poor outcome. The premise that there may be significant numbers of women with subclinical hypothyroidism that only becomes apparent during pregnancy should be viewed with caution.

Thyroid Function and Spontaneous Abortion. Since hypothyroid women experience increased fetal loss, serum thyroid hormone determinations were done in women who aborted; these values were found to be low.[141] Low values were not found, however, in patients who had induced abortions.[73] Follow-up studies indicated that patients with low thyroid hormone values who aborted were euthyroid and presumably had not been hypothyroid during pregnancy.[136] The data suggested that the low serum thyroid values were secondary to the abortion rather than the cause. Presumably, fetal death resulted in decreased estrogen production with a concomitant decrease in TBG and serum thyroid hormone concentrations.

The balance of evidence suggests that the great majority of women with early spontaneous abortions have normal thyroid function. Since the low serum thyroxine level merely reflects the decreased estrogen production and TBG, there is no reason to suppose that thyroid hormone would be helpful in these situations.[166] However, hypothyroidism does occur during pregnancy, and decreased thyroid function should be considered in pregnant women with low serum thyroxine concentrations. In one study of 31 women who had previously had at least one spontaneous abortion, half had some evidence of diminished thyroid reserve as determined by TSH responsiveness.[164]

Pregnancy in Hypothyroid Women. Myxedematous patients have been reported to carry their pregnancies to term successfully (Fig. 16–4).[104, 116, 127, 155] Any thyroid hormone necessary for fetal growth before the second trimester must come from the maternal side. In severely hypothyroid mothers this hormone would be lacking. Although the offspring have not been subjected to extensive developmental testing, they have been reported as normal.[179] Whether maternal thyroid hormone is absolutely necessary for fetal development is not clear; certainly, it is desirable. The suggestion has been made that fetal-maternal transfer of thyroxine may occur under these circumstances. In one patient the BMR increased from −40% to −4% with a subsequent decrease again to −28% after delivery of a euthyroid baby.[20] However, such changes are probably due to the pregnancy.

Diagnosis of Hypothyroidism

Hypothyroidism is most commonly iatrogenic following either surgery or radioactive iodine therapy. Idiopathic hypothyroidism is a more insidious cause of decreased thyroid function and is related to Hashimoto's disease. An interesting association has been noted in that mothers of children with Down's syndrome have sometimes been found to have high titers of thyroid antibodies.[64] The reason suggested

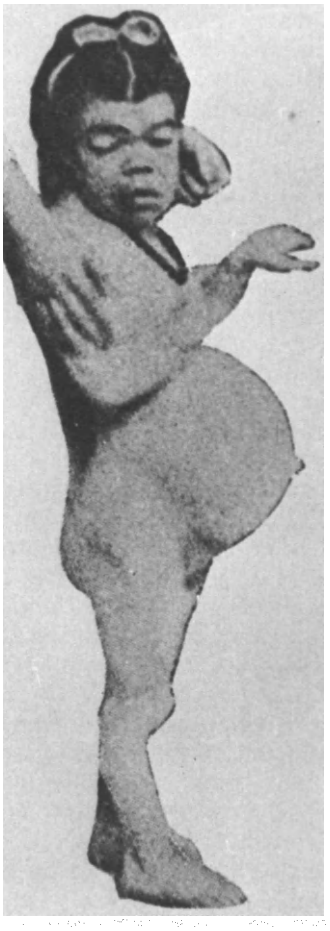

Figure 16–4. Pregnant cretin. (Reproduced from Osler, W.: Sporadic cretinism in America. Transactions of the Congress of American Physicians and Surgeons, 1897, with permission.)

is that maternal thyroid autoimmunity predisposes to aneuploidy in children and plays a major role in the birth of children with Down's syndrome in younger mothers. Hashimoto's disease is more common in patients with diabetes mellitus; in one study of 100 diabetic women, 20% of class D and F diabetics also had Hashimoto's disease.[205]

Regardless of the cause, hypothyroid patients may complain of cold intolerance, constipation, cool dry skin, coarse hair, inability to concentrate, and irritability. However, euthyroid pregnant women may also complain of the same symptoms, which makes the clinical diagnosis difficult. Parasthesia is an early symptom in about 75% of patients with hypothyroidism and may be helpful in the diagnosis. A pregnant woman is unlikely to present with gross myxedema, and the obvious signs, including a low body temperature, periorbital edema, large tongue, and hoarse voice, are the exception rather than the rule. The patient may feel more fatigued than she felt during previous pregnancies and may complain of coarse hair and dry skin. Delayed deep tendon reflexes would be very helpful in making the diagnosis. Postpartum amenorrhea and galactorrhea associated with hyperprolactinemia may be indicative of hypothyroidism.[117, 219]

The most sensitive indicator of primary hypothyroidism is an elevated serum TSH concentration in association with a low serum thyroxine concentration. Because of the elevated TBG in pregnancy, the serum thyroxine determination may not be as low as would be expected. The RT_3U is not very helpful because the greater number of unsaturated binding sites are difficult to quantitate. Representative thyroid function test results are shown in Table 16–3. Thyroid antibodies may provide supporting evidence for hypothyroidism, particularly in the absence of a history of surgery or radioactive iodine therapy. Elevated serum cholesterol and carotene concentrations occur in hypothyroidism but are not helpful in the diagnosis. During normal pregnancy, serum cholesterol concentration may increase 60% above prepregnancy values.

Therapy of Hypothyroidism During Pregnancy

Once the diagnosis of hypothyroidism has been made in the pregnant woman, full replacement doses of L-thyroxine should be given immediately, regardless of the degree of thyroid function. Although L-triiodothyronine may cross

Table 16–3. Initial Thyroid Function Tests in Nine Pregnant Hypothyroid Women*

	Normal Range (Nonpregnant)	Mean Hypothyroid (Pregnant)†
Serum T_4 μgm/100 ml	4.5–13.2	2.2
Serum T_3 ng/100 ml	100–200	84
RT_3U	0.88–1.19	0.61
Thyroid-stimulating hormone μU/ml	0–5	40

*Reproduced from Montoro, M. N., Collea, J. A., Frasier, S. N., et al.: Successful outcome of pregnancy in women with hypothyroidism. Ann. Intern. Med. 94:31, 1981, with permission.

†Pregnant hypothyroid values represent mean of initial values from nine hypothyroid women presenting between 8 and 30 weeks of gestation. (From Montoro et al., Ann. Intern. Med., 94:31, 1981)

the placenta with greater facility than L-thyroxine, serum T_3 concentrations in the mother rise to thyrotoxic values, and normal fetal serum T_3 concentrations are very low. In the young pregnant woman without other complications, therapy can be begun rapidly even if she experiences some initial discomfort. One reasonable schedule is 0.15 mg of L-thyroxine daily for 3 weeks and then readjustment of the dose, depending on the thyroid function test results. Thyroxine need be given only once a day because of the long half-life. With adequate treatment, the serum TSH concentration should decrease to values below 6 μU/ml, and the serum T_4 concentration should increase to normal values for pregnancy. If the values do not return to normal, the dose of L-thyroxine should be increased by 0.05 mg increments. In the study illustrated in Table 16–3, the dose of L-thyroxine administered ranged from 0.15 mg to 0.30 mg daily.[155]

Pregnant Women Receiving Thyroid Hormone. The number of women in whom hypothyroidism is diagnosed during pregnancy is small. More commonly, pregnant women are receiving thyroid hormone when first seen, and the initial diagnosis of hypothyroidism may have been obscure. The thyroid hormone therapy can be stopped for 5 to 6 weeks and the patient reevaluated, or full doses of thyroid hormone can be given for the rest of the pregnancy. Since hypothyroidism, if present, would represent a risk to the continuation of the pregnancy, giving full doses seems wiser because the possibility of several weeks of decreased thyroid function is obviated. To be sure the pregnant woman is receiving adequate thyroid hormone, full replacement doses must be given. During the postpartum period, thyroid hormone can be discontinued and thyroid function evaluated 5 to 6 weeks later. Normal thyroid function may be suppressed for a number of weeks after prolonged thyroid hormone therapy. The recommended replacement dose of L-thyroxine is about 0.15 mg daily, based on the amount of thyroxine necessary to suppress the elevated serum TSH concentration.[212] In subjects on estrogen who are receiving inadequate thyroid hormone replacement, no increase in the serum thyroid hormone iodine concentration occurred despite an increase in thyroxine-binding capacity.[59] Women on thyroid hormone therapy require follow-up during pregnancy to maintain optimal thyroid hormone concentrations. In a study of 34 hypothyroid women followed through 37 pregnancies, no change in the thyroxine dose was required in 27 women.[176] Seven women required an increase in thyroxine and one a decrease. There was no difference in the amniotic fluid thyroxine concentrations between treated hypothyroid women and normal pregnant controls.

NEONATAL HYPOTHYROIDISM

Thyroid hormone deficiency during the fetal and neonatal period results in generalized developmental retardation.[101] Both the severity of thyroid hormone deficiency and the time of onset during development play an important role in the degree and potential reversibility of the ensuing brain damage. Hypothyroidism beginning after the age of 2 years appears to exert little if any irreversible effects on mental development. The earlier hypothyroidism occurs during fetal development, the more likely it is that severe retardation will occur.

With the availability of exogenous thyroid hormone therapy, the possibility of reversing mental retardation in cretinous children appeared reasonable. Osler, in 1897, stated, "Not the magic wand of Prospero or the brave kiss of the daughters of Hippocrates ever effected such a change as that which we are now enabled to make in these unfortunate victims doomed heretofore to live in hopeless imbecility, an unspeakable affliction to their parents and their relatives."[174]

Availability in these cases is not sufficient; for thyroid hormone therapy to be effective it must be started early in life. If hypothyroidism is diagnosed and treated before 3 months of age, four fifths of affected children will have IQs above 90. Unfortunately, the early clinical diagnosis of congenital hypothyroidism is difficult.[51] Since early diagnosis and treatment of congenital hypothyroidism is important, yet early clinical diagnosis so difficult, the solution is to screen all newborns for congenital hypothyroidism.[23]

Etiology and Incidence

Neonatal thyroid screening programs have provided a great deal of information on the etiology and incidence of congenital hypothyroidism. Congenital hypothyroidism occurs once in about 4000 births. The various causes of congenital hypothyroidism are outlined in Table 16–4. Primary hypothyroidism is most com-

Table 16–4. Etiology and Incidence of Congenital Hypothyroidism

Primary hypothyroidism incidence
Thyroid dysgenesis, 1 in 4000
Inborn errors of thyroid function, 1 in 30,000
Drug-induced, 1 in 10,000
Endemic hypothyroidism, 1 in 7
Secondary and tertiary hypothyroidism, 1 in 60,000

mon, about two thirds of children having ectopic thyroids, one third having thyroid agenesis, and a few having dyshormonogenesis.[47] The presence of thyroid growth blocking antibodies has been reported in a large proportion of mothers who give birth to hypothyroid infants.[50] In most instances, the growth blocking immunoglobulins were found to be present in the absence of thyroid microsomal antibodies.[222] There is also a suggestion that the gene for susceptibility to congenital hypothyroidism due to thyroid dysgenesis is closely limited to the HLA-A locus of the mother.[152] However, seasonal variation in the incidence of congenital hypothyroidism suggests the possibility that environmental factors may play a role.[151] In developing countries where goiter is endemic, the incidence of congenital hypothyroidism may be as common as 14% of births.[60]

Thyroid Dysgenesis. The cause of thyroid dysgenesis is unknown, but there seems to be a hereditary predisposition. Although the term athyreotic cretinism has been used, some thyroid tissue is usually present.[133] Although it has been postulated that thyrocytotoxic factors are transferred across the placenta, most workers believe that thyroid antibodies that do cross the placenta represent a reaction to thyroid injury rather than a primary event.[32] However, transplacental transfer of a thyrosuppressive factor has been observed in one family.[82, 217]

Inborn Errors of Thyroid Function. Approximately one child in 30,000 is born with an inborn error in thyroid function that can result in goitrous cretinism. Usually these children do not have significant thyroid enlargement at birth, but the goiter develops subsequently.[15] These defects in thyroid hormone biosynthesis are inherited as an autosomal recessive trait with a biochemical defect to correspond to each step in hormone biosynthesis. A family history of goitrous cretinism should alert the physician to this possibility in the neonate.

Drug-Induced Hypothyroidism. A number of compounds ingested by the mother may adversely affect fetal thyroid function. Data from the neonatal thyroid screening programs suggest that transient hypothyroidism may occur in 1 in 10,000 births.

An important cause of neonatal goiter and hypothyroidism is maternal iodide ingestion during pregnancy. Although this has been most commonly reported in women receiving large doses of iodides for chronic lung disease, goiters may occur with the maternal ingestion of as little as 12 mg of iodide daily.[14, 29] The large amount of iodides in the radiopaque dyes used for amniography may result in transient neonatal hypothyroidism.[11, 189]

There have been several women who throughout pregnancy have received the antiarrhythmic drug amiodarone, which contains 75 mg of iodine in each 200 mg capsule. The offspring have not had goiters, which suggests that iodide goiter may not occur in all instances of chronic ingestion during pregnancy.[186] Maternal ingestion of iodides appears to produce a relatively greater enlargement of the fetal thyroid, and the goiter may be large and obstructive.[75, 230] Fetal ultrasonography can be used to delineate a large goiter *in utero*.[124] The major problem encountered is maintenance of an adequate airway. With a huge goiter, surgery may be necesssary in the neonatal period. The hypothyroidism is usually transient, but mental retardation has occurred. Children who have been exposed to propylthiouracil (PTU) *in utero* may be born with small goiters and transient hypothyroidism. Based on screening data, perhaps one infant in 100 who is exposed to PTU *in utero* will develop transient hypothyroidism.

Diagnosis

During the first weeks of life when the diagnosis is crucial, clinical features of hypothyroidism are so uncommon that the diagnosis is rarely suspected. The clinical features of neonatal hypothyroidism are variable and include prolonged gestation with large size at birth, feeding and respiratory difficulties, constipation, abdominal distention with vomiting, and protracted icterus. Hypothermia, cyanosis, a large posterior fontanelle, umbilical hernia, and rough dry skin have commonly been found.[118, 130, 204]

Cretinism may be mistaken for Down's syndrome because both diseases are characterized by short stature and mental retardation. How-

ever, the child with Down's syndrome is more active and has specific stigmata. Cretinism may also be confused with the Beckwith-Wiedemann syndrome, which includes umbilical hernia and macroglossia.[65]

A low serum thyroxine level (<4 μgm/100 ml) in combination with a very high TSH (>80 μU/100 ml) is diagnostic of hypothyroidism. Infants with borderline T_4 (4 to 7 μg/100 ml) or borderline TSH (20 to 80 μU/100 ml) or both detected in a screening program will require further assessment. Radiographic bone age estimation may be helpful because the low osteoblastic activity is reflected in a slow rate of skeletal gravity and maturation. In the neonate the lack of ossification of the distal femoral epiphysis on the proximal tibial epiphysis suggests thyroid hormone deficiency *in utero*.[137] Epiphyseal dysgenesis is commonly seen in the proximal femoral epiphysis but may affect any center of endochondral ossification.[235] The ossification center appears late and at first is not a single center but multiple small centers scattered throughout the epiphysis. These centers eventually coalesce to form a single center with an irregular shape and a stippled appearance.

Therapy of Congenital Hypothyroidism

If thyroid hormone is to be effective therapy must be started as soon as possible after birth. Early treatment will minimize the degree of mental retardation.[52, 120] In the most complete study, a 3-year follow-up of 59 hypothyroid infants and 40 controls demonstrated no statistically significant differences in the various psychologic test scores between the two groups.[81] However, there appears to be a relationship between lower IQ values and lower serum T_4 concentrations or retarded bone age at the time of diagnosis. Preliminary evidence has accumulated that suggests affected children may have normal IQ values but may also have speech disorders and problems with fine motor coordination.[191]

When an infant with possible hypothyroidism is identified by the screening program, a complete evaluation including a thyroid scan should be done. Infants with residual thyroid tissue, no signs or symptoms of hypothyroidism, and a normal bone age and serum T_3 concentration have an excellent prognosis for normal development. Infants without visible thyroid tissue, with detectable signs of bone age retardation, and with low serum T_4 and T_3 concentrations have a more guarded prognosis for entirely normal development, even with early treatment.

The preferred therapeutic approach is the prompt institution of oral L-thyroxine at an initial dosage of 50 μgm/day. Readjustment of the thyroxine dose on the basis of clinical signs and symptoms and thyroid function test results should be done within the first 4 weeks of therapy.[191] Available information suggests that adequate replacement therapy should maintain the serum T_4 concentration between 8 and 12 μgm/dl, with a normal growth rate. Serum TSH concentrations are often elevated in children with congenital hypothyroidism who receive adequate thyroid hormone replacement apparently because of a permanent aberration in TSH control.[66] Too much thyroid hormone is equally undesirable for the developing brain. In doubtful cases, when transient hypothyroidism has not been excluded, cessation of therapy and reassessment of thyroid function after the age of 1 to 3 years may be desirable.

***In Utero* Therapy.** Because of the possibility that irreversible central nervous system damage may occur before birth in congenital hypothyroidism, there has been interest in intrauterine treatment of fetal hypothyroidism. As previously discussed, the intrauterine diagnosis of hypothyroidism is difficult.[121, 129] In one instance, intramuscular T_4 was administered to the fetus because the mother had radioablation of the thyroid in the 13th week of pregnancy.[223] The last dose of T_4 was given 2 weeks before delivery. At birth, cord blood TSH determination was 340 μU/ml. Transabdominal transuterine injections of 120 μgm of L-thyroxine were given into the fetal buttock at 2-week intervals. The dose of thyroxine was inadequate, but the calculated dose of 500 μgm could not be given intramuscularly.

The fetus effectively absorbs L-thyroxine from amniotic fluid, which would obviate the problem of fetal intramuscular injections.[119] This approach was tried in a woman who inadvertently had received 150 mCi of ^{131}I during weeks 10 to 11 of pregnancy.[132] Because of the potential risks of fetal hypothyroidism, an amniocentesis with an injection of 500 μgm of thyroxine was performed weekly from week 33 until delivery. The concentration of T_4 in the cord serum was in the hypothyroid range, and the TSH concentration was low. However, the infant was not hypothyroid. Although these investigators suggested that the amniotic

fluid T_3 concentration might be useful in the intrauterine diagnosis of hypothyroidism, this has not been substantiated.[129] In addition, follow-up was necessary for the infant because of the possible results of exposure to large amounts of thyroxine. At present, both the intrauterine diagnosis and the therapy of congenital hypothyroidism are difficult.

MATERNAL THYROTOXICOSIS

Thyrotoxicosis occurs in about two of every 1000 pregnancies.[168] A total of 75 women with hyperthyroidism were identified in a prenatal research study, and while the relatively small numbers preclude definitive statements, hyperthyroidism during pregnancy appeared to be associated with a slight increase in the neonatal mortality rate and with a significant increase in the frequency of delivery of low birthweight infants. There is a suggestion that there is a higher incidence of congenital malformations in the offspring of mothers with untreated Graves' disease.[154] There is no evidence that pregnancy makes thyrotoxicosis more difficult to control. In fact, perhaps related to the immunology of pregnancy, hyperthyroidism tends to be more easily controlled during pregnancy, whereas relapses tend to occur post partum.

Convincing evidence is lacking that fertility is impaired in mild to moderate hyperthyroidism, and these is disagreement whether fetal mortality rate is greater once pregnancy is established. Early workers thought that there was a hazard to the fetus proportional to the degree of hyperthyroidism.[162] However, the fetal loss of 8.4% from 57 thyrotoxic pregnancies compared favorably with a total fetal loss of 17.2% in a group of normal euthyroid women. The women in this study had received treatment for hyperthyroidism, and there are no available data for untreated thyrotoxicosis during pregnancy. The balance of available evidence suggests that mild to moderate thyrotoxicosis is not inimical to the continuation of pregnancy. A higher incidence of toxemia has been reported in thyrotoxic pregnancies, but the studies were not well controlled, and this correlation appears doubtful.[149, 220] Down's syndrome has also been reported to occur more frequently in the offspring of thyrotoxic mothers, but these studies also were not well controlled.[163]

Etiology

Although there are a variety of possible causes of hyperthyroidism in pregnant women, including trophoblastic tumor and hydatidiform mole, toxic diffuse goiter (Graves' disease) and toxic nodular goiter (Plummer's disease) are of major importance. Plummer's disease is much less common during the childbearing years, since it arises in long-standing nodular goiter. Graves' disease is the most common form of hyperthyroidism in conjunction with pregnancy.

Patients with Graves' disease have tended to undergo remission during pregnancy and exacerbation in the postpartum period. Studies on the immunology of pregnancy have provided possible explanations for this observation. Pregnancy has been described as a successful allograft of foreign tissue, and maternal immunologic inertness has been postulated as the mechanism for protecting the fetal allograft.[12] Both humoral and cell-mediated immunity have been reported to be depressed during normal pregnancy. Thyroid antibodies have been observed to decrease during pregnancy in Graves' disease.[3, 6] The amelioration of Graves' disease during pregnancy with exacerbation after delivery is thought to be due to these immunologic changes. The increased fetal suppressor T cell function necessary to prevent immunologic rejection by the mother may cause a transient decline in the intensity of Graves' disease during pregnancy.[44] Delivery with loss of the fetal suppressor T cells could result in the clinically recognized postpartum exacerbation of Graves' disease.[207] Soluble factors produced by activated fetal suppressor cells presumably cross the placenta and decrease the maternal autoimmune process.[74] The disappearance of immunosuppression at delivery and transient enhancement of the immune reaction may be responsible for the transient recurrence of hyperthyroidism after delivery in patients with Graves' disease.[4, 78]

Diagnosis of Hyperthyroidism in Pregnancy

The clinical diagnosis of thyrotoxicosis in the pregnant woman may be difficult. The euthyroid woman may have a number of hyperdynamic symptoms and signs, including an increase in cardiac output with systolic flow murmur and tachycardia, skin warmth, and heat intolerance. Diagnostic difficulties are fur-

ther compounded because the usual laboratory tests of thyrotoxicosis may easily give rise to suspicion that is confirmed only with difficulty. Signs of hyperthyroidism such as weight loss may be obscured by the weight gain of pregnancy. The presence of the eye changes of Graves' disease or pretibial myxedema may be helpful but does not necessarily indicate thyrotoxicosis. A resting pulse above 100 is helpful, and if the pulse fails to slow during a Valsalva maneuver, thyrotoxicosis becomes more likely. The presence of onycholysis, or separation of the distal nail from the nailbed, may also be helpful in making the clinical diagnosis of thyrotoxicosis. A patient with thyrotoxicosis during pregnancy may present with hyperemesis gravidarum, which resolves only after successful therapy of the hyperthyroidism.[16] Interestingly, the serum T_4 concentration may be elevated but not the serum T_3 concentration, which suggests a block in deiodination.[49]

Although the serum thyroxine concentration is elevated in normal pregnancy, values above 15 μgm/100 ml are suggestive of hyperthyroidism. The exact cutoff value depends on the particular assay. Provided that the patient does not have TBG deficiency, an RT_3U in the euthyroid range during pregnancy is also suggestive of thyrotoxicosis. These two determinations can be combined mathematically to obtain a free thyroxine index that makes a comparison of thyroxine to thyroxine-binding protein into a single value. Unfortunately, because of the elevated TBG during pregnancy, the free thyroxine index is not an accurate measure of the actual free thyroxine concentration.[206] The newer nonequilibrium dialysis methods for the estimation of free thyroxine may be helpful, although some of them are also affected by changes in TBG.[236] If the patient is clearly thyrotoxic clinically but has normal values for serum thyroxine the possibility of T_3 toxicosis should be considered.[228]

Hydatidiform mole may produce enough hCG to stimulate the thyroid and produce thyrotoxicosis.[102] For reasons that are not totally clear, patients so affected may have few clinical signs of thyrotoxicosis despite elevated thyroid function.[76] Patients were clinically euthyroid with elevated serum thyroid hormone concentrations when the hCG level was 0.1×10^6 IU/L and thyrotoxic when the hCG concentration was 0.3×10^6 IU/L.[170]

Thioamide Therapy

Since radioactive iodine therapy is contraindicated during pregnancy, treatment involves a choice between antithyroid drugs and surgery.[154, 175] There are arguments for and against both forms of treatment, and in the final analysis the individual decision depends on the physician's treatment bias and recent past experience. Even if the decision is made to operate, the thyrotoxicosis must first be controlled by antithyroid drug therapy.

The mainstay of antithyroid drug therapy involves thioamides, which inhibit thyroid hormone synthesis by blocking iodination of the tyrosine molecule. Since these drugs block the synthesis but not the release of thyroid hormone, a clinical response to thioamides does not occur until the thyroid hormone stored in the colloid is utilized. Therefore, the time required to achieve control of the thyrotoxicosis will depend on the amount of colloid stored in the thyroid gland. Commonly, the patient will notice some clinical improvement after the first week of therapy and may approach euthyroidism after 4 to 6 weeks of therapy. Both propylthiouracil (PTU) and methimazole have a short duration of action. Although most patients can probably be maintained on a single daily dose,[88] some women may require the thioamides every 8 hours or even more frequently for adequate control of thyrotoxicosis.

PTU and methimazole (Tapazole) have been used interchangeably without evidence that one or the other had definite therapeutic advantages. However, PTU has the advantage of partially blocking the conversion of T_4 to T_3 in addition to inhibiting thyroid hormone synthesis. Furthermore, there is some evidence that methimazole therapy may be associated with aplasia cutis in the offspring (Fig. 16–5).[157, 209, 222a] For these reasons, PTU is the drug of choice in the therapy of thyrotoxicosis in pregnancy.

PTU Dose. Once the diagnosis of hyperthyroidism has been made, the patient should be given PTU, 100 to 150 mg, every 8 hours. After control of thyrotoxicosis has been achieved, as determined by an improvement in symptoms and signs as well as a fall in serum thyroxine level, the dose of PTU should be decreased to 50 mg, four times a day. If the patient remains euthyroid, the PTU could be decreased to 150 mg per day and then after 3

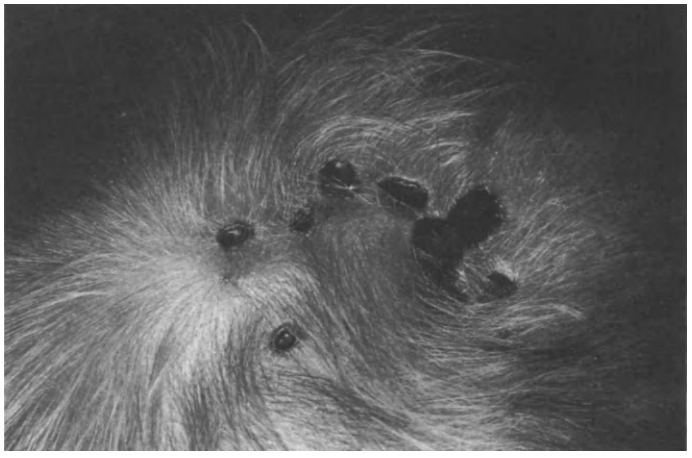

Figure 16–5. Aplasia cutis in a child whose mother had received methimazole. (Reproduced from Burrow, G. N., et al.: Yale J. Biol. Med. 51:13, 1978, with permission.)

weeks to 50 mg twice a day. Serial serum T_4 determinations obtained monthly may be helpful in monitoring the course of the disease. There appears to be a strong correlation between fetal and maternal free T_4 concentrations. Maintenance of the maternal free T_4 concentration in the range of the upper normal limit may be optimal for fetal thyroid function.[153] The serum thyroxine concentrations may increase before clinical signs of thyrotoxicosis recur. Pregnant women with thyrotoxicosis should be maintained on as low a dose of PTU as possible, preferably under 100 mg daily.[24]

If thyrotoxicosis recurs the PTU should again be increased to 300 mg per day. As mentioned previously in the chapter, a recurrence is particularly likely post partum, and PTU could be increased to 300 mg daily at that time. If control of thyrotoxicosis in the pregnant woman is not achieved on this treatment schedule, the PTU should be increased to 600 mg daily and given more frequently, e.g., every 4 to 6 hours. Rarely will it be necessary to give more than 600 mg PTU daily. The need for large amounts of PTU may relate to low serum concentrations of PTU.[198] In one study, serum PTU concentrations were consistently lower in the late third trimester compared with postpartum values.[77]

Complications of PTU Therapy. The most common complications of thioamide therapy include a mild, occasionally purpuric skin rash, pruritus, drug fever, and nausea, which occur in about 2% of patients taking PTU. These minor side effects tend to appear during the first 4 weeks of therapy. If a drug reaction occurs with PTU therapy may be continued with methimazole. However, the development of agranulocytosis requires that thioamide drug therapy be stopped immediately. Agranulocytosis usually occurs after 4 to 8 weeks of therapy in about 0.3% of the treated population and leads to a fatal outcome in less than 1 in 10,000 treated patients. A leukocyte count should be obtained before thioamide therapy, since about 10% of patients with Graves' disease have leukopenia. Weekly monitoring of the patient's leukocyte count during therapy is probably not helpful, because the white blood cell count may fall precipitously over several days.

Effect on the Fetus. The major concern with the use of PTU during pregnancy is the development of fetal goiter and hypothyroidism.[35] PTU crosses the placenta without difficulty[145] and blocks the fetal thyroid gland (Fig. 16–6). When the concentration of the fetal serum thyroid hormone decreases, stimulation of the thyroid gland by TSH might result in goiter formation.[24, 185] This effect may not depend solely on the amount of PTU. Many women have given birth to normal children even after receiving large amounts of antithyroid drugs during pregnancy. Four out of 400,000 children screened in the Quebec neonatal thyroid screening program had transient hypothyroidism due to PTU. The number of mothers receiving antithyroid drugs was unknown, but a crude estimate suggests that only 1 to 5% of children exposed to PTU develop transient hypothyroidism. Under ordinary circumstances, sufficient maternal thyroid hormone may cross the placenta to prevent fetal goiter.

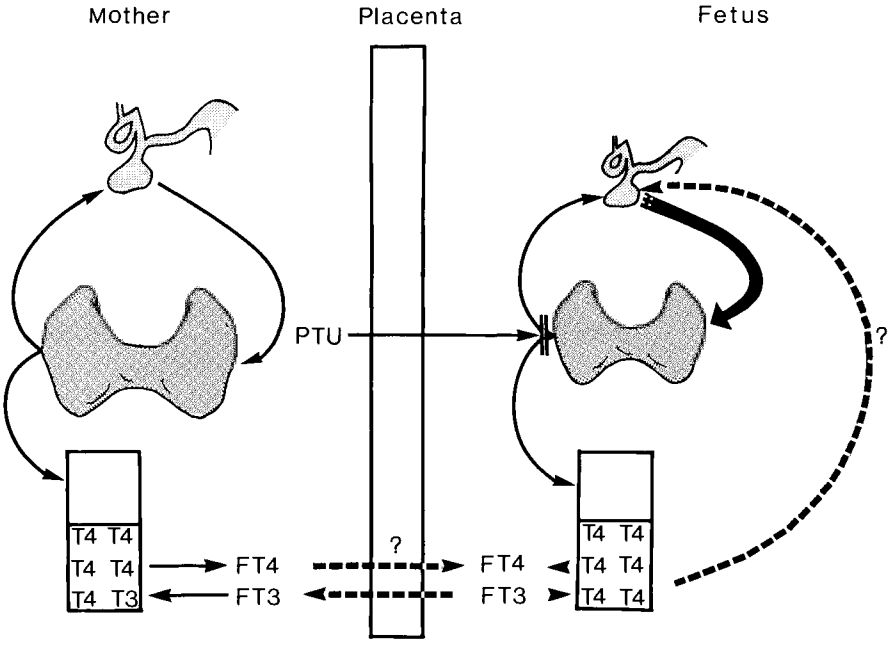

Figure 16–6. Effect of propylthiouracil (PTU) on fetal thyroid function.

Since a decrease in fetal thyroid hormone concentration may cause neonatal goiter, maternal thyroid hormone supplementation has been suggested.[199] However, the added hormone may increase the dosage of PTU that is needed to control thyrotoxicosis.[85, 109, 110, 123, 175] Certainly every effort should be made to avoid maternal hypothyroidism during pregnancy. PTU should be decreased or discontinued, and thyroid hormone should be administered if hypothyroidism is even suspected.

If the pregnant thyrotoxic woman on antithyroid medication can be followed at monthly intervals with laboratory determinations of thyroid function the thyroid hormone supplementation is probably unnecessary. If she cannot be followed closely, the thyroid hormone supplementation may ensure against the development of maternal hypothyroidism.[183] Certainly, an attempt should be made to treat the pregnant thyrotoxic woman with the lowest possible dose of antithyroid medication.[126] Pregnant women tolerate mild degrees of hyperthyroidism without great difficulty, and it would be better to err by giving too small a dose of antithyroid medication rather than too large. Finally, if early diagnosis and treatment of neonatal hypothyroidism prevent the sequelae of *in utero* hypothyroidism these affected children can be successfully treated after birth.

Breast-Feeding by a Mother Receiving PTU. A mother on antithyroid drug therapy may transfer thioamides in the breast milk. The amount of PTU transferred to the suckling infant is less than the amount of methimaxole and would not be expected to have any significant effect of neonatal thyroid function.[38, 124] Eleven thyrotoxic women were treated with carbimazole, which is similar to methimazole, or with PTU.[128]

The amount of thioamide did not exceed 15 mg of carbimazole or 150 mg of PTU. These investigators concluded that breast-feeding under these conditions does not pose a risk to the neonate. There is the theoretic risk of drug reaction, but none has been reported so far. In summary, if a mother receiving PTU has a strong desire to breast-feed, there should be a full explanation of the potential risks and a close monitoring of the neonatal thyroid function.

Possible Long-Term Effects. Unlike iodide-induced goiter, the neonatal goiter associated with PTU therapy is not large and obstructing. Although cretinism has been reported in the offspring of women treated with thioamides, there is no conclusive evidence that it was caused by the drugs. A more difficult question is whether children who were exposed to thioamides *in utero* attain full intellectual de-

velopment. To study this problem we compared 18 children exposed to PTU *in utero* with 17 nonexposed siblings.[22] The children ranged in age from 2 to 12 years, and there were no important differences in their physical or mental characteristics. Thyroid function test results were normal in both groups, and there was no evidence of abnormal physical development or delayed bone growth. Physiologic testing revealed no marked differences between the groups, either in overall intellectual development or in patterning of various mental skills.

The small size of the sample precluded definite conclusions, but in a subsequent study intelligence tests administered to 29 children who had been exposed to PTU *in utero* and 32 nonexposed siblings also showed no important differences between the groups.[25] Similar data were reported for 25 children aged 3 to 13 years who were exposed *in utero* to carbimazole, which is metabolized to methimazole.[147] These results indicate that with careful attention, thioamides can be given to pregnant women without interfering with subsequent intellectual development in the offspring.

Propranolol Therapy

Because the pregnant woman treated with PTU must be followed very carefully, there has been interest in alternative therapy, particularly with the beta blocking agent propranolol.[19] However, the pharmacologic actions of propranolol on the fetus and neonate have been associated with small placenta, intrauterine growth retardation, impaired responses to anoxic stress, and postnatal bradycardia and hypoglycemia.[79, 92, 181]

On the basis of these studies, which were often retrospective, beta blockers have not been recommended for long-term treatment of thyrotoxicosis during pregnancy. However, enough data have become available to suggest that pregnant women may be safely treated with beta-blocking agents, if indicated.[192] Whether selective beta blockers have any advantage in long-term therapy remains to be determined.[193, 197]

For rapid control of thyrotoxicosis, the combination of propranolol, 40 mg every 6 hours, with iodides, which block the release of thyroid hormone, will usually result in marked improvement within 2 to 7 days. Iodides act by inhibiting the secretion of thyroid hormone and by acutely inhibiting the uptake of iodide into the thyroid. Unfortunately, iodides cause similar effects on the fetal thyroid gland in doses as low as 12 mg daily. Five drops of Lugol's solution twice a day, which is equivalent to a total of 100 mg of iodide, may be added to the therapeutic regimen for no more than a week or so. Once control of the thyrotoxicosis has been achieved, the patient may undergo subtotal thyroidectomy, but the anesthesiologist should be made aware that the patient has received propranolol. Whether agents that inhibit the conversion of thyroxine to triiodothyronine, such as ipodate, have any role in the rapid control of thyrotoxicosis in the pregnant woman is not clear.[238]

Surgery

If a subtotal thyroidectomy is to be performed surgery is often delayed until after the first trimester. The rationale for this delay is that the spontaneous abortion rate is highest during the first trimester and surgery during this period might increase the risk of abortion. However, thyroid surgery probably need not be avoided during the first trimester, if indicated.[18] The argument against subtotal thyroidectomy for the treatment of the pregnant woman with thyrotoxicosis is twofold. First, there is a definite surgical risk, which is probably higher than that of fatal complications of medical therapy.[18, 231] Second, the surgical complications of hypoparathyroidism and recurrent laryngeal nerve paralysis are disabling and difficult to treat. Although uncommon, they do occur, and as fewer thyroidectomies are done because of medical therapy the complication rate rises.

In one study of 12 thyrotoxic women treated with subtotal thyroidectomies, all the patients were first controlled with PTU and a brief course of iodides.[218] Three women were operated upon during the first trimester, seven in the second and two early in the last trimester. Seven women received thyroid hormone postoperatively. Of the 12 patients, nine were delivered of normal children weighing more than 2500 gm. One patient had a spontaneous abortion 12 hours postoperatively, one intrauterine death occurred at 20 weeks, and one infant was premature. There was a total pregnancy wastage of 16% for this group compared with 33% for the medically treated group. The spontaneous abortion rate was 3.5% in the surgically treated group and 11.5% in the group receiving antithyroid medication. When

the difference in spontaneous abortion was eliminated, the overall fetal salvage was similar.

The difficulty with these studies is that patients must be controlled medically before they can undergo surgery.The spontaneous abortion rate is higher during early pregnancy and may be labelled a "medical failure" when the abortion is during preoperative preparation.[96] In a study in which subtotal thyroidectomy had been planned after control was achieved with antithyroid drugs, only seven patients were actually operated upon for various reasons.[237] With one exception, the more severe cases were treated medically. In spite of this, 70% of women treated with medical therapy had a successful pregnancy outcome, while 28% of women surgically treated were in the same category. Other workers have found that subtotal thyroidectomy after appropriate preparation resulted in no surgical complications.[58] If the patient is to have surgery she should be observed carefully for signs of hypothyroidism postoperatively. At the first chemical signs of hypothyroidism, the pregnant woman should be started on a regimen of 0.125 mg of L-thyroxine.[13]

Since surgical complications do occur and since the majority of patients who receive PTU have uncomplicated pregnancies, medical therapy seems to be preferred for hypothyroidism during pregnancy. Subtotal thyroidectomy should probably be reserved for antithyroid drug hypersensitivity, poor compliance, and the rare instance in which the drugs are ineffective.[110]

Thyroid Storm

The major risk to the pregnant woman with thyrotoxicosis is the development of thyroid storm. This uncommon but frightening complication occurs as a life-endangering augmentation of the signs and symptoms of hyperthyroidism. Thyroid storm is more likely when there is some precipitating factor such as labor, cesarean section, or infection.[91] The pregnant woman may present with a fever as high as 106°F, marked tachycardia, prostration, and severe dehydration, a course ending fatally in up to 25% of cases despite good medical management. Thyroid storm is more commonly seen in patients in whom the diagnosis of hyperthyroidism has not been recognized, but it also occurs in inadequately treated patients.

Precipitating factors should be alleviated if possible and specific therapy includes the following:

1. Propranolol, 40 mg by mouth every 6 hours to control the beta-adrenergic activity. (If necessary, the drug can be given intravenously in doses of 1.0 to 2.0 mg.)
2. Sodium iodide, 1 gm intravenously to block the secretion of thyroid hormone.
3. Lithium, 300 mg, three times a day, also to block thyroid hormone secretion.
4. PTU, 1200 mg orally in divided doses, to block the formation of thyroid hormone and the deiodination of T_4 to T_3.
5. Dexamethasone, 8 mg a day, to further block the deiodination of T_4 to T_3.
6. Five liters of fluids to replace severe fluid losses.
7. Hypothermia for malignant hyperpyrexia.[46, 109]

Plasma-exchange has been carried out successfully in three pregnant women with severe thyrotoxicosis and remains an option.[46] If hyperthyroidism is considered in the pregnant woman and adequate therapy initiated, this frightening complication of thyrotoxicosis should be virtually eliminated.

INADVERTENT ADMINISTRATION OF ^{131}I DURING PREGNANCY

As mentioned previously, radioactive iodine is absolutely contraindicated during pregnancy. However, occasionally a patient not known to be pregnant at the time is given a dose of radioactive iodine. Although all women in the childbearing age should have a pregnancy test before receiving therapeutic doses of radiation, this is not always done.

Maternal Irradiation. During a radioisotope thyroid uptake, both mother and fetus are exposed to radiation. The amount of radiation that the mother receives is insignificant in terms of a dose to the thyroid or ovaries. However, a treatment dose of ^{131}I for hyperthyroidism is 1000 times the dose required for an uptake study. Hyperthyroidism is the only common nonmalignant disease for which patients receive significant amounts of ionizing radiation. Although the incidence of leukemia and thyroid malignancy is not increased in thyrotoxic patients receiving radioactive iodine,[195] subsequent effects of the gonadal dose are less certain.[196] The radiation dose to the maternal and fetal thyroids as well as gonads

has been estimated.[93] In a similar study, a patient was estimated to receive a total body dose of 0.51 rad/mCi.[229] Therefore, a thyrotoxic patient receiving a therapeutic dose of 10 mCi of ^{131}I would receive a total body dose of about 5 rads. There are five sources of radiation to the gonads: beta radiation from blood flowing through the gonads and gamma radiation from the thyroid, extrathyroidal tissue, bladder, and colon. The mean gonadal dose has been estimated at about 0.3 to 0.45 rad/mCi of ^{131}I.[187, 229] Whether this radiation dose is sufficient to cause genetic effects is not clear. Persistent chromosomal abnormalities have been found in the white blood cells of patients who had received 5 mCi of ^{131}I for the treatment of thyrotoxicosis. There is no reason to suppose that similar changes do not also occur in gonadal chromosomes.

Fetal Irradiation. The fetal thyroid begins to concentrate iodine at about the 10th to 12th week of gestation and has an avidity for iodine 20 to 50 times that of the maternal thyroid. As a consequence, any dose of radioiodine will be more concentrated per gram of thyroid tissue in the fetus than in the mother. Congenital hypothyroidism has been reported in offspring of mothers who received therapeutic doses of radioiodine.[72, 120, 168, 214] A thyrotoxic mother who inadvertently received 14.5 mCi of ^{131}I at the end of the first trimester was estimated to have received a dose of 20,000 rads to the thyroid, while the fetus received an estimated dose of 250,000 rads to the thyroid with resulting hypothyroidism.

Not only is the fetal thyroid gland more avid for iodine, fetal tissues in general are more radiosensitive. Studies have demonstrated a causal relationship between prenatal irradiation and subsequent development of malignant disease, with indications that the risk is related to both the dose and the time of exposure.[138, 211] Microcephaly has also been noted in children who were heavily irradiated *in utero*.[84] Children who were *in utero* at the time of the atomic bombing of Hiroshima and Nagasaki have been studied intensively. Radiation *in utero* resulted in higher fetal and infant mortality and a higher prevalence of microcephaly and mental retardation. Microcephaly was most common when the child was irradiated between 7 and 15 weeks of gestation. Children exposed to ionizing radiation *in utero* also lagged behind nonexposed peers during adolescence in several aspects of growth and development.[24] These findings suggested that subtle defects that are not easy to detect may occur because of radiation. Most of the long-term effects occurred with whole body radiation above 50 rads. Whether there is a threshold effect for radiation damage or whether the damage is linear is important, since the whole body radiation dose to the fetus from a therapeutic dose of ^{131}I for thyrotoxicosis is well below 50 rads. At least some of the effects may be linear, and all radiation should be regarded as harmful.

Therapeutic Considerations

If a woman is subsequently found to be pregnant after a radioactive iodine uptake nothing further need be done. Although radiation is absolutely contraindicated in the pregnant woman, the amount received during a diagnostic thyroid study is not sufficient to cause concern. However, in a pregnant woman who has inadvertently received a therapeutic dose of ^{131}I for hyperthyroidism, the question of termination of the pregnancy arises. This situation tends to arise early in pregnancy when the fetal thyroid is not trapping iodine. The relatively low fetal whole body irradiation is probably not sufficient to justify pregnancy termination. If the fetal thyroid is trapping iodine hypothyroidism is a risk. However, the neonatal thyroid screening program is predicated on the supposition that prompt postnatal thyroid hormone therapy prevents the sequelae of *in utero* hypothyroidism.

If a pregnant woman with thyrotoxicosis is given a therapeutic dose of ^{131}I and this is discovered within a week of administration, she might be treated with PTU, 300 mg daily for 7 days, to block the recycling of the ^{131}I in the fetal gland. Iodides would dilute the uptake pool but would also inhibit the release of radioactivity. By 10 days after treatment, more than 90% of the dose of ^{131}I has been delivered, and such treatment would not be helpful. The mother will be thyrotoxic and will have to be treated, which will also complicate fetal thyroid function. Regardless of possible preventive measures, the condition of the infant should be carefully evaluated for hypothyroidism at birth and immediate treatment begun, if indicated.

Long-Term Management of Thyrotoxicosis. The long-term management of a young thyrotoxic patient who is planning subsequent preg-

nancies represents a difficult therapeutic decision. A medical remission in this age group is unlikely and thereby during the subsequent pregnancies will probably be indicated. A full 2-year course of PTU therapy seems indicated—possibly a second course, if necessary. If the patient is still thyrotoxic, then consideration should be given to radioactive iodine therapy. Long-term antithyroid drug therapy in such a situation may be undesirable.[157] Despite the attendant risks, subtotal thyroidectomy also deserves consideration in this situation.

NEONATAL THYROTOXICOSIS

Approximately 1% of pregnant women with a history of Graves' disease give birth to infants with neonatal thyrotoxicosis.[159] Although the mothers themselves were not necessarily thyrotoxic during pregnancy, virtually all of them had some clinical or laboratory manifestations of Graves' disease. The neonatal thyrotoxicosis was transient, usually lasting 2 to 3 months or less. Hyperthyroidism has been a most frequent problem in the infant; the eye changes of Graves' disease have not always been present. Neonatal thyrotoxicosis is not a benign condition and has been associated with a mortality rate of 16%.[106] Perhaps the most serious, long-term complication in surviving infants is premature craniosynostosis that might result in inadequate cerebral development.[150]

Etiology

Neonatal thyrotoxicosis is most likely due to the placental transfer of thyroid-stimulating immunoglobulins from the mother with Graves' disease to the fetus (Fig. 16–7). The pathogenetic role for thyroid-stimulating immunoglobulins in this condition is demonstrated by the correlation between high concentrations in maternal and cord serum with a subsequent decline in immunoglobulin activity accompanied by resolution of the neonatal thyrotoxicosis.[148, 159] After great initial enthusiasm, the inability to detect thyroid-stimulating immunoglobulins in the sera of all cases of neonatal thyrotoxicosis raised questions about the etiologic agent.[105, 106] Much of the confusion has arisen because different assay procedures have been used, and data obtained by one procedure may not be comparable to data obtained by another.[152] The process is even more difficult because there may be immunoglobulins present that are inhibitory as well as stimulating with different binding affinities.[239]

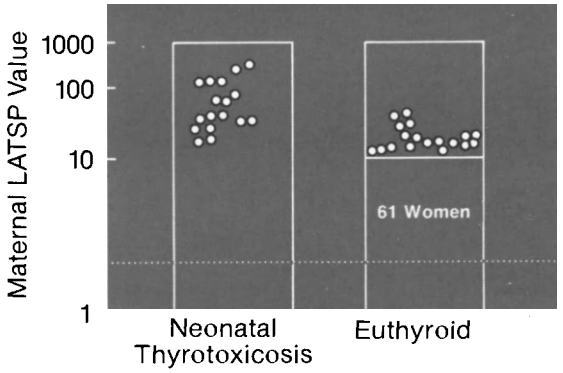

Figure 16–7. Maternal serum concentrations of long-acting thyroid stimulator (LATS) protector (P) in 93 pregnant women with Graves' disease. Sixty-one euthyroid women with LATS P values of less than 10 are represented by the dotted area. (Modified from Munro, D. S., et al.: Br. J. Obstet. Gynaecol. 85:837, 1978, with permission.)

An infant has been described who did not develop neonatal thyrotoxicosis until levels of the inhibitory immunoglobulin declined and then had a prolonged course because of the presence of a third antibody that presumably facilitated binding of the thyroid stimulating immunoglobulin to the TSH receptor.[242, 243] The equal sex incidence in neonatal thyrotoxicosis also favors the concept of placental transfer of the etiologic agent when compared with the female-to-male ratio of 3:1 or 4:1 for Graves' disease. The duration of neonatal thyrotoxicosis appears to be a function of the initial neonatal serum concentration of the thyroid stimulating immunoglobulin and the rate of degradation. The half-life of the antibody in the serum of the thyrotoxic neonate, based on studies with long-acting thyroid stimulator (LATS)-protector, is between 4 and 10 days.[139, 158]

The question has been raised whether neonatal thyrotoxicosis is transient.[106, 107] A number of children have been described in whom the hyperthyroidism persisted beyond 1 year of age. These patients, particularly females, appear to inherit congenital Graves' disease as an autosomal dominant trait. Certainly, the majority of patients with neonatal thyrotoxicosis appear to have the disease only transiently.

Diagnosis

The diagnosis of neonatal thyrotoxicosis can usually be made on the basis of the total

clinical picture (Fig. 16–8). If the mother has Graves' disease, then the possibility of neonatal thyrotoxicosis should be carefully considered. The presence of goiter, exophthalmos, and tachycardia in a hyperirritable infant with an elevated serum thyroxine level is sufficient to enable the diagnosis to be made with a reasonable degree of certainty.[201] Infants with neonatal thyrotoxicosis have also presented with other signs, such as cardiac failure, hepatosplenomegaly, jaundice, and thrombocytopenia.[57] Such patients can be detected in the population screening programs for neonatal hypothyroidism if the T_4 radioimmunoassay is used.[227] The diagnosis would be greatly strengthened by a positive assay for thyroid stimulating immunoglobulin. A high titer is diagnostic in the infant and virtually predictive in the mother. Unfortunately, this assay is only available in research laboratories.

Although a history of Graves' disease is the rule, the mother may not have active thyrotoxicosis and may be euthyroid or hypothyroid. Another problem in the diagnosis of neonatal thyrotoxicosis may occur in children exposed to antithyroid drugs *in utero*. These children may not be thyrotoxic at birth but may develop the disease subsequently.[157] Presumably, the antithyroid drugs block the clinical expression of thyrotoxicosis in the neonate so that it may not become evident until after the child has left the hospital.[234] The neonatal narcotic withdrawal syndrome could be confused with neonatal thyrotoxicosis with irritability and tremulousness, and the serum thyroxine level may also be elevated.[112]

Therapy

If neonatal thyrotoxicosis is mild no specific antithyroid therapy is necessary because the disease is self-limited. Otherwise, the infant can be treated with Lugol's solution (8 mg iodine) one drop three times a day, and propranolol, 2 mg/kg/day.[97, 203] PTU, 10 mg every 8 hours, can also be added but is perhaps better reserved for children whose condition cannot be controlled with iodides or propranolol. Sodium ipodate has also been used for therapy.[114a] Patients with neonatal thyrotoxicosis who have died have usually been premature and have had severe hyperthyroidism accompanied by congestive heart failure. However, most children recover without incident. The majority of infants who require treatment do so for 3 to 6 weeks, depending upon the serum titer of thyroid stimulating immunoglobulin. Some children with neonatal thyrotoxicosis have been considered to be premature because of low birth weight. However, these children may actually have accelerated maturity on the basis of bone age, the low birth weight being secondary to the thyrotoxicosis.[62]

Figure 16–8. Neonatal thyrotoxicosis.

FETAL THYROTOXICOSIS

Thyrotoxicosis due to the placental transfer of thyroid stimulating immunoglobulins may begin *in utero*, particularly if the mother with Graves' disease is not receiving antithyroid medication. This situation occurs when the maternal thyroid has been ablated because of Graves' disease but high levels of thyroid-stimulating immunoglobulins persist.[39, 146] One woman who was euthyroid on thyroid hormone replacement after surgical treatment for active Graves' disease with the subsequent development of hypothyroidism had twins who died of thyroid storm *in utero* (Fig. 16–9).[146]

The possibility of fetal thyrotoxicosis should be considered in all pregnant women with a history of Graves' disease regardless of current thyroid status. Elevated concentrations of maternal thyroid stimulating immunoglobulins and persistent fetal tachycardia above 160 beats/minute strongly suggest the diagnosis. Because of increased fetal morbidity and mortality, consideration should be given to thioamide treatment directed specifically towards the fetus in doses of 50 to 100 ml daily.[34, 188] After delivery the neonate should continue to be treated for thyrotoxicosis.[225]

POSTPARTUM THYROIDITIS

The immunologic changes that occur during pregnancy may influence the course of autoimmune thyroid disease. The syndrome of postpartum thyroiditis is much more common than previously supposed.[63] A survey of the Japanese population revealed that 5.5% of women had postpartum thyroiditis, with hyperthyroidism, hypothyroidism, or both.[5] A similar prevalence was reported from Scandinavia.[111] Some of the women so affected, in the past, may have been misdiagnosed as having postpartum anxiety or depression. Postpartum thyroiditis usually occurs 3 to 6 months after delivery and manifests itself as transient hyperthyroidism followed by transient hypothyroidism with spontaneous recovery in 90% of cases. Physical examination reveals a small goiter in half the cases. Laboratory test results show elevated thyroid hormone concentrations during the thyrotoxic phase with a low radioactive iodine uptake. Microsomal antibodies are commonly present. In the Swedish study, half the patients with positive microsomal antibody titers during pregnancy developed postpartum thyroiditis.[111]

The signs and symptoms of postpartum thyroiditis are often subtle and difficult to detect. Perhaps pregnant women should be screened for microsomal antibodies, and patients with positive antibody titers followed closely during the postpartum period. The thyrotoxic phase usually lasts for 1 to 3 months but occasionally longer with about the same length of time for the hypothyroid phase. Postpartum thyroiditis tends to recur with subsequent pregnancies.[226]

THYROID NODULE AND THYROID CARCINOMA

The presence of nontoxic diffuse goiter in response to relative iodine deficiency during pregnancy is discussed earlier in this chapter. A more difficult problem concerns the pregnant woman who is discovered to have a solitary thyroid nodule.

Management of the Thyroid Nodule

One study reported 26 women who had been operated upon for a clinically solitary nodule that had arisen during, or was affected by, pregnancy.[42] At surgery most of the nodules represented nontoxic nodular goiters, but there were nine true adenomas. Parity seemed to have no influence on the development of thyroid nodules.

Evaluation of the solitary thyroid nodule during pregnancy is limited because the radioisotope thyroid scan is contraindicated. However, it is possible to obtain ultrasound evaluation of the nodule to determine whether it is solid or cystic and to follow that procedure by a fine needle aspiration biopsy of the nodule. If the biopsy specimen does not reveal suspicious cells the thyroid should be suppressed with 0.15 mg of L-thyroxine for the duration of the pregnancy. Following delivery, the thyroid nodule should be reevaluated. If the biopsy specimen is suggestive of malignancy, surgery is indicated even though the patient is pregnant.

Management of Thyroid Carcinoma

Pregnancy apparently has no effect on the natural history of thyroid carcinoma, nor does thyroid carcinoma have any significant effect on pregnancy. In one study of 60 women who had thyroid carcinoma in association with pregnancy, 38 women had been treated and were free of disease for 2 to 15 years before becom-

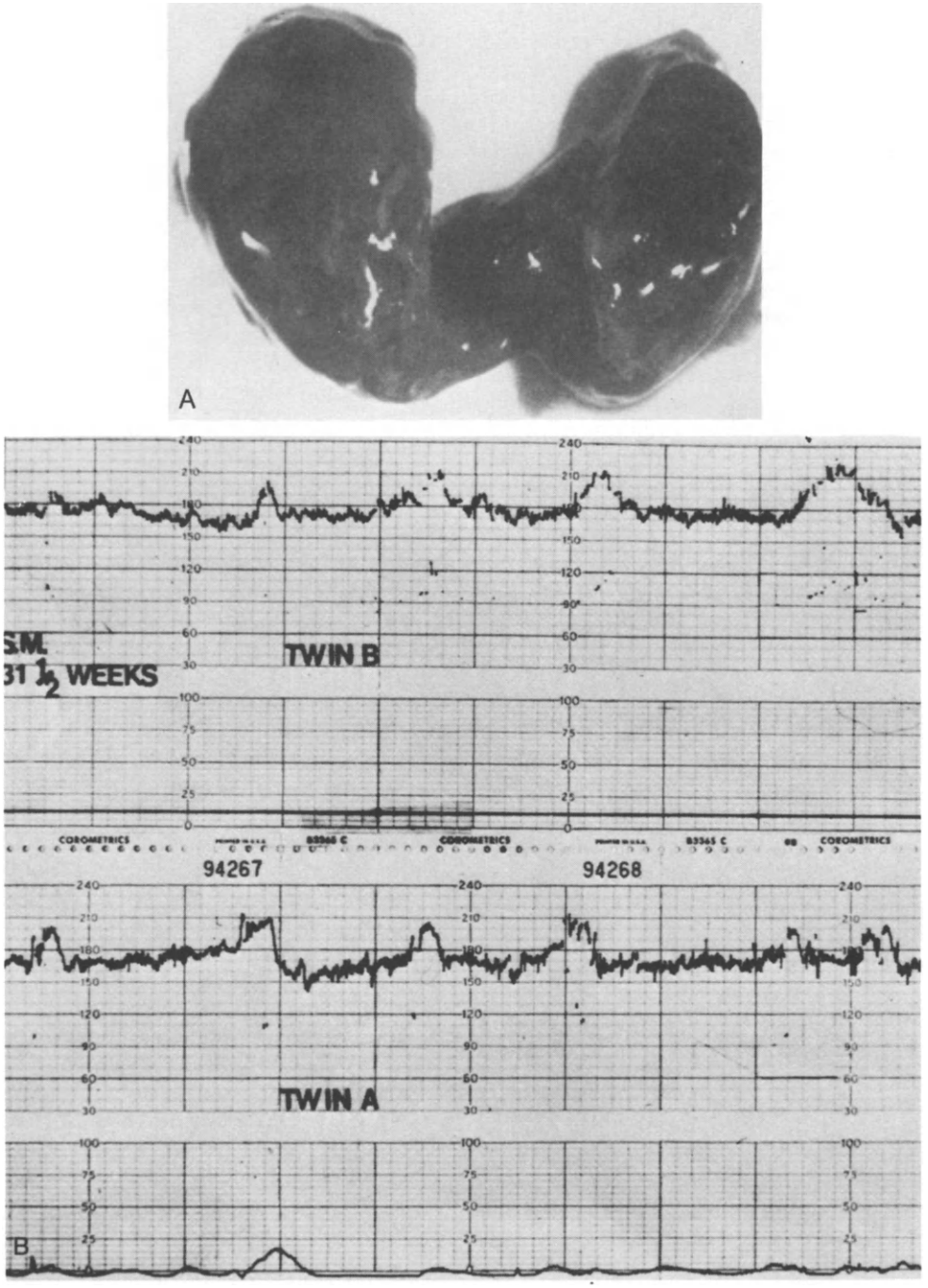

Figure 16–9. *A,* Fetal thyrotoxicosis. At autopsy the thyroid gland of twin B (see *B*) was grossly enlarged and weighed 5 gm. *B,* Oxytocin challenge test performed at 32 weeks of gestation on twins A and B with tachycardia. (Reproduced from Maxwell, K. D., et al.: Obstet. Gynecol. 53:188, 1980, with permission.)

ing pregnant.[190] The second group in that study included 22 women with thyroid carcinoma who were pregnant one to five times with this condition. Two therapeutic abortions were performed, one because of extensive metastases and one because of radioactive therapy. In another study, 70 women with thyroid carcinoma who became pregnant were compared with 109 women with thyroid carcinoma who did not.[103] There was no significant difference in the overall recurrence rate between the two groups, and the investigators concluded that pregnancy subsequent to the diagnosis of thyroid carcinoma had no effect on the course of the disease.

The diagnosis of carcinoma during pregnancy is not an absolute indication for terminating the pregnancy, nor is pregnancy a contraindication to necessary thyroid surgery. Radioactive iodine therapy should be withheld until after delivery but is rarely indicated.

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