CONTEMPORARY ENDOCRINOLOGY™

Diseases of the Thyroid

SECOND EDITION

Edited by

Lewis E. Braverman, MD

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CONTEMPORARY ENDOCRINOLOGY™

P. Michael Conn, Series Editor

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Lewis E. Braverman, MD

Chief, Section of Endocrinology, Diabetes, and Nutrition,
Boston Medical Center, Boston, MA

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In this second edition of his popular and widely acclaimed book, Lewis Braverman and a panel of international authorities have thoroughly updated and revised the first edition with fresh perspectives, many new authors, and the latest scientific and clinical developments in thyroid disease. New to this edition are the clinical relevance of the sodium/iodide symporter (NIS), broad coverage of autoimmune thyroid disease, and expanded coverage of thyroid cancer, including recombinant human TSH. Other chapters discuss such important topics as thyroid dysfunction during pregnancy and postpartum, thyroid disease in children and the elderly, the evaluation and management of nodular goiter, the worldwide problem of iodine deficiency and its eradication, and environmental goitrogens.

Authoritative and up-to-date, *Diseases of the Thyroid, Second Edition,* provides internists, family physicians, obstetricians and gynecologists, and endocrinologists alike with a highly readable guide not only to understanding thyroid disorders, but also to their optimal clinical management.

FEATURES

- **Authoritative guide to the scientific and clinical aspects of thyroid disease**
- **Ready access to up-to-date information on the management of thyroid disorders**
- **Coverage of thyroid dysfunction during pregnancy and postpartum**
- **Review of thyroid disease in children and the elderly**

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Second Edition

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Diseases of the Thyroid

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PREFACE

In the past five years, so much additional progress has been made in our understanding of both the basic and clinical aspects of a wide variety of thyroid disorders that a second edition of *Diseases of the Thyroid* was considered a necessary addition to the *Contemporary Endocrinology* series. Fresh perspectives also seemed warranted, so we recruited new authors for many of the chapters and believe that this edition will provide the reader with further insights into the pathophysiology and clinical presentation and treatment of thyroid disease. It includes topics ranging from neonatal thyroid screening, thyroid dysfunction during infancy and childhood, peripartum thyroid disorders, thyroid disease in the elderly, and the clinical relevance of the sodium/iodide symporter (NIS) to the pathogenesis and treatment of nodular goiter, thyroid cancer, thyrotoxicosis, and hypothyroidism. The worldwide problem of iodine deficiency and its eradication is also discussed, along with environmental goitrogens and iodine-induced thyroid disease.

I am indebted to all the contributors for their cooperation and expertise in providing their chapters in an extremely timely fashion, to Mr. Thomas Moore and Mr. Craig Adams from Humana Press for their expert assistance, and to Ms. Christine Archung for providing superb administrative assistance.

Lewis E. Braverman, *MD*

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1 Molecular Mechanisms of Nuclear Thyroid Hormone Action

William W. Chin, MD, and Paul M. Yen, MD

CONTENTS

INTRODUCTION

The role of thyroid hormone (L-triiodothyronine, T_3; L-tetraiodothyronine, T_4; TH) in the regulation of diverse cellular activities, including normal growth and development, and in general metabolism, is well established *(1–4)*. TH exerts its major effects at the genomic level, although action at nongenomic sites such as the plasma membrane, cytoplasm, and mitochondrion is also evident (*see* Chap. 2). Much work in the field, especially over the past decade, has provided a better understanding of the molecular mechanisms involved in TH action and gene transcription *(5,6)*. As illustrated in Fig. 1, circulating free TH enters the cell by either passive diffusion or other, yet poorly described mechanisms. In addition, the more biologically active T_3 (triiodothyronine) may be generated from T_4 (thyroxine) in some tissues by iodothyronine 5′-deiodinases, and both T_3 and T_4 may be subject to further intracellular inactivation. TH then enters the nucleus, where it binds to the nuclear thyroid-hormone receptor (TR) with high affinity and specificity with K_d values in the nanomolar range). TR is a ligand-regulated transcription factor that is intimately associated with chromatin, and also associates with other nuclear proteins to form heterodimers. These in turn are bound to target DNAs known as TH-response elements (TREs). The formation of a liganded TR/DNA complex leads to activation of its associated gene, and to consequent

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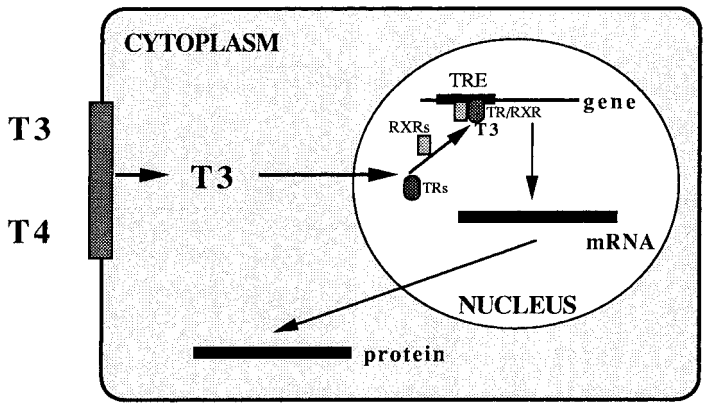

Fig. 1. TH action at the nuclear level. Thyroid hormone (T_4 and T_3; TH) exerts numerous effects on the cell. Whereas many of its actions involve regulation of gene expression, thyroid hormone may also act at the plasma membrane, cytoplasm, mitochondrion, and other non-nuclear sites. T_3 and T_4 may enter the cell by passive diffusion or other poorly defined pathways. In addition, T_4 may be deiodinated to more active T_3 by iodothyronine 5′-deiodinases. Furthermore, T_3 may be subjected to degradation within the cell. T_3 then enters the nucleus to bind to the thyroid hormone receptor (TR). The TR, in collaboration with a number of other nuclear proteins including the RXRs, form heterodimers that are bound to target DNA sites known as thyroid hormone-response elements (TRE). The liganded TR/RXR/TRE complex initiates alterations in gene expression among genes containing such TREs, and these alterations in turn alter their corresponding mRNA and protein levels.

changes in messenger RNA (mRNA) and protein. Thus, the central role of TR in the nuclear action of TH is evident.

THYROID HORMONE RECEPTORS

TRs are ligand-regulated transcription factors that are members of the steroid hormone receptor superfamily, which also includes the glucocorticoid, estrogen, progesterone, androgen, aldosterone, vitamin D, and "orphan" (unknown ligand and/or DNA target) receptors. TRs are encoded by a protooncogene, *c-erb*A, and are represented by two genomic loci (α and β), located on human chromosomes 17 and 3, respectively *(5,7–10)* (Fig. 2). Each TR contains a DNA-binding domain (DBD) with zinc finger motifs, and a ligand-binding domain (LBD). In the carboxy-terminal region of the TR, dimerization and major transactivation domains are found in addition to the LBD. The region between the DBD and LBD is called the hinge region, and contains the nuclear localization signal, typically a basic amino acid-rich sequence, first described in viral nuclear proteins. Recent X-ray crystallographic studies of liganded rat TRα-1 show that TH is buried in a hydrophobic "pocket" lined by discontinuous stretches of protein sequences of the LBD, and that additional hydrophobic interfaces exist that may contribute to the dimerization potential of TRs *(11,12)*. Twelve amphipathic helices comprise the LBD, and specific helices provide the contact surfaces for protein–protein interactions with coactivators and corepressors (helices 3, 5, 6, and 12 and 3, 4,5, and 6, respectively) *(111,112)*. Ligand binding causes major conformational changes in the LBD, particularly in helix 12, that allow TRs to discriminate between coactivators and corepressors.

The TRα gene produces an RNA transcript that is alternatively spliced to TRα-1 and *c*-erbAα-2 mRNAs, whereas the TRβ gene encodes TRβ-1 and TRβ-2 mRNA by alternate promoter choice *(13)*. As a result, TRα-1/c-erbAα-2 and TRβ-1/TRβ-2 are C-terminal- and

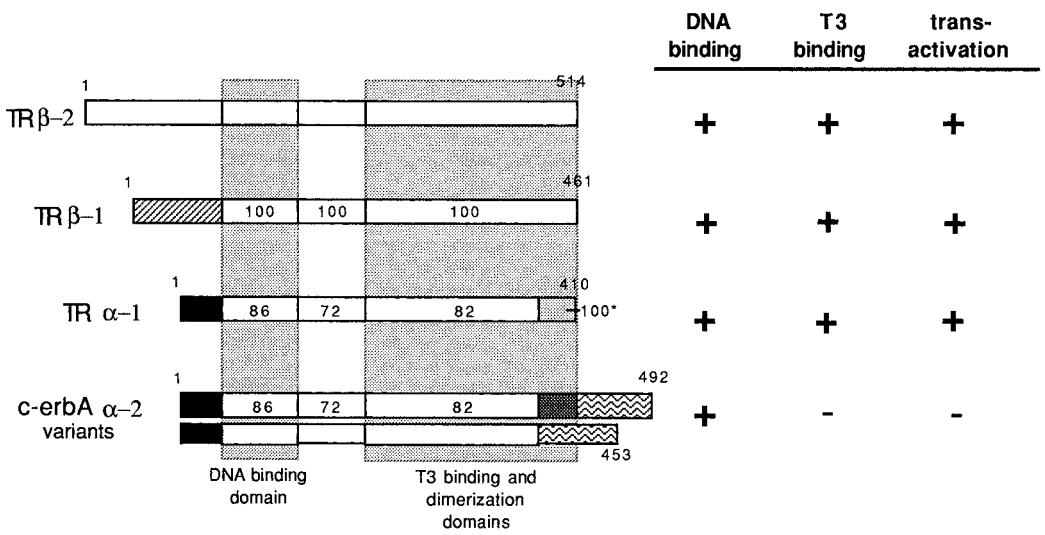

Fig. 2. Thyroid hormone-receptor (TR) isoforms. A number of TR and TR-related isoforms are depicted. Each TR contains a DNA-binding and T_3-binding and dimerization domains; the former is located in the center, and the latter are located at the carboxy-terminus. There are two human TR genes, α and β, located on different chromosomes. As a result of alternative promoter use and RNA splicing, two TRβ transcripts are formed, which encode TRβ-2 and TRβ-1. They are identical except for their NH_2 termini. The TRα gene is transcribed into a single precursor RNA, which is then subjected to alternative RNA splicing to yield TRα-1 and c-erbAα-2 mRNAs and their variants, which are identical from the NH_2-termini to a point near the carboxy-termini. Since this region encodes amino acids that are critical for TH binding, c-erbAα-2 and its variants do not bind TH. The end result is that TRβ-2, TRβ-1, and TRα-1 are "true" TRs by virtue of their abilities to bind DNA and T_3, and to initiate transactivation. C-erbAα-2 and its variants manifest weak DNA binding, dependent on phosphorylation of their carboxy-terminal tail, absent T_3 binding and failure to transactivate. Instead, they may counteract the effects of the active TRs.

N-terminal (A/B)-region variants, respectively. TRα-1, TRβ-1, and TRβ-2 are authentic TRs by virtue of their abilities to bind TH and transactivate TREs. In contrast, c-erbAα-2 and related variants, which have different carboxy-terminal sequences, do not bind TH, cannot transactivate TREs, and may serve as antagonists of TH action, probably depending on their phosphorylation state *(5,6,10,14)*

The differential function of the TR isoforms in gene transcription has been shown but not extensively documented *(15–17)*. The A/B region of the TR isoforms differs, and each probably contains a constitutive activation function-1 (AF-1) transactivation domain *(18–19)*. Mutations in helices 3, 4, 5, and 12 result in receptors that bind DNA and TH, and which dimerize appropriately, but which fail to activate TREs *(20)*. These regions of the TR LBD interact with coactivators that enhance ligand-dependent transcriptional activity (*see* below). The TR isoforms are expressed in a tissue-specific manner, so that TRα-l, TRβ-1, and c-erbAα-2 mRNAs are widely distributed but variably present. TRα-1 mRNA is expressed in skeletal and cardiac muscle and in brown fat, whereas TRβ-1 mRNA is predominant in liver, kidney, and brain. c-erbAα-2 mRNA is most prevalent in brain and testis. In contrast, TRβ-2 mRNA is most readily detected in the anterior pituitary gland, and less so in the hypothalamus and other tissues *(13,21–23)*.

The TRs are also expressed in a development stage-specific fashion, and are subject to regulation by hormones and other factors *(5,6,10)*. For instance, TRα-1 mRNA is expressed

at an early stage whereas TRβ-1 mRNA is expressed later in the brain during embryonic development *(24)*. In the pituitary gland, TH decreases TRβ-1, TRα-l, and c-erbAα-2 mRNAs without much effect on TRβ-2 mRNA. However, in most other tissues, TH decreases TRα-1 and c-erbAα-2 mRNA but not TRβ-1 mRNA *(25)*.

The generation of laboratory animals with isoform-specific knockouts of each of the TR isoforms has produced distinct phenotypes (*see* "Transgenic and Knockout Models Involving TRs" following). However, it is not currently known whether tissue-specific and development stage-specific expression of the TR isoforms or gene-specific regulation by TR isoforms accounts for these differences in phenotype. Additionally, TRβ isoform-specific ligands have been developed, and may be useful in distinguishing TR-isoform-specific pathways *(26)*.

THYROID HORMONE-RESPONSE ELEMENTS

The DNA-binding targets of the TRs have been recently identified and characterized among TH-responsive genes *(27)*. Insofar as there is considerably more information on the molecular mechanisms involved in the positive than in the negative regulation of gene transcription by TH, the remainder of this chapter will focus on this positive regulation. However, it should be emphasized that negative TH-response elements (nTREs) also play critical roles in TH physiology. For example, the rat growth hormone gene promoter contains a classic positive TRE; in contrast, the rat thyrotropin, β-subunit gene promoter contains a putative nTRE with an apparent TRE half-site *(28)*.

The characteristic feature of TREs that mediate the stimulation of gene expression is a TR-binding half-site with a consensus hexamer sequence of AGGTCA, usually arranged in two or more repeats. Hence, like the steroid-hormone receptors, TRs bind as dimers to TREs. TREs in their simplest forms are represented by direct or inverted repeats, with specific spacer lengths, or in more complex arrangements in natural settings (Fig. 3). For instance, a direct repeat with a spacer of four nucleotides (DR + 4) *(29,30)*, an inverted palindrome with a spacer of six nucleotides (IP + 6) *(31,32)*, and a palindrome without an intervening base (TREpal0) *(33)* are each functional positive response elements. Of importance is that the flanking and spacing sequences all have critical effects on TRE activity *(34)*. In addition, the precise sequence of the half-site may be important, especially since the consensus sequence is seldom observed in natural TREs. These variations are highlighted in recent studies by Spindler *(35)* and Koenig *(36,37)*, which indicate that decamer and octamer TR binding sites, respectively, may be optimal. Indeed, Koenig and coworkers have shown that a single TAAGGTCA site may be sufficient to mediate a TH-mediated transcriptional response *(38)*. Of the 20–30 known positive TREs, most are direct repeats, with the next most frequent being inverted palindromes and then palindrome. The palindrome TRE is exceedingly rare among known TH-regulated genes. As a further point, TREs were originally thought to be enhancers that function largely independently of position or orientation. However, recent data indicate that specific TREs are active only in the appropriate basal or minimal promoter context *(39)*.

Recently, complementary (cDNA) microarrays have been used to study the TH regulation of hepatic genes in mice. Fifty-five genes, of which 45 were not previously known to be TH responsive, were identified *(39a)*. Surprisingly, 41 of these genes were negatively regulated, whereas most of the previously known target genes were positively regulated. These studies showed that TH affected gene expression in a wide range of cellular pathways and functions, including gluconeogenesis, lipogenesis, insulin signaling, adenylate cyclase signaling, cell proliferation, and apoptosis. With completion of the sequencing of the human genome,

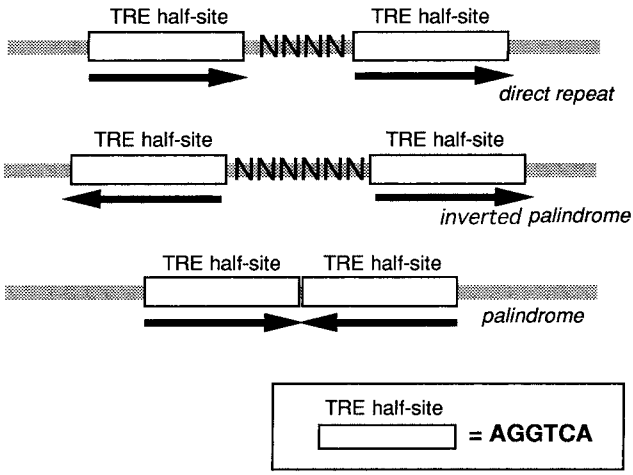

Fig. 3. Thyroid hormone-response elements (TREs). TREs that mediate positive responses to TH have several forms. The basic component of a TRE is a half-site with a consensus sequence, AGGTCA (its asymmetric sequence is indicated by a bold arrow), that is generally arranged in pairs, either as direct or inverted repeats or palindromic sequences, as shown. For the direct repeat, optimum spacing of the half-sites is four nucleotides (NNNN); for the inverted palindrome, optimal spacing is six nucleotides (NNN-NNN); and for the palindrome, 0 nucleotides.

a combination of microarray technology and the promoter sequence database will allow the identification of putative TREs and study of the transcriptional regulation of a large number of new target genes.

TR/PROTEIN COMPLEXES ON TREs

In vitro DNA binding studies initially indicated that TRs became bound to TREs as either monomers or homodimers, with TRβ having a greater propensity than TRα to form the latter on a number of response elements *(40)*. However, Murray and Towle, and Burnside et al. *(41,42)*, then showed that TRs interact with other nuclear proteins to form heterodimers or heteromultimers on TREs, with enhanced binding properties (Fig. 4). These factors were called TR auxiliary proteins (TRAPs) *(43)*. Soon thereafter, it was established that the majority of TRAPs are represented by the isoforms of the retinoid X receptors (RXRs) *(44–49)* (α, β, and γ) *(50–52)*, each with a different tissue distribution. However, other nuclear proteins, such as the retinoic acid receptors (RAR), vitamin D receptor (VDR), peroxisomal proliferator activated receptors (PPARs), and COUP-TFI/TFII were shown to interact with the TRs and exert regulatory functions. RXRs can activate certain elements either as a homodimer or as a heterodimer with RARs, VDR, and TRs (6), and can also bind to their own specific RXR ligand, 9-*cis*-retinoic acid *(53,54)*. Hence, because the RXRs are nearly ubiquitous, it is likely that TR action, at least in part, requires the interaction of TRs with these nuclear factors. In addition, data suggest that the TR:RXR heterodimer binds with a specific orientation on DR + 4, so that the TR is bound to the downstream or 3′ TRE half-site *(55–57)*. This polarity of heterodimer binding may have important implications for mechanisms of TH-mediated transactivation.

Although it is not certain which TR complex (monomer, homodimer, or heterodimer) is the primary mediator of TH action, evidence has been provided in support of the TR:RXR heterodimer as an important player in this role. First, the TR/RXR heterodimer binds intrinsically with greater affinity to most TREs than do other TR complexes. In addition,

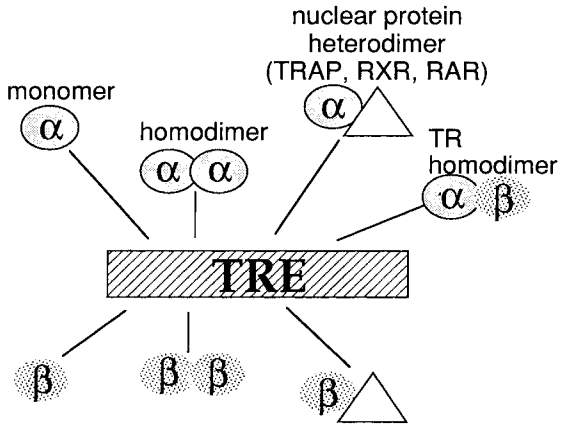

Fig. 4. Formation of TR complexes on a TRE. This diagram illustrates the many combinations of TR monomer, homodimer, and heterodimer that may form on a TRE. α, TRα-1; β, TRβ-1 or TRβ-2.

TH, in physiological concentrations, can decrease the amount of TR homodimer bound to certain TREs while yet leaving the heterodimer intact. Second, transfection studies with animal-cell cultures *(58,59)* and yeast *(60)* show that TR and RXR can synergize the effect of TH. Additionally, Lee et al. *(61)*, using an in vitro transcription system, have shown that TR and RXR also exhibit this cooperative behavior. Heterodimerization of TR with RXR requires the ninth heptad hydrophobic repeat in the LBD of the TRs. It is noteworthy that whereas TR:RXR heterodimers generally initiate a positive response to TH, they may also exert a negative effect *(58,62)*. Also, TR:RXR binding to a TRE may initiate these effects by bending DNA *(63)*. Thus, the DNA target, the bound TR monomers and dimers, and the presence or absence of ligand are all likely to participate in altering the overall conformation of the bioactive TR/TRE complexes to produce activation of the basal transcriptional machinery *(64)*.

Lastly, TR/TRE complex formation is involved in the syndrome of resistance to TH (RTH). RTH is a condition that is inherited as an autosomal dominant disorder. The affected individuals are generally clinically euthyroid and have thyroid function tests that show increased circulating concentrations of free TH with concomitant, inappropriately normal or increased levels of TSH. There is no evidence of a pituitary tumor. The mutations in TRβ are ones that generally reduce the affinity of the TR for TH. A defective TR acts in a dominant negative manner to abrogate TH-regulated transcription; dimerization and DNA binding of the mutant TR is required for this abnormal activity, suggesting that the mechanism of action involves the formation of inactive heterodimers or homodimers on TREs, which actively compete with wild-type complexes *(65,66)*.

EFFECT OF LIGAND ON TR COMPLEX FORMATION

Both TH binding of TR, and posttranslational modifications, particularly phosphorylation, greatly influence the conformation of the receptor *(67,68)* and dictate the nature of the TR complexes bound to TREs. Multiple TR complexes can potentially form on different TREs. As indicated, TR monomer, homodimer, and heterodimer binding may occur with the relative amounts of each complex depending on a number of factors, including the TR and RXR isoforms, TRE orientation and sequence, and the presence of other putative interacting TRAPs *(6)*. Also, experiments done to examine TR dimerization in solution

show that TR monomer and heterodimer, but not the homodimer, are observed off DNA. The homodimer is then observed in the presence of the TRE, and the amount of heterodimer is augmented *(69)*.

It has been shown that unliganded TRs, in various complex forms, can bind efficiently to target DNAs *(6)*. However, using an electrophoretic mobility shift assay (EMSA), it has been shown that TH, in physiologic concentrations, can decrease the binding of TR homodimer, but not of the monomer or heterodimer, to either DR + 4 or IP + 6 *(70,71)*. Other efforts have confirmed this initial finding, but have found that the TREpal0 did not permit this ligand effect *(72–74)*. Further, this ligand effect is probably a result of rapid dissociation of the TR homodimer from DNA ($t_{1/2}$ < 2 min) *(69)*. This result has possible implications for the mechanism by which unliganded TR relieves the repression of transcription, and suggests that the unliganded TR homodimer may serve a silencing role that is relieved by the addition of TH. In addition, the inability of TH to reduce mutant TRβ (those mutants that bind TH poorly) homodimer binding to TREs, and the inherently increased binding of the mutant TRβ homodimer, may partly explain the ability of the mutant TRβ to mediate its dominant negative effect in RTH *(66)*.

EFFECT OF TR PHOSPHORYLATION ON TR COMPLEX FORMATION

Phosphorylation has been shown to play a role in steroid-hormone action *(75)*. However, such posttranslational modification has not been shown convincingly to participate in the action of TH. Early work on the in vitro phosphorylation of chicken TRα showed that the A/B region of the receptor is modified but on a Ser residue that is not conserved among different species *(76)*. In addition, data from Swierczynski et al. *(77)* have demonstrated the ability of serine–threonine kinase inhibitors to blunt TH regulation of a number of genes in chicken liver. Also, inhibitors of both cellular kinases and phosphatases were used to show that TH-regulated transfected (CV-1 fibroblasts) or endogenous (GH_3 somatolactotropes) genes depend on the cellular phosphorylation state *(78)*.

Phosphoamino acid analyses of in vitro-phosphorylated, bacterially expressed rat TRβ, done with HeLa cytoplasmic extracts, show the presence of modified Ser, Thr, and, rarely, Tyr residues. Most notably, phosphorylated TR exhibited increased binding as a homodimer, but not as either a heterodimer or monomer, to TREs *(79)*. Recent work by Cheng and coworkers *(80)* has confirmed this homodimer effect, although it also shows an augmentation of the heterodimer binding. It is not yet clear whether the phosphorylation of TR is the critical step in the modulation of TH action, or whether RXRs or other proteins are also important targets. In any case, such coregulatory events, if they do occur, are likely to permit crosstalk to participate in activation of the receptor.

A CURRENT MODEL OF BASAL AND ACTIVATED TRANSCRIPTION

Facilitation of the binding and activity of RNA polymerase II (pol II) to the promoter of expressed eucaryotic genes in the basal state requires the assembly of at least 20 proteins. Recent data from the molecular cloning of several constituent components, and detailed biochemical and functional studies using reconstituted in vitro transcription systems, have provided a glimpse into the nature of the transcription-initiation complex *(81)*. The first step in this process is the binding of TFIID to the TATA box, or alternatively to the Inr, of the promoter. TFIID is composed of a TATA-binding protein (TBP) and TBP-associated factors (TAFs). TFIIB is then recruited, with pol II and TFIIF binding following shortly thereafter. The pol II/D/B/F complex constitutes the minimal initiation complex. TFIIA may participate

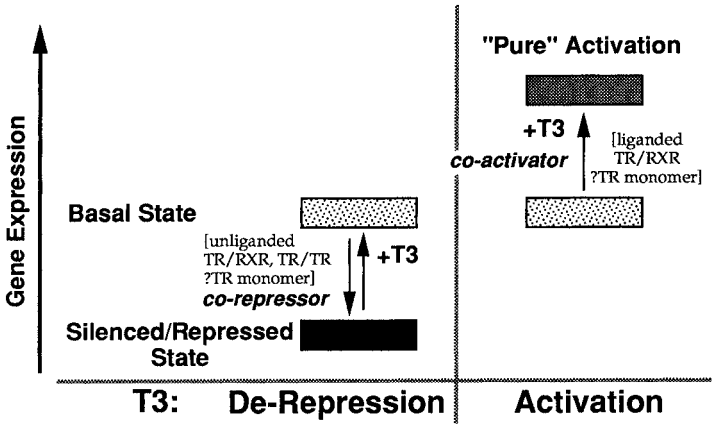

Fig. 5. States of TH action; silencing and transactivation on a positive TRE. This schematic diagram illustrates the two major features of TH action on a positive TRE. In the absence of TR and T_3, a TRE initiates basal-level gene expression (basal state). The addition of TR in the absence of T_3 results in silencing of the gene (silenced/repressed state). The addition of T_3 results in the phase of gene activation called transactivation or "pure" activation.

at this stage and maintain the minimal complex under physiological conditions, but is not required for the activity of the promoter. The addition of TFIIE and TFIIH results in a complete initiation complex. Although the details of the precise protein : protein interactions remain to be defined, it is clear that the pol II/TBP/B/F(E/H) initiation complex is sufficient to ensure basal activity of the promoter in vitro.

It is important to note that whereas TBP alone can support basal promoter activity, the presence of TAFs is critical for expression of the activated promoter by a number of enhancer-binding proteins, such as Spl, CTF, and Gal4-VP16. To date, eight TAFs have been identified (250-, l50-, 110-, 80-, 60-, 40-, 30α-, and 30β-kDa proteins); several are required to reconstitute the activity of the activator proteins in vitro. Hence, these coactivators or adaptors have been postulated to connect the information inherent in an enhancer-binding protein interacting with its target DNA with the basal transcriptional machinery *(82–84)*. For example, some research shows direct contact of Spl with TAF110 *(85)*, and of VP16 with TAF40 *(86)*, each being necessary for activation. Note that VP16 also "touches" TFIIB and TBP *(87)*, so that multiple contacts may be important for the full function of activator proteins. In addition, the required activator–TAF interactions may occur after activator–TFIIB interactions. Hence, Tjian and Maniatis *(88)* suggest that TAFs may play multiple roles in transcription, including initiation, promoter clearance, elongation, and termination. Alternatively, Kornberg and coworkers *(89)* have recently identified a non-TBP-associated factor complex in yeast, which they termed "mediator," and which may also be an important participant in activator-regulated transcription. Finally, there is increasing evidence of repressors that act in these systems, an example of which is the TBP-binding repressor DR1 *(90)*.

The major challenge in studies of the molecular mechanisms of action of TH is to determine the components and events involved in the activation of a gene by TH. Figure 5 illustrates the key steps in the stimulation of gene expression mediated by TR, positive TRE, and TH. As noted, TR is located in the nucleus, even in the absence of ligand *(5,6,10)*. Noteworthy is the ability of the unliganded TR to silence positive target gene activity

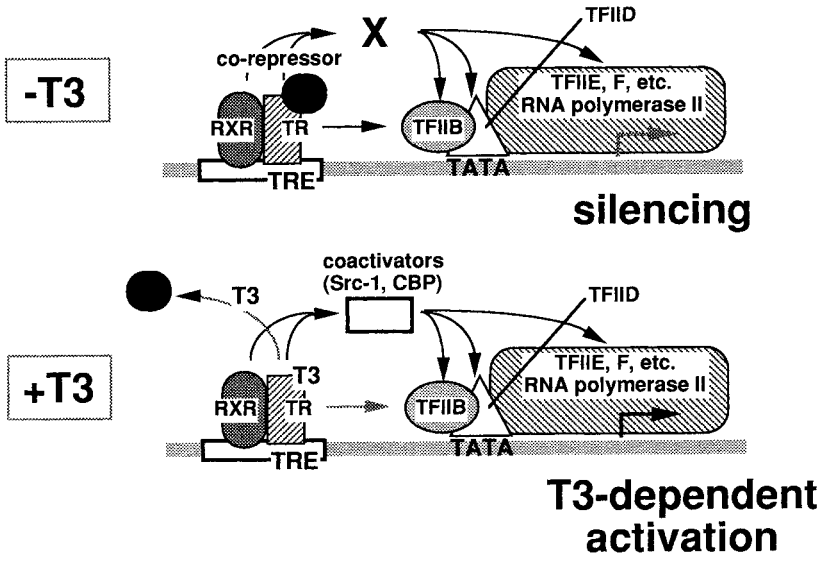

Fig. 6. Molecular mechanisms of TH action. This diagram illustrates the factors involved in TH action on a TRE in the absence ($-T_3$ and presence ($+T_3$) of TH. In the $-T_3$ state, TR:RXR heterodimer is bound to a TRE that is, in turn, associated with a corepressor. The presence of the corepressor in this configuration results in silencing of the associated gene. The direct interaction of unliganded TR with the basal transcription factor, TFIIB, may also participate in this silencing function. The addition of T_3 results in dissociation of the corepressor and subsequent association with putative coactivators such as SRC-1 and CBP to result in activation of the basal transcription machinery (TFIIB, TFIID, TFIIE and F, RNA polymerase II, etc.) and stimulation of the associated gene by T_3.

(6,91–94). The binding of unliganded TR to a positive TRE results in decreased gene expression or silencing. This is depicted as a transition from a basal to a lower activity state. TH then "desilences" or derepresses the gene in step one, and further activates ("pure activation") the gene in step two.

Recent work utilizing a two-hybrid cDNA screening technique to identify proteins that interact with TRs and other nuclear proteins has identified a set of proteins known collectively as TR-associated corepressors (TRACs), which bind TR in the absence of ligand. NCoR (270 kDa) *(95)*, SMRT (170 kDa) *(96)*, and the related TRIP-13 *(97)* have been so isolated and characterized as TRACs. NCoR contains two amino-terminal repressor regions and a carboxy-terminal TR-interacting domain. This latter domain binds to the so called "NCoR box" sequence located in the hinge region of TR. The TRACs, in turn, recruit other nuclear factors, such as sin3 and histone deacetylases (HDACs), which may enzymatically modify chromatin to maintain it in its repressed or silenced state. TR binding of TH facilitates release of the TRACs (an event that may require an intact AF-2 region) and the binding of additional nuclear proteins. TRs with mutations in the NCoR box fail to silence TRE activity in the absence of TH, but are fully capable of mediating TH-regulated pure activation, providing evidence for the separable silencing and activation functions of TR. The unliganded TR has also been shown to interact with the basal transcription factor TFIIB *(98,99)*, which has suggested a role of this contact in silencing. However, it is not clear whether this interaction is required or physiologically relevant (Fig. 6).

In step 2 of the action of TH on a positive TRE, TH binding to TR initiates the binding of additional nuclear factors called coactivators. Earlier studies of viral and hormone regulatory

systems have suggested the existence of such factors to "bridge" or "adapt" the transcription factor complexes formed on TREs to the basal transcriptional machinery. For instance, titration of a putative coactivator with the LBD of TR could abrogate the TH-induced transactivation of a TRE-containing reporter gene but not affect basal gene activity *(100)*. Again, using the two-hybrid cDNA screening technique and other techniques, several groups have identified the first putative coactivator for the nuclear receptor (NR) family known as steroid receptor coactivator-1 or SRC-1 *(101,102)*. It is a ubiquitous, 160-kDa protein that possesses NR interaction (LXXLL motifs) and other protein-binding domains, and synergizes the actions of TRs and other nuclear receptors. Recent work reveals that SRC-1 and its homologs, such as TIF2/GRIP1 and p/CIP/TRAM-1/AIB1/ACTR/RAC, interact largely with the C-terminal activation function-2 (AF-2) domain of NRs in a ligand-dependent manner. This large complex—also called an enhanceosome *(102a)*—has also been found to contain general coactivators such as cAMP response element binding (CREB) protein, or CBP *(103,104)*, and p/CAF *(104a)*, both of which have histone acetyltransferase activities that serve to modify chromatin to derepress its gene-regulation function; TRBP/ASC2/Rap250/NRC/PRIP *(104b)*; and CoAA *(104c)*. In addition, another complex, called DRIP/TRAP/ARC/SMCC, may interact with the liganded TR and other NRs, possibly serving to engage the RNA polymerase holoenzyme for increased transcriptional activity *(2,3,104a)*. Additionally, it is likely that further coactivators for TR, as has been seen with estrogen receptor *(104a,105)* and other NRs, will be identified, with tissue-specific expression, and may therefore permit selective action of TR in different tissues.

TRANSGENIC AND KNOCKOUT MODELS INVOLVING TRs

Recently, a number of genetically-engineered mouse models have provided information on the mechanisms of RTH and the isoform-specific functions of TRs in vivo. Among these models are those for the transgenic expression of dominant-negative mutant TRs, tissue-specific expression of dominant-negative mutant TRs, targeted gene inactivation or knockout (KO) of TR isoforms, and insertion or "knock-in" of mutant TRβ into the native TRβ gene locus.

Wong et al. first generated transgenic mice that overexpressed a natural human mutant TRβ (PV) that had a frame-shift mutation. The mice exhibited increased serum levels of T_3, inappropriately normal TSH, behavioral abnormalities, decreased fertility, and decreased weight *(106)*. These findings recapitulated some of the major clinical features observed in RTH patients harboring this mutation. Tissue-specific promoters have been used to introduce dominant-negative mutant TRs into the pituitary and heart, and adenovirus vectors have been used to express mutant TRs in liver *(107,108)*. These mice exhibited RTH in a specific target tissue but maintained TH sensitivity in other tissues.

Two lines of TRα KO mouse have been generated that display different phenotypes *(109,110)*. This difference is probably due to the different sites in the TRα gene locus used for homologous recombination to generate the KO mice. The TRα gene is complex, in that it encodes TRα-1, c-erbAα-2 (which cannot bind T_3), and rev-erbA (generated from the opposite strand encoding TRα) *(111,112)*. KO mice in which expression of both TRα-1 and *c-erb*Aα-2 mRNA was abolished (TRα -/-) had a more severe RTH phenotype, with hypothyroidism, intestinal malformation, growth retardation, and death shortly after weaning *(109)*. T_3 injection prevented the early death of pups. KO mice that lacked only TRα-1 (TRα-1-/-) had a milder phenotype, with decreased body temperature and prolonged QT intervals on electrocardiograms *(110)*. Samarut and coworkers have reported generation

of short TRα isoforms from internal translation start sites, which have dominant negative activity on TR function *(111)*, and it is likely that these short TRα isoforms are responsible for the more severe RTH phenotype of TRα -/- mice. In this connection, TRα KO mice that did not express either TRα-1 or c-erbA α2 (TRα o/o) had a milder phenotype than TRα -/- mice that expressed only the short TRα isoforms *(113,114)*. Interestingly, TH stimulation of some target genes was increased, perhaps due to the absence of c-erbAα2, which inhibits normal TR-mediated transcription *(115)*.

TRβ KO mice (TRβ -/-) have been generated and showed increased levels of TSH and T_4, thyroid hyperplasia, and hearing defects *(114)*. These findings involving the hypothalamic/pituitary/thyroid (HPT) axis resemble those found in RTH patients *(116)*. Moreover, deafness was observed in the index cases of RTH that had deletion of TRβ *(116)*, and in some RTH patients with mutant TRs. TRβ-2-selective KO mice also have been generated, and exhibited increased levels of TH and TSH, suggesting that TRβ-2 plays the major role in regulating TSH *(117)*. TRβ-2-selective knockout mice also have abnormal color discrimination, and suggest that TRβ-2 may be involved in cone development in the retina *(118)*.

The relatively mild phenotypes of the TRα-1 and TRβ KO mice suggest that the two isoforms have redundant roles in the transcriptional regulation of many target genes. In order to test for the role of TRs in general, both TR isoforms were abolished, and surprisingly, the resultant double-KO mice (TRα-1-/- TRβ -/-) were viable *(119)*. These mice had markedly increased levels of T_4, T_3, and TSH, as well as large goiters. They also showed decreased growth, fertility, and levels of heart rate, as well as diminished bone density and development, features consistent with hypothyroidism. These findings thus demonstrate that the total absence of TRs is not incompatible with life in mice. It is possible that a certain amount of "leaky" transcription of target genes, or functional redundancy by other nuclear receptors or transcription factors, may partly compensate for the loss of TRs.

Recently, Cheng et al. generated a knock-in mouse model in which a mutant TRβ (PV) was introduced into the endogenous TRβ gene locus *(120)*. These mice have a phenotype similar to that of patients with RTH, in that the heterozygous mice show increased concentrations of T_4 and TSH, mild goiter, hypercholesterolemia, impaired weight gain, and abnormal bone development. Homozygous mice had markedly increased serum levels of T_4 and TSH, and a much more severe phenotype. Wondisford et al. also have generated a knock-in mouse expressing mutant TRβ *(121)*. These mice had abnormal cerebellar development and function, and learning deficits. Both of these studies suggest that expression of mutant TRβ under the control of endogenous TRβ promoter recapitulated many of the clinical features of RTH syndrome in mice.

RESISTANCE TO THYROID HORMONE

RTH is a condition that in almost all cases is inherited as an autosomal dominant disorder *(116,122)*. Affected individuals are generally clinically euthyroid and have thyroid function tests that show increased circulating levels of free TH with concomitant, inappropriately normal or increased levels of TSH. There is no evidence of a pituitary tumor. Patients with RTH often have mutations in three different regions of the TRβ LBD ("hot spots"), which generally reduce the affinity of the TR for TH *(112,116)*. The mutant TR acts in a dominant negative manner by blocking TH-regulated transcription of target genes. Dimerization and DNA binding of the mutant TR are required for this abnormal activity, suggesting that the mechanism of action involves the formation of inactive heterodimers or homodimers on TREs that actively compete with wild-type complexes *(123)*. Recently, several studies

have shown that TRβs containing mutations in the AF-2 region of the LBD have dominant negative activity *(124,125)*. These mutants typically have normal T3-binding affinity but cannot interact with coactivators. Thus, mutant TRs that cannot interact with coactivators also can cause RTH. Additionally, mutant TRs that have defective release from corepressors in the presence of TH have strong dominant negative activity *(126–128)*. In general, decreased T_3 binding affinity has correlated with defective corepressor release, although a few exceptions have also been described. Taken together, these findings suggest that transcriptionally inactive TRs, whether they are bound to corepressor or are incapable of binding coactivator, are able to mediate dominant negative TR activity.

Several patients with clinical features of RTH have not shown mutations in TRβ or TRα *(129)*. It is possible that mutations in cofactors, or their inappropriate expression, may cause the RTH phenotype observed in these patients. In this connection, Weiss et al. have shown that loss of a coactivator, SRC-1, can lead to mild RTH in mice *(130)*. Another possible mechanism for RTH without mutations in TRβ or TRα could be posttranscriptional regulation of TRs in some patients *(131)*.

Several somatic mutations in TR have also been described in tumors. Somatic mutations in TRα and TRβ from a human hepatoma cell line have been described *(132)*. It is not known whether these mutant TRs contributed to oncogenesis, although both exhibited dominant negative activity on wild-type TR in cotransfection studies. Recently, a somatic mutation in TRβ and an aberrantly spliced TRβ have been identified in TSH-secreting adenomas *(131,133)*. These aberrant TRs have dominant negative activity, and defective negative regulation of TSHβ and glycoprotein hormone α-subunit transcription.

SUMMARY

The molecular mechanisms of the nuclear action of TH have become clearer in the past decade. In particular, the nature of the TRs and their heterodimeric partners, the target DNAs (TREs), and corepressors and coactivators has been elucidated. Further, the biochemistry of the interplay of these components on the TRE has been studied. The results are summarized in Figs. 5 and 6. What is now required is a deeper understanding of the interactions of the unliganded and liganded TR : RXR with corepressor/coactivator complexes, respectively, and of the linkage of these components with the basal transcriptional machinery. It is expected that this new knowledge will spring from ongoing studies utilizing techniques of structural biology and in vitro transcriptional systems. Ultimately, such information should provide insight into the mechanisms of disease caused by abnormal levels of circulating TH, and into the actions of TH agonists and TH antagonists in pathologic and physiologic states.

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2 Nongenomic Actions of Thyroid Hormone

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CONTENTS

INTRODUCTION

Nonnuclear or nongenomic cellular actions of thyroid hormone (TH) are those that are independent of intranuclear liganding of the hormone and traditional nuclear thyroid-hormone receptors (TRs) *(1,2)*. There are a number of such actions (Fig. 1), at least in part because they involve various organelles, specialized functions of the plasma membrane, and biochemical events in cytoplasm. Further, TH can nongenomically activate signal-transduction pathways in the cell that culminate in phosphorylation of certain nucleoproteins *(3,4)*. Recognition of the existence of nongenomic actions of TH has provided a complex picture of the roles of TH in the cell beyond critical functions in regulating gene expression *(5,6)*.

Nongenomic actions of TH have several functional qualities that distinguish them from nucleus-mediated actions of iodothyronines. Such qualities may include structure–activity relationships of the hormone—e.g., the dominant activities of L-thyroxine (T_4) *(7)* or 3,3′,5′-triiodo-L-thyronine (rT_3) *(8)* in several nongenomic actions over that of 3,3′,5-triiodo-L-thyronine (T_3), or, in some cases, onsets of action that are apparent in seconds *(9,10)* or minutes *(11)*. The signal-transduction pathways on which TH acts nongenomically, such as the mitogen-activated protein kinase (MAPK) cascade *(3,4,12)* and cyclic adenosine monophosphate (cAMP)-dependent protein kinase (PKA) *(3,13)* or inositol phosphate pathways *(14)*, have not been implicated in genomic actions of the hormone. Additionally, the calmodulin–Ca^+ complex can play a role in certain nongenomic effects of T_4 and T_3 *(15,17)*.

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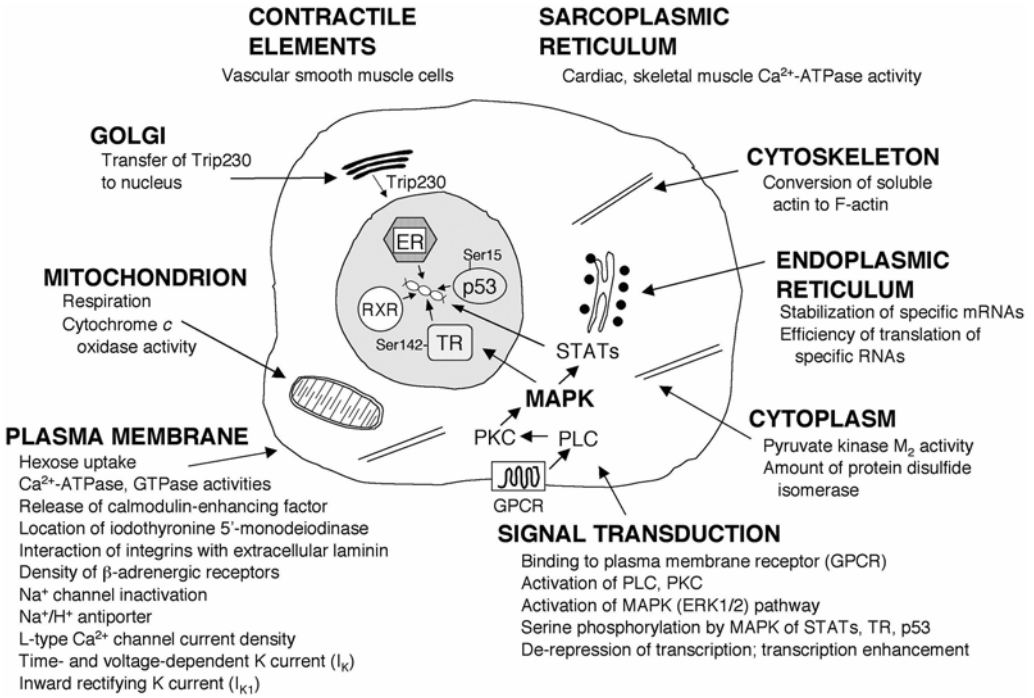

Fig. 1. Nongenomic actions of TH classified by site in an idealized cell. See text for specific cells in which actions have been described. The amount of plasma-membrane iodothyronine-5′-monodeiodinase activity and integrin–laminin interaction, as well as the distribution of protein disulfide isomerase between cytoplasm and endoplasmic reticulum, reflect the action of TH on the cytoskeleton. TH regulates the solubility of actin. The action of T_4 on cellular signal transduction is initiated at a cell-surface G protein-coupled receptor (GPCR), leading to activation of the MAPK (ERK1/2) pathway and serine phosphorylation of the nuclear thyroid-hormone receptor TRβ-1 (TR) and p53. In the nucleus of T_4-treated cells, the proteins TR, RXR, p53, and estrogen receptor (ER) are coimmunoprecipitated with activated MAPK (Lin H-Y, Davis PJ and Davis FB, unpublished observations), forming an "enhanceosome" that may then bind to DNA.

Certain nonnuclear actions of TH appear to be homeostatic. That is, hormonal action on plasma membrane ion pumps or channels *(18)*, on the compartmentalization of ions within the cell *(19,20)*, or on the rate of transport of nonionic solutes, such as glucose *(21)*, may contribute to the intracellular content of specific ions or glucose. The modulation of ion-channel or pump activities involves a large number of factors, and any role of the iodothyronines in such modulation is likely to be modest. Interestingly, nongenomic actions of TH can interface with genomic effects of the hormone. For example, enhancement of the antiviral activity of interferon-γ by TH is achieved genomically and nongenomically *(22)*, and MAPK, nongenomically activated by T_4, can serine-phosphorylate TRβ-1, the nuclear receptor for T_3 *(3)*. The hormone can also modify by an apparently nonnuclear mechanism the half-lives of specific messenger RNAs (mRNAs) *(23)*, the transcription of whose genes may be affected by iodothyronines. The abundance of certain cell-surface adrenergic receptors may be regulated acutely and nongenomically by TH *(24)* or by nucleus-dependent processes *(25)*. The plasma-membrane content of iodothyronine-5′-monodeiodinase is regulated nongenomically in astrocytes by T_4 and rT_3 *(26)* through a mechanism that involves a change in the solubility of actin *(8)*.

The physiologic significance of nongenomic effects of TH observed at the cellular level in most examples remains to be determined in the intact organ or organism. Recent observations by Schmidt et al. *(27)*, however, have substantiated acute cardiovascular effects of T_3 in human subjects, including effects on myocardial contractility and on vasomotor tone. These effects may be manifestations of hormone actions on cell-membrane ion channels (Na^+-channel inactivation) *(9,10)*, on sarcoplasmic-reticulum (SR) ion-pump activity (Ca^{2+}-adenosine triphosphatase [Ca^{2+}–ATPase]) *(16,19)*, or on contractile elements in vascular smooth muscle *(28)*. The principal cardiovascular actions of TH nonetheless are seen to be genomic in mechanism *(29,30)*. There are several other nonnuclear cellular effects of the hormone whose systemic roles appear to be of interest. Among such effects are the action of 3,3'-diiodothyronine (T_2) on cytochrome *c* oxidase activity in mitochondria *(31,32)*, an effect that may contribute to modulation by TH of cell and organ respiration, and which raises the possibility that deiodination of T_3 may result in a biologically important analogue. The effect of T_3 and T_4 on synaptosomal Ca^{2+} uptake *(33)* may provide insight into the effects of hypothyroidism on central nervous system (CNS) function.

The cellular or molecular mechanisms of most nongenomic actions of iodothyronines are incompletely understood, and specific extranuclear binding sites ("receptors") that are relevant to these actions have not yet been isolated or cloned for a number of such actions. However, evidence has been presented for the existence of a plasma-membrane receptor site for TH that is linked to the MAPK pathway *(3,4)*. This putative site has characteristics of a G protein-coupled receptor that preferentially binds T_4 and tetraiodothyroacetic acid *(3,12,34)*. A mechanism by which TH regulates the activity of cytoplasmic pyruvate kinase M_2 has been defined *(35)* and involves direct interaction of the hormone with an enzyme subunit. The relevant hormone-binding site *(36)*, probably a signal-transducing protein kinase *(37,38)*, and the role of the calmodulin–Ca^{2+} complex *(15)*, have been described for the stimulation by TH of human erythrocyte Ca^{2+}–ATPase activity.

EXPERIMENTAL DEFINITION OF NONGENOMIC ACTIONS OF THYROID HORMONE

Secure definition of nongenomic effects of TH requires exclusion of the participation of TRs in hormonal action. Strategies for such exclusion include the experimental: (1) use of enucleated cell models, notably the human mature red cell *(7,15)*; (2) use of cell lines in which the nuclear TR is not present *(12,34)*; (3) use of cellular systems in which inhibition of protein synthesis *(22)* or of gene transcription *(34)* is achieved; (4) use of model systems whose rapid time courses of hormone action (seconds or a few minutes) preclude mediation by the cell nucleus *(9,10)*; and *(5)* use of cell organelles. Examples of the last that have been used to study hormone action are mitochondria *(39)*, synaptosomes *(33,40)*, cytosol *(35,37)*, and SR *(20)*. Because certain nongenomic actions of TH require several hours to be manifested *(41)*, a time course consistent with a nucleus-mediated effect does not eliminate the possibility of a nongenomic contribution of TH to a cellular event.

It is desirable in models of the nongenomic action of TH to link a particular effect—such as prolonged inactivation of the myocardial Na^+ channel *(9,10)* or stimulation of red-cell Ca^{2+}–ATPase activity *(15)*—to relevant physiologic events, such as, respectively, changes in myocardial Ca^{2+} content (via Na^+–Ca^{2+} exchange) or myocardial contractility, or to red-cell Ca^{2+} content *(18)* or Ca^{2+} efflux *(42)*. It is also desirable to identify relevant hormone-specific binding sites in the system in which a nongenomic event has been described. We have also excluded a genomic contribution in certain of our own studies by using TH analogs, such as

tetrac, that have virtually no intrinsic biologic activity at TRs, but are capable of inhibiting putative nongenomic actions of the hormone *(3,12,22)*.

SPECIFIC NONGENOMIC ACTIONS OF THYROID HORMONE

Actions of Thyroid Hormone at the Cell Membrane

Functions of the plasma membrane include solute transport and regulation of selective permeability (ion channels), reception of primary messenger molecules at specific receptors, signal transduction, presentation of molecules with metabolic roles (such as enzymes) at the cell surface, and interaction of the cell surface with molecules in the extracellular matrix (ECM). These latter molecules are structural/adhesion substances of the interstitium that are important determinants of cell–cell relationships *(43)*.

At the outset, it should be noted that the cell membrane contains several energy-dependent transport systems for TH *(44,45)*. These systems are a mechanism for cellular uptake of iodothyronines, and are linked to the transport of specific amino acids *(44,46)* and to Na^+–H^+ exchange *(47)*. The hormone-uptake systems themselves are not considered in this review as sites of action of TH. It would be of interest if these transport/uptake systems were initial participants in specific intracellular nongenomic actions of TH, but no such association has yet been established.

Action of Thyroid Hormone on the Distribution of Specific Cell-Surface Proteins

The extrathyroidal ("peripheral") conversion of T_4 to T_3 is accomplished by deiodinases. Iodothyronine-5'-monodeiodinase Type II (5′-DII) is located in the plasma membrane of astrocytes. The abundance of this enzyme in the cell membrane has been shown by Siegrist-Kaiser and coworkers to be under the control of T_4 and rT_3 *(8)*. The nongenomic mechanism involved, as reported by Farwell et al. in the same laboratory, is the regulation by TH of actin polymerization and thus of the amount of F-actin stress fibers in the microflament network that are available to interact with the plasma membrane *(26)*. Unique features of this model are that T_3 is ineffective, and that rT_3, usually seen to have little or no biologic activity, is equipotent to T_3 in causing actin polymerization *(8)* and the transfer of 5′-DII to endosomes *(26)*. The molecular basis of this action of TH on actin is not yet known. The possibility that calmodulin is involved in the mechanism merits examination because of the importance of the calmodulin–Ca^{2+} complex to the interaction of F-actin with actin-associated proteins *(48)*, and because calmodulin is implicated in three other nongenomic, membrane-associated actions of TH: (1) hexose uptake; (2) Ca^{2+}-ATPase activity; and (3) the release of a calmodulin-enhancing factor (*see* next subheading).

Of particular interest is that the effect of T_4 on actin also regulates the interaction of glial cells with laminin in ECM *(49)*. Laminin is the principal component of ECM in the developing brain, and is bound to astrocytes by integrins. Integrins are plasma membrane-spanning molecules stabilized in the plasma membrane by actin microflaments *(43)*. It is possible that TH acts via this route to influence neuronal migration, synapse formation, and morphogenesis *(40)*. It is not yet known whether control of actin polymerization by iodothyronines occurs in cells other than glial cells, nor is it understood whether T_4 and rT_3 control the cell-surface presentation of proteins other than 5′-DII.

It has recently been observed that the density of beta-adrenergic receptors (BAR) on the surface of chick embryonic cardiac myocytes can be increased nongenomically by T_3 *(24)*. The change is small (10–15%) and is followed by a nucleus-dependent response that increases BAR density by 40%. It is not known whether rT_3 is effective in the nongenomic

component of this process; T_4 was shown to affect BAR density, albeit less well than T_3. The nonnuclear TH response is inhibited by colchicine *(24)*, indicating that the mechanism of the hormone-induced change in surface BAR density requires an intact microtubule system. The possibility exists that the acute BAR response to T_3 might contribute, via an enhanced response to endogenous beta-agonists, to the improvement in cardiac output that can be observed clinically in humans with administration of T_3.

From the above observations it is clear that TH nongenomically influences the function of both microflament (actin) and microtubule networks that are important to the function of cell-surface proteins. TH may also affect cell-membrane adenylate cyclase activity; this signal-transduction action is linked to solute (glucose) transport, and is described in the next section.

Action of Thyroid Hormone on Plasma-Membrane Transport Functions

The myocardial Na^+ channel is a sarcolemmal feature essential to development of the cell action potential. It also regulates Na^+–Ca^{2+} exchange by gating Na^+ entry into myocardiocytes. Huang et al. *(9)* have shown in neonatal rat myocardial cells that inactivation of the Na^+ channel (i.e., the duration of opening of the channel after depolarization) is prolonged within seconds after application of T_3. This prolongs the action potential of these cells and, by promoting cellular uptake of Na^+, may activate the Na^+–Ca^{2+} exchanger, increase intracellular Ca^{2+} content, and cause an inotropic effect. Huang et al. have also described the structure–activity relationships of TH analogues on the Na^+ channel *(9)*. Acute inotropic effects of TH have been reported in human subjects *(27)*. The nongenomic effect of T_3 on rabbit cardiac Na^+ channels has been confirmed by Dudley and Baumgarten *(10)*. These investigators have shown that the hormonal effect must occur at or near the extracellular face of the Na^+ channel, since application of T_3 to the cytoplasmic face of sarcolemmal patches was ineffective. It should be emphasized that a variety of factors in addition to TH, including second-messenger pathways *(10)*, can influence channel gating.

It should also be noted that TH can acutely enhance the action of isoproterenol on peak L-type Ca^{2+} channel current density *(50)*. Such an alteration may promote Ca^{2+} entry into the cell and enhance Ca^{2+}-induced Ca^{2+} release from SR stores. Interactions of effects of adrenergic agonists and nongenomic effects of TH action are also described below in regard to hexose uptake and to Ca^{2+}–ATPase activity.

Action-potential duration (APD) is prolonged in ventricular myocytes obtained from hypothyroid rats *(51)*. Incubation of hypothyroid cardiomyocytes with 100 n*M* T_3 normalized APD. No hormonal effect on APD was observed when ventricular cells from euthyroid animals were exposed to TH. The action of TH can be viewed as a contribution to the basal APD. Sun has interpreted the mechanism of hormone action to be an effect on the time- and voltage-dependent potassium current (I_K), an important determinant of repolarization *(51)*.

The myocardial inward rectifying potassium current (I_{K1}) maintains the resting membrane potential of ventricular myocytes. I_{K1} is acutely increased by T_3 (1 n*M*–1 μ*M*), as shown by Sakaguchi et al. *(52)*. In studies they conducted on guinea pig ventriculocytes, these investigators reported onset of the effect at 5–15 min and a maximum effect at 25 min. This effect may contribute to the prolongation of APD caused by TH. It is not known whether this action of the hormone contributes to cardiac arrhythmias in the clinical setting of hyperthyroidism; these arrhythmias are largely atrial, rather than ventricular, in origin.

It should also be noted that T_3 has recently been shown to activate one class of voltage-regulated potassium channel by a wortmannin-inhibitable mechanism *(53)*. This is the first description of a nongenomic action of TH that is apparently mediated by a

phosphatidylinositol-3′-kinase-dependent signal-transduction pathway. Rac, a Rho-guanosine-triphosphatase (GTPase), is also implicated in this potassium-channel effect of TH *(53)*.

Another membrane-transport system nongenomically affected by TH is the Na^+/H^+ exchanger or antiporter. Experiments done on a rat skeletal myoblast cell line by Incerpi et al. *(54)* showed that T_3 or T_4 in nanomolar concentrations shortened the time of recovery of these cells from an acid load. Propylthiouracil blocked the action of T_4 in this model system, indicating that T_3 in fact mediates the effect on the antiporter. Protein kinase C (PKC) is involved in this action of TH, as is MAPK[1] (S. Incerpi, personal communication). In studies not involving iodothyronines, other laboratories have shown that MAPK modulates activity of the Na^+/H^+ exchanger *(55)*.

Glucose transporters (GLUTs) are a family of membrane-spanning proteins that facilitate glucose uptake by cells. The abundance of GLUT1 in a liver-derived cell line (ARL 15) has been shown by Weinstein and Haber *(56)* to be genomically regulated by TH. However, the stimulation by T_3 of glucose uptake (transporter activity) in this model cannot be fully explained by the increased abundance of GLUT1 protein induced by T_3. Thus, it is postulated that iodothyronines nongenomically activate preexisting GLUT1 molecules in the cell membrane *(57)*.

A series of studies by Segal and coworkers has substantiated a nongenomic effect of iodothyronines on 2-deoxy-D-glucose uptake in rat myocardium *(58)* and in thymocytes *(21)*. T_3 is more effective than T_4 in the glucose-uptake models *(21)*. Enhancement of glucose uptake by up to 20% above basal levels is achieved with nanomolar concentrations of T_3 *(59)*. As described in the thymocyte, the action of TH involves an increase in calcium uptake ($^{45}Ca^{2+}$ accumulation) and in the cytoplasmic free-calcium concentration within 15–30 s after exposure of intact cells to T_3 (10^{-9} *M*) *(60)*. A subsequent increase in thymocyte plasma-membrane adenylate cyclase activity occurs at 1–2 min *(61)*. In the absence of extracellular Ca^{2+}, T_3 has no effect on glucose uptake, and cyclic adenosine monophosphate (cAMP) is required for hormone action. Further, the effect of TH is inhibited by β-adrenergic antagonists, specifically, β_1 antagonists *(62)*, but not α-adrenergic antagonists. The Ca^{2+}-binding protein calmodulin is also implicated in this model *(17)*.

This plasma-membrane effect of TH on glucose transport thus involves signal transduction (involvement of cAMP, calmodulin–Ca^{2+}), and can be modified by specific adrenergic receptors. The binding site ("receptor") for TH in this model has not been described. Adenylate cyclase activity may also be increased nongenomically by iodothyronines in cat myocardial membranes *(63)* (*see* "Additional Membrane Actions of Thyroid Hormone" following).

The action of TH on membrane Ca^{2+}-ATPase (calcium pump) activity has been documented by several laboratories in erythrocytes *(7,15,64)*, thymocytes *(65)*, and cardiac *(16,19)* and skeletal muscle *(20)*. This enzyme is a factor that maintains the erythrocyte intracellular calcium concentration ($[Ca^{2+}]_i$) at less than 10^{-6} *M (66)*. In the human red cell, the enhancement of Ca^{2+}–ATPase activity by physiologic concentrations of TH has been associated with increased $^{45}Ca^{2+}$ efflux *(42)* and decreased $[Ca^{2+}]_i$ *(18)*. Several characteristics of the membrane-binding site ("receptor") for the hormone that are relevant to Ca^{2+}–ATPase activity have been described *(36,67)*. The structure–activity relationships for hormone analogues in the erythrocyte model have been extensively studied *(7)*; T_4 is more active than T_3, rT_3 and D-analogues are without effect, and 3,5-dimethyl-3′-isopropyl-L-thyronine (DIMIT) is active. Tetraiodothyroacetic acid (tetrac) is inactive, but blocks the action of T_4 *(3,12,36)*.

The calmodulin–Ca^{2+} complex is the principal intracellular regulator of Ca^{2+}–ATPase activity *(68)*. The TH effect on Ca^{2+}–ATPase requires the presence of calmodulin *(15)*, and pharmacologic antagonists of calmodulin inhibit the stimulation of the enzyme by TH *(15,16)*. One of the products of the phosphoinositide (PI) signal-transduction pathway, D-myoinositol-1,4,5-trisphosphate ($InsP_3$), has been shown to inhibit the stimulation by TH of red-cell Ca^{2+}–ATPase activity *(14)*. Two mechanisms of this inhibition of hormone activity are known: $InsP_3$ inhibits T_4- and T_3-binding to erythrocyte membranes *(14)*, and $InsP_3$ interferes with the binding of calmodulin to Ca^{2+}–ATPase *in situ* in the red-cell membrane *(69)*. Thus, factors that activate the PI pathway can modify this nongenomic action of iodothyronines. Further, the presence of α_1-adrenergic receptors on red-cell membranes has been shown, and α_1-adrenergic agonists can inhibit the action of T_4 on red-cell Ca^{2+}–ATPase activity *(70)*.

The mechanism by which T_4 stimulates the activity of Ca^{2+}–ATPase is believed to depend on PKC. PKC has been shown to activate Ca^{2+}–ATPase *(38)*, and we have described the stimulation of red-cell PKC activity nongenomically by T_4 *(37)*. We have also shown non-nucleus-mediated enhancement of PKC activity by T_4 in HeLa cells *(13)*. Red-cell membrane Ca^{2+}–ATPase activity has been shown to be increased in hyperthyroidism and decreased in hypothyroidism *(71)*. This is thought primarily to be a genomic effect. However, stable circulating levels of TH in eumetabolic subjects may contribute nongenomically to the set-point of enzyme activity. Selected plasma-membrane actions of TH are summarized in Table 1.

Action of Thyroid Hormone on Synaptosomes

Bellabarba et al. have described T_3-binding sites in synaptosomes of developing rat brain *(40)*. Recently, this group has shown that these hormone-binding sites are linked to G protein and GTPase activity in synaptosomes *(72)*. These observations raise the possibility that via this nongenomic mechanism, TH may modify neurotransmission. Studies of the model that involve neurotransmitter and Ca^{2+} release/reuptake in the presence and absence of iodothyronines are required to define the possible physiologic role of TH in synaptosomes.

Effects of Thyroid Hormone on Signal-Transduction Pathways

Acting at the plasma membrane, iodothyronines can stimulate the MAPK (ERK1/2) signal-transduction cascade in several human and animal cell lines *(3,4,12)*. That the action is initiated at the cell membrane is supported by observations that agarose-T_4 is as effective as T_4, that pertussis toxin blocks the hormonal effect, and that tetrac, a hormone analogue known to block binding of T_4 to the plasma membrane *(36)*, inhibits activation by T_4 of MAPK *(3,12)*. T_3 is less effective than T_4 in this model of hormone action *(3)*. We have shown that upstream of MAPK itself, inhibition at the individual steps of MAPK kinase (MEK), Raf-1, Ras, PKC or phospholipase C (PLC) serves to block action of iodothyronines on the MAPK pathway *(3,4,12)* (Fig. 2).

The significance of this nongenomic effect of TH has been explored by examining possible substrates of MAPK. It has been shown that TH-directed MAPK phosphorylates specific serines in a number of important cellular proteins. For example, T_4-activated MAPK phosphorylates serine-727 (Ser-727) of the signal-transducer and activator of transcription (STAT)-1α *(12)*. This effect potentiates the signal generated at the cell surface by interferon (IFN)-γ—tyrosine phosphorylation of STAT-1α at Tyr-701 *(73)*—and results in significant enhancement by TH of the antiviral *(12)* and immunomodulatory *(34)* effects of IFN-γ in

Table 1
Selected Plasma Membrane Actions of Thyroid Hormone

Action	*Cell*	*Result*	*Mechanism of action*	*Analogue*	*Ref. no.*
Na^+/H^+ antiporter stimulation	Rat myoblasts	Increased intracellular pH 5–10 min	PKC	$T_3 > T_4$	*54*
Sodium current (I_{Na})	Neonatal rat ventricular myocytes	Increased peak I_{Na}; slight prolongation of I_{Na}, inactivation 5 min	PKC; independent of β-blockade	$T_3 = T_4 = 3,5\text{-}T_2 = DIT$; rT_3 blocks T_3, T_4 action	*9*
Inward rectifier potassium current (I_{K1})	Guinea pig ventricular myocytes	Increased open probability of channel, shortened action potential, 5–25 min	?G protein	T_3, triac	*52*
Action-potential duration	Hypothyroid rat ventricular myocytes	Shortened action potential	?G protein	$T_3 > T_4$	*51*
Ca^{2+}–ATPase activity	Human erythrocytes, rabbit myocardial sarcolemma	Increased calcium pump activity	Calmodulin, PKC	$T_4 = T_3$	*7,15,19,37*

PKC, protein kinase C.

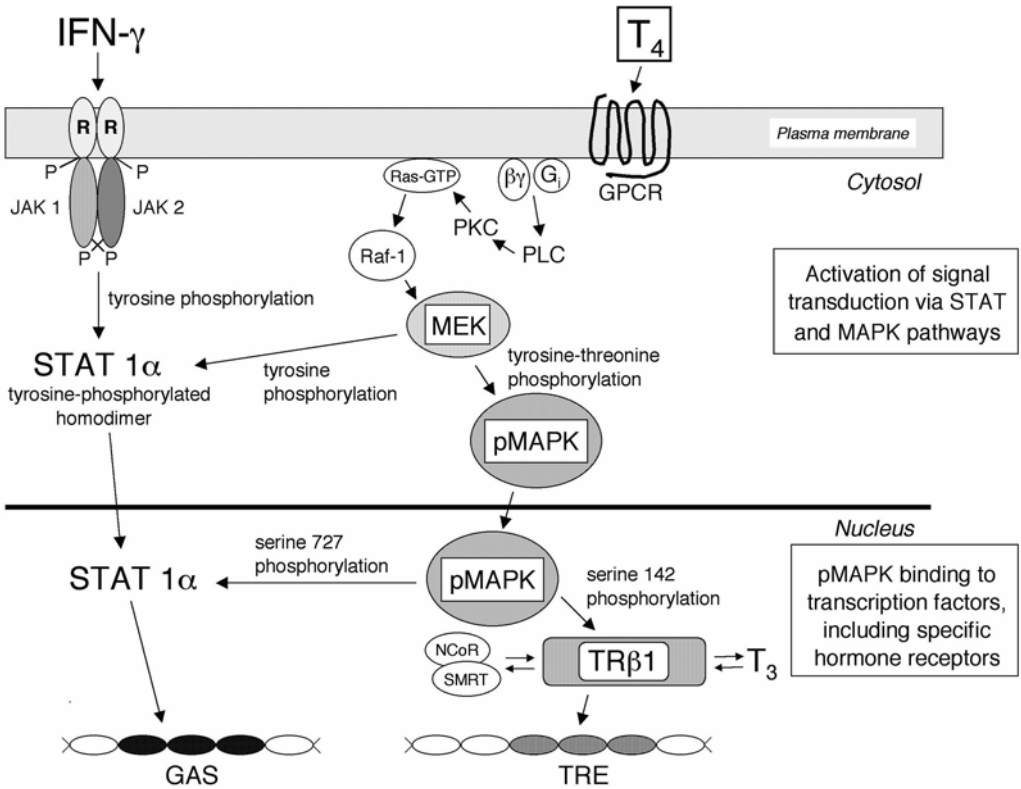

Fig. 2. Signal-transduction pathways by which T_4 affects specific nuclear proteins. T_4 interacts with a plasma-membrane G-protein-coupled receptor (GPCR) to initiate a protein kinase cascade including activation of PLC, PKC, Ras, Raf-1, and MAPK kinase (MEK). Phosphorylated by MEK, pMAPK translocates to the nucleus, forms an immunoprecipitable complex with TRβ-1, and serine-phosphorylates this nuclear receptor at residue 142. Serine phosphorylation of TRβ-1 is associated with dissociation of the corepressors NCoR and SMRT, previously bound to the unphosphorylated receptor. This T_4-directed MAPK action is thought to be sufficient to permit binding of receptor to thyroid-hormone response elements (TREs), but it is thought that substantial transcriptional activity results only in the presence of T_3. Cells treated with IFN-γ respond with phosphorylation and dimerization of the IFN-γ receptor (R), tyrosine phosphorylation of Janus kinases (JAK) 1 and 2, and tyrosine phosphorylation of STAT-1α. This protein dimerizes, translocates to the nucleus, and binds to IFN-γ-activated sequences (GAS) on DNA. In the presence of T_4, STAT-1α is serine-phosphorylated on residue 727 by pMAPK, enhancing by up to 100-fold the effect of submaximal concentrations of IFN-γ. Even in the absence of IFN-γ, T_4 also causes tyrosine phosphorylation of STAT-1α by MEK, but this hormone action alone is not sufficient to cause increased expression of antiviral or other IFN-γ-responsive proteins.

human cell lines. T_4-activated MAPK phosphorylates Ser-15 of the transcription factor, p53, an oncogene-suppressor protein *(4)*. This alters the transcriptional activity of p53, inferred from its modulation of expression of an immediate-early gene, c-*Jun (4)*. MAPK activated by T_4 also phosphorylates Ser-142 of TRβ-1, the nuclear receptor for T_3 *(74)*. The significance of this phosphorylation step is that dissociation of TR from the corepressor proteins, NCoR (nuclear corepressor) and SMRT (silencing mediator of retinoid and thyroid hormone receptors) takes place with T_4 treatment *(3)*.

Unactivated MAPK resides in cytoplasm *(75)*. When activated by T_4 at the cell surface, MAPK translocates to the nucleus and phosphorylates the substrates mentioned above. Interestingly, TH-activated MAPK is recovered from nuclear fractions of treated cells in

a coimmunoprecipitated complex that includes the substrate proteins as well as retinoid X receptor (RXR) (Fig. 1), a member of the nuclear superfamily of hormone receptors that heterodimerizes with TR *(76)*. In cells not exposed to physiologic concentrations of T_4 in vitro, no such association of nucleoproteins occurs, and activated MAPK is not found in the nucleus. Such nucleoprotein complexes have been described in signal-transduction models as "enhanceosomes" *(77,78)* when transcriptional activity of a specific transactivator is increased by protein–protein interactions. In the cellular models we describe, the increased activity of STAT-1α complexed with MAPK, as promoted by T_4 in interferon-treated cells *(12,34)*, would qualify as an enhanceosome. Undetermined in such a complex is whether inclusion of factors such as TR influence the behavior of STAT-1α, or whether the latter, in the presence of T_4, may modulate the activity of TR. It is thought that such associations of nucleoproteins enhance the ability of transactivators to bind to DNA *(77)*.

The ability of TH to activate PKC *(37)* and MAPK may provide a mechanism by which the hormone can change the activity of certain membrane ion channels and pumps, as suggested above. It is known, for example, that PKC can modulate the activity of the plasma membrane Na^+/H^+ exchanger *(79)*, Na^+ channel *(80)*, and Ca^{2+}–ATPase *(38)*, and that MAPK may modulate the activity of the Na^+/H^+ antiporter *(81)*.

Additional Plasma-Membrane Actions of Thyroid Hormone

Stimulation of myocardial adenylate cyclase by TH in a membrane (particulate) fraction was described more than 25 yr ago by Levey and Epstein *(63)*. They showed that T_4 (5×10^{-6} *M*) increased cAMP accumulation in this system by 20–45% within 3 min of exposure of membranes to the hormone. RT_3 was active, but less so than T_4, and the action of T_3 was equivalent to that of T_4. The dependence of the model on greater than physiologic levels of TH, and the apparent responsiveness of the cyclase to D-T_4, make this report difficult to interpret.

T_4 promotes the release from human erythrocyte membranes of a soluble, heat-stable calmodulin-enhancing factor that binds to calmodulin and increases by sixfold the ability of native calmodulin to stimulate 3′,5′-cyclic nucleotide phosphodiesterase activity *(82)*. This enhancing factor resembles a "calmodulin activator" reported to occur in an animal model of hypertension *(83)*. The possibility that such a factor might relate to the actions of TH on the cytoskeleton, and that this might involve participation of the calmodulin–Ca^{2+} complex, is attractive, but has not yet been studied.

Action of Thyroid Hormone on the Cytoskeleton

Regulation of Actin Polymerization by Iodothyronines

The regulation by TH of actin polymerization in astrocytes is intimately related to the effects of the hormone on specific plasma-membrane proteins (e.g., iodothyronine-5′-monodeiodinase *(26)* and the interaction of membrane integrin molecules with laminin in the ECM *[49]*), and has been described above. Another feature of the nongenomic actions of iodothyronine on the cytoskeleton is regulation of the cellular distribution of protein disulfide isomerase *(84)*, an enzyme that is important to the processing (folding) of nascent proteins in the endoplasmic reticulum (ER) (*see* "Actions of Thyroid Hormone at the Endoplasmic Reticulum" following). It is important to determine whether these actions are general or are limited to the specialized cells (astrocytes) in which they have been initially described.

Because of its actions on these structural elements of cells that are linked to the plasma membrane, TH may be speculated to regulate cell shape. This possibility has not yet been explored.

Action of Thyroid Hormone on Sarcoplasmic Reticulum

Stimulation of Ca^{2+}–ATPase Activity of Sarcoplasmic Reticulum

The Ca^{2+}–ATPase of the plasma membrane exports Ca^{2+}; the SR of striated and cardiac muscle also contains a Ca^{2+}–ATPase, which is directed vectorially to import Ca^{2+} into SR *(85)*. These enzyme activities are different structurally and biochemically, but, like the Ca^{2+}–ATPase of the plasma membrane, that of the SR of skeletal muscle *(20)* and of myocardium *(16,19)* is stimulated in vitro by physiologic concentrations of T_4 and T_3. Increased SR uptake of Ca^{2+} enhances muscle relaxation by decreasing the Ca^{2+} concentration in the sarcoplasm around actin–myosin bundles. Enhanced uptake of Ca^{2+} by SR can also promote inotropy by increasing the pool of Ca^{2+} potentially available before muscle excitation. Because hyperthyroidism is associated with a shortened muscle-relaxation time and increased force of contraction, the nongenomic effects of TH on SR Ca^{2+} metabolism may be implicated in these clinically observed actions of the hormone. However, such a speculation should be limited to very acute effects of the hormone on muscle. TH is known to induce the expression of genes for Ca^{2+}–ATPase *(86)*, and such effects probably explain what is observed clinically in terms of skeletal- and cardiac-muscle function. Further, chronic TH treatment in animal models is associated with an increase in Ca^{2+} concentration in sarcoplasm, rather than a decrease *(87)*.

Actions of Thyroid Hormone at the Endoplasmic Reticulum

Actions of the Hormone on Stability of Specific mRNAs

Vandenbrouck et al. have shown that T_3 increases the half-life ($t_{1/2}$) of apolipoprotein AI (apoAI) mRNA by two-to-threefold over that of control mRNA in HepG2 cells not exposed to the hormone *(23)*. Stabilization by TH of specific mRNAs has been reported by other investigators *(88)*, whereas shortening of mRNA $t_{1/2}$ by TH has also been reported *(89)*. Although such changes may be genomic in origin, Puymirat et al. have shown that T_3 stabilizes specific mRNA by a mechanism that is independent of gene transcription and the processing of transcripts, and which requires activation of a serine–threonine kinase-dependent pathway *(90)*. Because we know that TH can nongenomically stimulate such kinase activity (e.g., PKC) *(37)*, the stabilization of mRNAs by T_3 may in some cases work through a nongenomic mechanism.

Possible Action of Thyroid Hormone on Efficiency of Translation of mRNA

T_3 induces apolipoprotein B (apoB) gene expression in rat hepatocytes (HepG2 cells); the hormone enhances this genomic effect by increasing the efficiency of translation of apoB mRNA *(91)*. These several effects of TH are complicated by the ability of T_3 to increase the degradation rate of the apoB protein. Thus, the influence of iodothyronines on abundance of apoB protein is the algebraic sum of three rate effects. Whether the change in efficiency of translation is nongenomic is unclear. However, the ability of the TH to regulate compartmentalization of factors important to the ER, such as protein disulfide isomerase (PDI) *(84)*, by nongenomic mechanisms increases the likelihood that the change in efficiency of translation of mRNA induced by TH does have a nongenomic component. About 25% of astrocyte PDI monomeric subunit (glial-p55) is cytoplasmic, but via a hormone-induced mechanism related to actin polymerization, cytoplasmic glial-p55 redistributes to the particulate fraction (ER) and becomes associated with actin *(84)*. PDI is catalytic in the synthesis of disulfide-bond-containing proteins, and is also a component of a triacylglycerol transfer protein (MTP) that facilitates insertion of lipids into nascent lipoproteins within the ER *(92)*. Such effects could increase the efficiency of protein synthesis.

Actions of Thyroid Hormone in Cytoplasm

Regulation of Pyruvate Kinase Activity by T_3

TH is bound by several cytosolic proteins *(93–95)*. The functions of these proteins and of the hormone–protein interaction are largely undefined. However, Ashizawa et al. have shown that T_3 binds reversibly to cytoplasmic pyruvate kinase M2 (PKM_2) monomer (p58), and that this hormone–protein interaction prevents the association of monomers into the enzymatically active PKM_2 tetramer *(35)*. The hormone does not bind to tetrameric PK. Fructose-l,6-diphosphate promotes tetramer formation, thereby activating the kinase and stimulating glycolysis. The affinity of PKM_2 monomer for T_4 is about 50% that of T_3, and rT_3-binding is negligible. These findings were originally made in a human epidermoid carcinoma-cell line, but the interaction of T_3 with human erythrocyte PK has also been reported *(96)*. Thus, this cytoplasmic glycolytic enzyme system is a definitive example of nongenomic regulation by TH of enzyme activity. To the extent that T_3 diminishes PK activity, the hormone limits ATP generation by the glycolytic pathway and the availability of pyruvate to other cellular pathways, such as the citric-acid cycle.

Regulation of Access of Thyroid Hormone to the Cell Nucleus by Cytosolic Proteins

We have shown that cytosolic binding proteins for iodothyronines can restrict nuclear uptake of T_3 and T_4 *(93)*. We do not know whether these hormone–protein complexes maintain a steady-state intracellular pool of unbound TH whose availability is determined by stable off/on kinetics, or whether the binding of TH might be varied by interaction of intracellular factors with cytosolic binding proteins that would adjust the intracellular free hormone concentration. However, a cytosolic thyroid hormone-binding protein (CTBP) described in rat kidney by Hashizume et al. can facilitate nuclear uptake of T_3 in the presence of nicotinamide adenine dinucleotide phosphate (NADP) *(95)* and in association with the formation of a T_3-CTBP–(NADP) complex. The T_3–CTBP–(NADPH) complex is not associated with nuclear uptake of the hormone, and may serve to stabilize the cytoplasmic pool of T_3. The NADP : NADPH ratio thus appears to be a regulator of nuclear uptake of T_3. The activity of the pentose-phosphate shunt, source of the NADPH, may by this mechanism modulate the availability of TH to the nucleus.

Action of Thyroid Hormone on Cytosolic Protein Kinase Activities

PKC activity in rabbit mature erythrocyte cytosol is stimulated in vitro by physiologic concentrations of TH *(37)*. T_4 is more active than T_3 in this model. When these observations were made, only diacylglycerol (DAG) and DAG-like compounds (phorbol esters) were recognized as stimulators of PKC. Other laboratories have confirmed that TH stimulates PKC *(97)*. It has been reported that PLC activity is stimulated by iodothyronines *(12)*, an effect by which PKC activity could be enhanced via elaboration of DAG. 1,25-Dihydroxyvitamin D3 *(98)* and a variety of phospholipids have been shown to modulate PKC activity. PKC is a serine–threonine kinase *(99)*. As noted, Puymirat, Etongue-Mayer, and Dussault have recently shown that T_3 stimulates cytosolic serine–threonine kinase activity in neuroblastoma cells, and that such activity is involved in stabilizing acetylcholinesterase mRNA *(90)*. It is not yet clear whether this kinase activity is indeed PKC.

We have shown that TH potentiates the antiviral activity of homologous recombinant IFN-γ in HeLa cells by more than 100-fold *(41)*. This action of the hormone has both genomic and nongenomic components *(22)*. The nongenomic component represents a recently recognized postnuclear pathway that is independent of protein synthesis and that involves the action of

two signal-transduction enzymes, PKC and PKA *(13)*. That is, inhibition of either PKC or PKA activity prevents postnuclear-pathway enhancement by TH of IFN-γ action. In addition, the potentiating effect of TH in the IFN model can be wholly reproduced by the addition of both phorbol ester (to stimulate PKC activity) and 8-Br-cAMP (to stimulate PKA), but not by the addition of only one of these agents *(13)*. To our knowledge, this is the first observation of the reproduction of an action of TH by components of a signal-transduction pathway and supports the concept that nongenomic actions of TH can involve such pathways.

Because the IFN–HeLa cell model described above has a genomic component that is activated by rT_3 *(22)*, it has been possible to show, using sequential submaximal concentrations of rT_3 and T_4 in a time paradigm that allows separation of nucleus-dependent and postnuclear effects of thyroid hormone, that the nongenomic action of the hormone can potentiate its genomic effect *(22)*. The molecular mechanism by which TH stimulates PKC and PKA activities in the IFN-γ system is not yet known. We believe that the physiologic relevance of the system is potentiation, by circulating levels of T_4, of the early cytokine response in host defense.

It should also be noted that TH can enhance the immunomodulatory activity of IFN-γ, i.e., HLA-DR antigen expression *(100)*. This action of TH, however, appears to involve both nongenomic (signal transduction) and genomic mechanisms.

Actions of Thyroid Hormone on Mitochondria

Stimulation of Respiration by Thyroid Hormone

The stimulatory action of TH on cell and tissue respiration has been widely acknowledged. Respiration in isolated mitochondria has been shown to be enhanced by TH *(39,101)*. There are a number of mechanisms by which iodothyronines could stimulate mitochondrial respiration. These include: (1) enhanced activity of ADP–ATP (adenine nucleotide) translocase (AdNT), promoting the uptake of ADP; (2) stimulation of the tricarboxylic acid (TCA) cycle, producing intermediates that are sources of electrons; (3) promotion of phosphate uptake; (4) stimulation of F_oF_1–ATPase (ATP synthase), perhaps by dissipation of the H^+ gradient across the inner mitochondrial membrane; and (5) stimulation of the electron-transport chain. The precise site of action of TH on mitochondria has not been firmly established, but a substantial body of evidence is consistent with an action of the hormone on AdNT *(39,102)*.

Of interest is the recent finding that analogs of TH can stimulate the activity of cytochrome *c* oxidase isolated from rat liver *(31)* and bovine heart *(103)* mitochondria. T_3 has no effect on the activity of this enzyme, whereas diiodothyronines (3,3′-T_2, 3,5-T_2) are active. Diiodothyronines are apparently capable of rapidly stimulating mitochondrial respiration *(104,105)*, but do not have an important effect on the whole-organism metabolic rate. A *bona fide* action of iodothyronines on cytochrome *c* oxidase activity is capable of enhancing electron transport. Arnold, Goglia, and Kadenbach *(32)* have recently reported that T_2 binds to subunit Va of the cytochrome *c* oxidase of bovine heart mitochondria, and as a consequence reduces ATP binding by subunit IV of the oxidase. ATP binding promotes an allosteric change in the oxidase that inhibits respiration, so that the cellular affect of T_2 binding is the stimulation of mitochondrial respiration.

Thyroid Hormone Action on Contractile Elements of Cells

Ojaama, Klemperer, and Klein have shown that application of T_3 to isolated rat aortic vascular smooth-muscle cells (VSMCs) causes relaxation of the cells *(28)*. This occurs sufficiently rapidly to exclude a genomic mechanism. Such an action could contribute importantly to the reduction in peripheral vascular resistance that is associated with

hyperthyroidism or with increased cardiac output when T_3 is administered in the setting of weaning patients from cardiopulmonary bypass *(29)*. The molecular mechanism by which TH can alter VSMC tone is not yet known.

PRINCIPLES OF MECHANISMS OF NONGENOMIC ACTIONS OF THYROID HORMONE

From the foregoing discussion, it is clear that our understanding of the mechanisms of nongenomic actions of iodothyronines is incomplete. However, there are several interesting features of what is known about the highly varied mechanisms so far implicated in the nongenomic actions of these hormones. First, there are examples of the "receptor" as "effector." In the case of PKM_2 monomer, for example, the binding site for T_3 must be linked to an allosteric change in the cytosolic protein that prevents the self-association of monomers into the enzymatically active PKM_2 tetramer. That mitochondrial AdNT itself is a receptor for T_3 is another example of the receptor as effector, albeit open to contention. The stimulation by T_2 of mitochondrial cytochrome *c* oxidase also appears to be of a receptor–effector nature.

Second, the apparent receptors for nongenomic actions of iodothyronines can have features remarkably different from those of nuclear receptors for T_3. The human red-cell receptor for TH that is linked to Ca^{2+}–ATPase activity binds T_4 and T_3 equally well *(67)*; it also binds tetrac in a manner that precludes binding of T_4 and T_3, but that does not lead to the activation of ATPase *(36)*. The mitochondrial cytochrome *c* oxidase binding site recognizes diiodothyronines, but not T_3. The unknown mechanism by which iodothyronines regulate the polymerization of actin is activated by rT_3 and T_4, rather than by T_3.

Third, there are examples in which complex interactions of signal-transduction-pathway components are interposed between putative TH receptors and nongenomic actions of the hormone. The stimulation of the MAPK pathway and its consequences on nucleoprotein activity is a primary example of the action of TH on signal transduction. The effects of the hormone on plasma-membrane hexose uptake and on Ca^{2+}–ATPase activity appear to reflect the action of TH on signal-transducing kinases. We have also suggested, as described earlier, that several of the nonnuclear effects of iodothyronines on plasma-membrane ion channels and on the Na^+/H^+ exchanger may be mediated by hormonal actions on MAPK or PKC.

Fourth, genomic and nongenomic mechanisms may interface in certain models of TH activity. Examples of this are the effects of T_4-directed MAPK on serine phosphorylation of TR, and the observations that TH can affect both expression of the SR Ca^{2+}–ATPase gene and, by a nongenomic effect, the activity of the gene product. Another possible example of interfacing of genomic and nongenomic mechanisms in the activity of TH is the stimulation of transcription of the apoB gene in association with the posttranscriptional stabilization of apoB mRNA.

SUMMARY

A number of nongenomic actions of TH have been substantiated in the past decade. Experimental conditions in which the existence of nongenomic actions of TH is suspected must securely exclude contributions from nuclear TRs. The physiologic importance of nongenomic actions of iodothyronines is largely speculative, but contributions by such actions to the basal activity of certain homeostatic mechanisms, such as ion-channel transport or the activity of the Na^+/H^+ exchanger, appear likely. For most of these actions, relevant

specific hormone receptors and mechanisms remain to be identified. Actions of TH on signal-transduction pathways do offer possible mechanisms for action of the hormone at the plasma membrane and on the activity of nucleoproteins. Serine phosphorylation of TRβ-1 and p53 by T_4-directed MAPK, for example, alters the behavior of these nucleoproteins, and specific serine phosphorylation of STAT-1α by MAPK explains the potentiation by TH of the antiviral effects of IFN-γ on human cells in vitro. It appears likely that nongenomic effects of the hormone contribute to the regulation of intracellular protein trafficking and to the state of the cytoskeleton in certain cells. Recent evidence for binding of T_2 by a subunit of mitochondrial cytochrome *c* oxidase is one explanation for the effect of TH on cellular respiration.

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3 Thyroid Testing

A Clinical Approach

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Contents

INTRODUCTION

Diseases of the thyroid are among the most common endocrine disorders encountered in clinical paractice. They are typically detected and managed on the basis of the results of thyroid-function tests, the diversity and multiplicity of which can be confusing to some clinicians. Ongoing changes and laboratory pitfalls in thyroid-related testing add to this complexity. Moreover, the recent introduction of sensitive laboratory tests has led to the early detection of thyroid disorders and to the detection of mild disorders. For these reasons, expertise is often needed to determine what tests to order and how to interpret them, bearing in mind that results can often be affected by concomitant diseases or medications. Beyond this, cost considerations dictate that physicians be selective and order specific laboratory tests for specific disorders.

This chapter is intended to assist endocrinologists and other physicians in ordering and interpreting thyroid-function tests. The first part of the chapter presents a concise review of thyroid testing, and the second part considers the interpretation and management of thyroid-test abnormalities in symptomatic and asymptomatic patients.

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DIAGNOSTIC TESTS

Biochemical Tests

Thyroid-Stimulating Hormone

The current sensitive thyroid-stimulating hormone (sTSH) assays are a type of immunometric assay (IMA) *(1)*, and have greater sensitivity than the earlier radioimmunoassay (RIA) methods, which were unable to distinguish low from normal values because their sensitivity limit was 0.5–1 mIU/L *(2)*. IMAs have replaced RIA methods for TSH.

The newer IMA technique involves a sandwich-assay configuration in which two anti-TSH antibodies are used, rather than the single antibody used in RIA methods. Usually, one or both of these antibodies are monoclonal and thus have a high degree of specificity for the epitopes of TSH. The first antibody is directed at the specific β-subunit of TSH. It selectively extracts virtually all TSH molecules from the serum sample. This bound TSH is then quantified by a second, labeled anti-TSH antibody, which is usually directed at the α-subunit of the TSH molecule. This sandwich IMA method offers improved assay sensitivity in addition to enhanced specificity, faster turnaround, and a wider working range in comparison with RIA methods *(2)*.

The IMAs for TSH, most of which can be referred to as "sensitive" TSH assays, have been classified according to the concept of assay generation. First-generation assays can measure to 1.0 mIU/L. Each subsequent assay generation can measure to a level 10-fold lower than this. Thus, a second-generation assay can measure to 0.1 mIU/L, and a third-generation assay can measure to 0.01 mIU/L. Thus, in theory, only third-generation assays (immunochemiluminometric assays [ICMAs]) can differentiate mild TSH suppression caused by nonthyroidal illness from the profound TSH suppression (TSH less than 0.01 mIU/L) typically seen in hyperthyroid patients *(3,4)*. However, current ICMA methods have varying sensitivities, and it is recommended that laboratories using ICMAs establish a realistic functional sensitivity limit independent of the manufacturer's claims of "generation" *(5)*. Currently, most clinical laboratories use second-generation assays because the clinical utility of ICMAs for TSH has not been defined.

Indications for a TSH-centered strategy are the following:

1. When assessing primary hypothyroidism or hyperthyroidism during early diagnosis of either condition or during maintenance therapy of hypothyroidism.
2. When persons require thyroid-function evaluation because of (1) a family history of thyroid disease; (2) nonthyroidal autoimmune conditions; (3) hypercholesterolemia; (4) advanced age; or (5) symptoms (such as fatigue or palpitations) for which thyroid disease should be excluded.
3. In monitoring TSH-suppressive therapy in differentiated thyroid cancer.

In a few clinical conditions, measuring the serum level of TSH is not recommended as an initial test because it can be diagnostically misleading. These include the following:

1. Acute psychiatric illness in inpatients. From 2 to 3 wk should elapse after admission before TSH values are determined.
2. Pituitary or hypothalamic disease.
3. During changes in thyroid status in which TSH levels adjust slowly.
4. During the first trimester of pregnancy, because the serum level of TSH may be low owing to the effect of human chorionic gonadotropin (HCG), whereas free thyroxine (free-T_4[FT_4]) is normal.

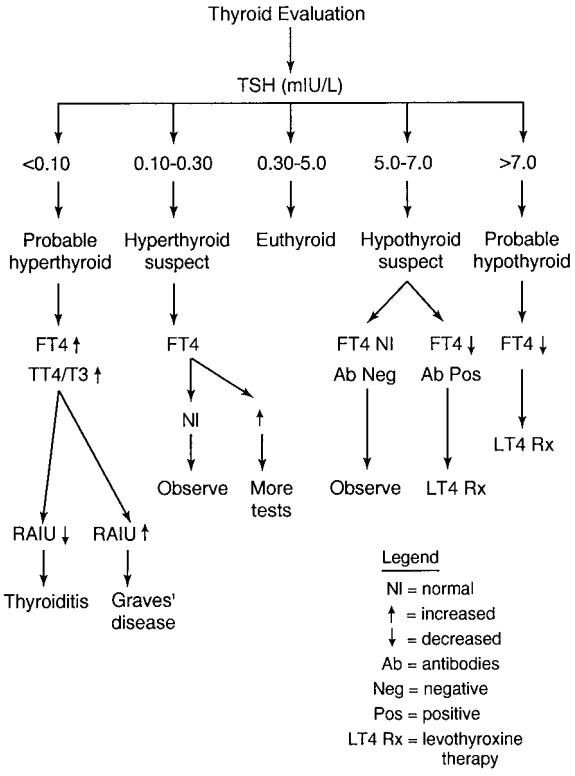

Fig. 1. Suggested algorithm for evaluation and management of thyroid disease when TSH is the initial diagnostic test.

When patients have pituitary or hypothalamic disease or when changes occur in thyroid status, FT_4 estimate is preferred.

In hospitalized patients with intact hypothalamic–pituitary function, a normal level of TSH usually excludes the diagnosis of primary thyroid disease. However, because nonthyroidal illness may cause either TSH values or tests for estimated FT_4 to be diagnostically misleading, a panel approach (TSH plus FT_4) is recommended for sick patients in whom thyroid dysfunction is suspected clinically.

The general approach and subsequent testing recommended on the basis of the initial TSH testing results are illustrated in Fig. 1.

Free Thyroxine

Many approaches have been taken to the measurement of free or active thyroid-hormone (TH) concentrations, mainly because quantitative and qualitative variations in TH-binding proteins can affect the serum level of both total T_4 and triidothyronine (T_3). Although almost all methods represent an improvement over older methods for determining total hormone values, most are limited in some respect. Ekins *(6)* has summarized the methods available for measuring FT_4 and has reviewed the limitations of each method. This has also been reviewed in modified form *(7)*; the reader is referred to these sources for full details on all techniques for measuring free T_4. Two of the most common measurement techniques, dialysis and ultrafiltration, are considered below.

Table 1
Causes of Euthyroid Hyperthyroxinemia

Binding abnormalities
TBG excess
Familial dysalbuminemic hyperthyroxinemia
Transthyretin-associated hyperthyroxinemia
Autoantibody-associated binding abnormalities
Generalized TH resistance
Drug effects
Amiodarone
Oral cholecystographic contrast agents
Propranolol
T_4 administration
Transient acute illness
Hyperemesis gravidarum

Dialysis assays for T_4 can be done by indirect, symmetric, or direct methods, with the following limitations, respectively: radiochemical purity of ^{125}I bound to T_4 or the dilution of competitors for T_4 in the dialysate; on the radiochemical purity of ^{125}I bound to T_4; and on the sensitivity of the assay system caused by antibody affinity or dilution of competitors into the dialysate. Ultrafiltration can be performed by indirect or direct methods, with the following potential limitations, respectively: the radiochemical purity of ^{125}I bound to T_4 and the sensitivity of the assay system limited by antibody affinity *(6)*.

Many clinical laboratories estimate FT_4 by an FT_4 index method that combines a measurement of total T_4 and T_3 resin uptake (T_3RU) to calculate the free-T_4 index (FT_4 index = total $T_4 \times T_3RU$) *(6)*.

The FT_4 estimate is usually indicated in conjunction with measurement of the serum level of TSH in the evaluation of suspected hyperthyroidism or hypothyroidism. Measurements of total T_4 may suffice in place of FT_4 assessments, but the potential effects of abnormalities in T_4-binding protein must be considered.

Total Thyroxine

Since the early 1970s, the total T_4 concentration has been measured in serum with RIA methods. The measurement of total T_4 includes both free and bound T_4. T_4 is bound to albumin, transthyretin (prealbumin), and T_4-binding globulin. Therefore, changes in the various T_4-binding proteins will cause the total T_4 to change secondarily. The serum level of T_4 is usually (but not always) increased in hyperthyroidism and decreased in hypothyroidism. Serum T_4 is unchanged with age *(1–12)*. The disadvantage of total-T_4 determination is that high or low levels of T_4 may occur in euthyroid subjects because of changes in binding proteins, binding of T_4 to antibodies, increases in T_4 binding to albumin, the use of various medications, or nonthyroidal illness *(13–17)* (*see* Table 1 for causes of elevated total T_4 associated with the euthyroid state). The causes for decreased total T_4 associated with the euthyroid state are listed in Table 2.

Free Triiodothyronine

Only a minute fraction of T_3 is free and thus, together with FT_4, is capable of mediating biologic activities *(18)*. Commonly, levels of FT_4 and free T_3 (FT_3) are high in hyperthyroidism, low in hypothyroidism, and normal in euthyroidism. Patients with various nonthyroidal

Table 2
Causes of Euthyroid Hypothyroxinemia

Binding abnormalities, congenital or acquired
Phenytoin therapy
Iodine deficiency
T_3 treatment

illnesses have low levels of FT_3 because of decreased conversion of T_4 to T_3. Direct measurements of absolute FT_4 and FT_3 are technically demanding and expensive. Thus, the FT_4 index and FT_3 index are calculated instead. However, in routine clinical thyroid-function testing, the FT_3 or FT_3 index is seldom used.

Total Triiodothyronine

Total T_3 has usually been measured with RIA methods, although other, related nonisotopic methods are now more commonly used. Total T_3 may be helpful in assessing hyperthyroidism, especially when a patient has T_3 toxicosis, a condition in which T_3 is increased but T_4 is not. In hyperthyroidism, T_3 may increase disproportionately to T_4 through augmented peripheral conversion as well as increased thyroidal secretion. T_3 may decrease with age, but the decrement is less marked in persons selected for extreme health or when nonthyroidal illness is excluded *(19)*. T_3 poses the same problem as total T_4—a high or low total concentration may exist because of alterations in binding proteins.

Thyroglobulin

Thyroglobulin (Tg) is a large (660 kDa) dimeric glycoprotein that is secreted uniquely by thyroid follicular cells. In most clinical situations, thyroglobulin concentrations are determined by three factors:

1. Thyroid cell mass.
2. Physical damage to the thyroid (by biopsy, surgery, hemorrhage, radioactive iodine [RAI], external irradiation, or inflammation), with thyroiditis being the most common clinically encountered effect.
3. Activation of TSH receptors by TSH, hCG, or thyroid-stimulating antibody (TSAb)/thyroid-stimulating immunoglobulin (TSI).

Serum Tg measurement is technically challenging; Tg is measured either with a double-antibody immunometric assay or a single-antibody immunoassay *(20)*. The greatest limitation to these methods is the potential for interference by anti-Tg autoantibodies, which are detected in approximately 10% of normal subjects, 20% of patients with thyroid cancer, and the majority of patients with autoimmune thyroid diseases. In the immunometric assays, the serum Tg concentration can be falsely decreased by autoantibodies, whereas in RIA, anti-Tg autoantibodies cause falsely high values.

Although there has been a trend toward standardization, the variability of results using differing assays remains at about 25%.

The main clinical usefulness of Tg measurements is for following the status of patients with differentiated thyroid carcinoma who have had thyroidectomy and remnant ablation.

Antithyroid Antibodies

The spontaneous development of antibodies to various antigenic components of the thyroid gland is a well-established phenomenon of autoimmune thyroid disease. Several

Table 3
Thyroid Autoantibodies and Their Use

Antibody	*Use*[a]
Anti-TPO	Hashimoto's thyroiditis
	Postpartum thyroiditis
	High-risk pregnancy
	Polyglandular autoimmune disease
TSI	Graves' disease
	Euthyroid ophthalmopathy
	Neonatal hyperthyroidism

[a]See text for details on indications for use.

antithyroid antibodies have been described, but the most clinically useful assays are for antibodies to Tg, the thyroid receptor (TSI), and thyroid peroxidase (TPO) (previously known as the "microsomal antigen") *(21–23)*. The general indications for autoantibody testing are described in Table 3. Antibodies to TPO are usually assayed with a hemagglutination technique *(24–26)*. Currently, it appears that antibodies to TPO are a good reflection of lymphocytic infiltration of the thyroid (i.e., autoimmune thyroiditis) but are not clearly part of the cytotoxic process *(27)*.

Anti-TPO or anti-Tg antibodies occur in more than 90% of patients with autoimmune thyroiditis; consequently, their detection helps in defining the cause of primary hypothyroidism. Other settings in which these assays are clinically useful include the following:

1. Predicting the progression of subclinical hypothyroidism *(28)*.
2. Increasing the suspicion of underlying thyroid disease in hypothyroxinemic patients with nonthyroidal illness.
3. Predicting postpartum thyroiditis *(29)*.
4. Evaluating polyglandular autoimmune syndromes.

TSH receptor-stimulating antibodies were first recognized in 1956 and have had various names and acronyms. Initially they were called "long-acting thyroid stimulator" (LATS), but are now called TSAb or TSI, the term used here.

Although the determination of TSI is not needed in the evaluation of a typical case of Graves' disease (GD), it may be of clinical value in selected situations, including the following:

1. In establishing the diagnosis of GD when an RAI uptake test cannot be performed (e.g., during pregnancy or after recent exposure to iodine), or perhaps in some cases of euthyroid ophthalmopathy *(30)*.
2. In estimating the prognosis of GD, because patients with high TSI titers at the time of initial diagnosis are likely to have persistently detectable TSI and therefore persistent or recurrent disease if the thyroid gland is not ablated *(31)*. Also, assessment of TSI may be useful in determining when to stop antithyroid drug treatment of GD.
3. In forecasting neonatal GD, because this can be predicted on the basis of a high maternal titer of TSI in the third trimester of pregnancy *(32)*.

IMAGING

Thyroid Scintigraphy

Radionuclide scanning uses technetium-99 pertechnetate (^{99m}Tc) or radioactive iodine ^{123}I. Theoretically, iodine is the superior isotope because it is both transported and organified by thyroid follicular cells, whereas technetium is only transported into the cells and not organified. However, technetium scanning is probably used more in the United States because it is less expensive and more convenient.

Radioisotope scanning allows further assessment of thyroid-functional anatomy. Traditionally, radioisotope scanning was the first diagnostic test in the evaluation of thyroid nodular disease, but fine-needle aspiration (FNA) has recently become the recommended initial step in the evaluation *(33–35)*. The rationale for using radionuclide scanning is the clinical observation that malignant thyroid tissue does not organify iodine. Radioisotope scanning divides thyroid nodules into hypofunctioning ("cold"), isofunctioning ("warm"), and hyperfunctioning ("hot") nodules. Functioning nodules are less likely to be malignant. Approximately 1–20% of cold nodules are malignant, as compared with less than 4% of hot nodules *(36)*. Thus, radionuclide scanning is neither very sensitive nor very specific. Although most thyroid cancers are cold on scanning, most cold nodules are benign.

One indication for radionuclide scanning may be to differentiate a homogeneously hyperfunctioning thyroid, as in GD, from a multinodular goiter or toxic adenoma when physical-examination findings are not optimal for doing this.

Ultrasonography

Ultrasonography is a noninvasive, sensitive, radiation-free procedure. High-resolution ultrasonography has exceptional ability to delineate thyroid anatomy and locate small lesions within the gland *(37)*. When thyroid nodularity is indeterminate by palpation, ultrasonography can be used for its delineation. Thyroid scanning defines the functional status of a nodule, and ultrasonography differentiates solid from cystic thyroid lesions by evaluating their echogenicity. Hypoechoic nodules usually represent benign colloid nodules. High-resolution ultrasonography has shown that virtually all cystic thyroid lesions have some solid component and that there is no true "pure" cyst. The presence of calcification has been detected in about 13% of thyroid nodules; peripheral calcification is considered benign, but internal or punctate calcification throughout the nodule is suggestive of papillary carcinoma *(38)*.

Currently, high-resolution ultrasonography is a very sensitive test, but it is not specific for thyroid malignancy. Most radiologists believe that no ultrasonographic criteria reliably differentiate a benign lesion from a malignant one; consequently, the use of ultrasonography in thyroid practice is limited.

RAI Uptake

The thyroid RAI uptake test involves the oral administration of ^{123}I, followed by a 6- and 24-h determination of radioactivity over the gland. The normal range of radioactivity is inversely proportional to dietary iodine intake. Thus, lower values are noted in the United States than in most western European countries or other areas of relative iodine deficiency.

The test is used most often to determine the cause of thyrotoxicosis or to assist in dosimetry for ^{131}I treatment of GD or Plummer's disease. The test is also used in assessing thyroid remnant activity after thyroidectomy for thyroid cancer. In this case, ^{131}I is used.

Thyrotoxicosis with a high uptake of RAI is consistent with GD or toxic nodular thyroid disease, whereas thyrotoxicosis with a low uptake of RAI usually results from inflammatory disease (thyroiditis), exogenous intake of TH, or iodine-induced thyrotoxicosis.

Fine-Needle Aspiration Biopsy

FNA biopsy of the thyroid has been performed as a diagnostic test for more than 50 yr. Its main purpose is to differentiate benign nodules from malignant ones. The FNA biopsy procedure, when performed by an experienced clinician and cytologist, is relatively simple, safe, expedient, cost-effective, and accurate *(39,40)*. The Mayo Clinic experience *(39)* suggests that cytologic findings are satisfactory (diagnostic) in approximately 85% of cases, and that the results are unsatisfactory (nondiagnostic) in the other 15%. When a satisfactory aspirate is obtained, the diagnostic cytologic categories are "benign" in 75% of cases, "suspicious" or "indeterminate" in 20%, and "malignant" in 5%. Approximately 25% of suspicious lesions are found at operation to be malignant *(37,41)*. The overall accuracy of FNA biopsy approaches 95%, the overall sensitivity is 83%, and the overall specificity is 92%, according to a review of the literature *(42)*.

CLINICAL APPLICATIONS OF THYROID-FUNCTION TESTS

The following cases and discussions are intended to demonstrate the clinical utility, interpretation, and limitation of thyroid-function tests.

Case 1: Elevated TSH

An asymptomatic patient has an increased TSH level of 9.0 mIU/L (normal, 0.3–5.0 mIU/L). What is the significance of this, and what management strategy is recommended?

In an asymptomatic patient with an increased TSH level (but less than 15 mIU/L), the diagnosis of subclinical hypothyrodism should be considered. This condition is defined by a slightly increased level of TSH, normal serum levels of total and free T_4, and the absence of overt symptoms of hypothyroidism. Subclinical hypothyroidism is extremely common, with a prevalence as high as 17% among elderly women *(43)*. The causes are the same for subclinical hypothyroidism and symptomatic hypothyroidism; these are listed in Table 4 and considered below. Subclinical hypothyroidism may progress to overt hypothyroidism, with autoimmune thyroid disease being a known risk factor for this progression. Prospective studies have shown that in patients with subclinical hypothyroidism and positive anti-TPO antibody titers, the disease may progress to frank hypothyroidism at a rate as high as 10%/yr, depending on the population studied *(42,44)*. Issues related to the treatment of subclinical hypothyroidism are discussed elsewhere in this text.

Case 2: Elevated TSH

A patient is found to have an increased TSH level of 22 mIU/L during a medical evaluation for fatigue and hypersomnolence. What should be done?

A moderate increase in the TSH value is usually due to primary hypothyroidism. The serum levels of total or FT_4 should be low or low-normal; typically, symptoms of hypothyroidism are present. The most common cause of primary hypothyroidism in the United States is chronic autoimmune thyroiditis (Hashimoto's thyroiditis [HT]). Other causes are surgical removal of the thyroid gland, RAI ablation of the thyroid, external irradiation, and organification defects of the gland. Secondary causes include pituitary and hypothalamic disease (Table 4). Rare causes of increased TSH include the recovery phase

Table 4
Causes of Hypothyroidism

Chronic autoimmune thyroiditis (Hashimoto's thyroiditis)
Thyroidectomy
^{131}I Therapy
External irradiation
Iodine organification defects
Iodine induced
Pituitary or hypothalamic disease

of nonthyroidal illness (TSH should not be greater than 20 mIU/L), TH-resistant states, and the presence of heterophile antibodies. The latter can cause a false increase in TSH levels in some assays. Recent nonthyroidal illness should be obvious from the patient's history; resistance to TH should always be considered when T_4 is increased and TSH is normal or increased. Also, TSH may be increased in patients with hypothyroidism who are receiving T_4 replacement therapy, in whom the circulating level of T_4 is too low because of noncompliance, malabsorption, interference with T_4 absorption by medications (such as ferrous sulfate, sucralfate, aluminum hydroxide found in some antacids, or cholestyramine), or because of enhanced biliary excretion of conjugated T_4 caused by agents such as phenytoin, carbamazepine, and rifampin.

The appropriate laboratory evaluation is critical for establishing the diagnosis and cause of hypothyroidism. A TSH assay should always be the primary test; other tests may include total T_4 or FT_4, antithyroid antibody titers (to help confirm autoimmune thyroid disease), and possibly thyroid scanning or ultrasonography (if necessary to evaluate suspicious structural abnormalities).

Management must be tailored to the individual patient. This is considered in detail elsewhere in this text. Overt hypothyroidism should be treated with replacement levothyroxine.

Case 3: Low TSH

An asymptomatic patient has a low TSH level of 0.2 mIU/L (normal, 0.3–5.0 mIU/L). What is the significance of this, and what is the recommended management?

TSH levels between 0.1 and 0.3 mIU/L (determined with an assay sensitive enough to detect levels below 0.3 mIU/L) are considered equivocal and often warrant repeat or further testing, whereas levels below 0.1 mIU/L predict an excessive level of circulating TH sufficient to suppress the hypothalamic–pituitary axis *(4)*.

Further evaluation in this patient should include an assessment of FT_4. If this is normal, the patient has subclinical hyperthyroidism, which is defined as an asymptomatic state associated with normal serum levels of total T_4, FT_4, and T_3, but subnormal serum levels of TSH.

Subclinical hyperthyroidism is often due to exogenous TH replacement or suppressive therapy. Other causes include autonomous adenoma, multinodular goiter, and early GD. Rare causes of low TSH levels (not associated with early or evolving hyperthyroidism) include nonthyroidal illness, glucocorticoid or dopamine therapy *(45)*, persistent TSH suppression after treatment or spontaneous resolution or overt hyperthyroidism, and secondary hypothyroidism; at times, low TSH levels are also seen during the first trimester of a normal pregnancy *(46)*.

Patients who are taking levothyroxine (L-thyroxine) as T_4 replacement therapy and are found to have suppressed serum levels of TSH should have the dose of levothyroxine adjusted to restore the serum level of TSH to within the normal range. Subclinical hyperthyroidism cannot be avoided in patients taking suppressive as opposed to replacement levothyroxine therapy, such as, for goiter reduction or thyroid cancer. When used to suppress benign disease, the goal of levothyroxine therapy should be to have the TSH value in the low normal or slightly subnormal range. Patients with thyroid cancer require more complete TSH suppression.

Although previously considered controversial, the biologic significance of subclinical hyperthyroidism has been supported by many recent studies. The adverse consequences of this condition include decreased bone density in women, especially postmenopausal women, and cardiac arrhythmias.

Treatment of endogenous subclinical hyperthyroidism is a difficult clinical problem and is considered elsewhere in this text.

Case 4: Low TSH

A patient has features typical of thyrotoxicosis and a TSH level of 0.01 mIU/L. What is an appropriate evaluation?

A TSH level below 0.1 mIU/L is predictive of excessive levels of TH sufficient to suppress the hypothalamic–pituitary axis. The causes of thyrotoxicosis are listed in Table 5.

Further evaluations that may help discern the underlying cause of a low TSH include palpation of the thyroid gland and the following laboratory and isotope studies: FT_4 or total T_4 and T_3 resin uptake, T_3 (especially if T_4 is normal), tests for thyroid autoantibodies including TSIs, RAI uptake, and thyroid scanning. The differential diagnosis and test interpretation require expertise; the evaluations of three different cases of thyrotoxicosis (cases 5–7) are described below.

Case 5: Low TSH

A patient presents with a TSH of 0.01 mIU/L (normal, 0.3–5.0 mIU/L); FT_4 of 4.0 ng/dL (normal, 0.7–2.0 ng/dL); TSI index of 10 (normal, 0.0–1.3); and radioactive iodine uptake of 52% (normal, 8–24%). The patient has many signs and symptoms consistent with thyrotoxicosis but no findings to suggest ophthalmopathy or dermopathy. The thyroid gland is diffusely enlarged and approximately twice its normal size, without nodularity.

This patient has findings typical of GD. All of the tests described above were helpful and the findings sufficient to make the diagnosis. The TSI index may not have been needed because the clinical presentation and the results of initial laboratory testing were all consistent with the diagnosis. Also, the relatively high cost and effort needed for the TSI index test argue against its use in such straightforward cases. There is no role for radionuclide scanning or ultrasonography in this case.

Case 6: Low TSH

A patient presents with a TSH of 0.01 mIU/L (normal, 0.3–5.0 mIU/L); FT_4 of 4.0 ng/dL (normal, 0.7–2.0 ng/dL); and RAI uptake of 4% (normal, 8–24%). The patient was experiencing palpitations, tremulousness, weight loss, and frequent bowel movements. Examination showed a moderately large, nontender thyroid.

This patient has findings typical of silent lymphocytic thyroiditis. The TSH and T_4 assessments established the diagnosis of thyrotoxicosis. The very low RAI uptake value is most consistent with the diagnosis of thyroiditis, although other conditions should also

Table 5
Causes of Thyrotoxicosis

Exogenous TH
Overzealous TH-replacement therapy
TH-suppressive therapy
Endogenous causes
Graves' disease
Toxic nodular goiter (multiple nodules or solitary toxic nodule)
Neonatal hyperthyroidism (owing to transplacental passage of thyroid-stimulating antibodies)
Inappropriate secretion of TSH—pituitary tumor or pituitary resistance to TH
Exogenous iodide
Choriocarcinoma, hydatidiform mole, embryonal testicular carcinoma
Struma ovarii
Thyroid cancer

be considered, as mentioned above. The patient should be questioned about the ingestion of TH and exposure to iodine (possibly in the form of vitamin or mineral supplements, radiographic contrast agents, or medications). FNA or core biopsy showing lymphocytic thyroiditis would confirm the diagnosis but is rarely needed. Assessment of antithyroid antibodies could be helpful because anti-TPO antibodies are almost always positive in lymphocytic thyroiditis *(47)*.

Case 7: Low TSH

A patient presents with a TSH of 0.01 mIU/L (normal, 0.3–5.0); free FT_4 of 2.8 ng/dL (normal, 0.7–2.0 ng/dL); and T_3 of 220 ng/dL (normal, 80–180 ng/dL). This patient had a goiter with a right thyroid nodule measuring approximately 2.5 cm on examination. Symptoms include nervousness, irritability, heat intolerance, frequent bowel movements, and insomnia. Therefore, a toxic nodule was strongly suspected and further evaluation is tailored to this probable diagnosis. The evaluation included thyroid scannning that shows a hyperfunctioning (hot) nodule in the right thyroid lobe, measuring 2.5 cm, with suppression of the remaining thyroid gland.

This patient has a hyperfunctioning or toxic right thyroid nodule that is causing hyperthyroidism. The TSH and FT_4 assessments established the diagnosis of thyrotoxicosis. The T_3 assessment probably was not necessary in this case because the level of FT_4 was increased (ruling out T_3 toxicosis). Treatment in cases such as this must be individualized and is considered elsewhere in this text.

Case 8: Low TSH

Thyroid function tests were performed on a very ill male patient in an intensive care unit to help evaluate his hyperdynamic cardiovascular status. Sepsis was subsequently confirmed. The patient had no history of thyroid disease, and physical examination did not detect any thyroid abnormalities. Thyroid testing showed a TSH of 0.1 mIU/L (normal, 0.3–5.0 mIU/L); T_3 of 70 ng/dL (normal, 8–180 ng/dL); and total T_4 of 7.0 μg/dL (normal, 5–12 μg/dL).

This is an example of nonthyroidal illness. The most common aberrations in thyroid-function testing associated with such illness are low T_3, normal T_4, and normal or near-normal TSH levels. The cause and biologic significance of this syndrome (also known as "sick euthyroid syndrome") are considered in detail elsewhere in this text.

Generally, thyroid-function testing should not be performed in very ill hospitalized patients unless there is concern that thyroid disease contributes to their current status. In the patient in this case, sepsis was confirmed, explaining the compromised hemodynamic status. The patient should be followed clinically for thyroid status, and if thyroid disease is suspected in the future, repeat thyroid-function testing can be performed.

Case 9: High T_4

Screening tests show that an asymptomatic young woman has an increased serum level of T_4 (14.4 μg/dL; normal, 5–12 μg/dL). A subsequently measured TSH level is normal. The history and examination findings are consistent with euthyroid status. What could cause the abnormality noted on thyroid testing?

Euthyroid hyperthyroxinemia has various potential causes, as listed in Table 1. In this case, the patient was taking oral contraceptive pills, which caused an increase in serum levels of thyroxine-binding globulin (TBG) and hence in serum T_4.

Case 10: Low T_4

An asymptomatic patient is found to have low total T_4 (3.2 μg/dL; normal, 5–12 μg/dL) and normal TSH levels on screening tests. Euthyroid status is confirmed clinically by a thorough history and examination. What could be the cause of the low level of total T_4 in this patient?

Euthyroid hypothyroxinemia is usually due to a low level of TBG, with a secondary depression of total T_4 (Table 2). This patient was found to have a congenitally low TBG. No further evaluation or treatment is needed.

Case 11: Follow-Up T_4 Therapy

A patient with recently diagnosed hypothyroidism caused by autoimmune thyroiditis has been treated with levothyroxine for the past 8 wk. What is the best way to assess the adequacy of the T_4 replacement dose?

Approximately 3–4 mo after levothyroxine therapy has been initiated, the serum level of TSH should be measured to assess correctness of the levothyroxine dose. The goal of therapy is to normalize the serum level of TSH. The levothyroxine dose can be adjusted as needed, and the serum level of TSH can be measured again, approximately 3–4 mo later. After the correct dose of levothyroxine has been established, it is good practice to evaluate the patient and to measure the serum level of TSH annually, not only to ensure compliance but also to determine whether a dose adjustment is needed.

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4 Neonatal Screening for Thyroid Disease

Marvin L. Mitchell, MD, and Cecilia A. Larson, MD

CONTENTS

INTRODUCTION

There is every reason to believe that physicians choose their profession because of a desire to contribute to improving the health of individuals, and to benefit society. Without question, this is the overriding goal of most physicians in public health, whose actions can have a profound impact on society as a whole and, additionally, can affect the welfare of generations to come. The implementation of newborn screening for congenital hypothyroidism (CH) is a prime example of a successful public-health initiative. Before the mid-1970s, babies with CH had a high likelihood of mental retardation, not because there was no treatment for the disorder, but because the disorder was largely clinically silent until irrevocable central-nervous-system (CNS) damage had occurred. The absence of conspicuous clinical features of CH in the neonate contributed to a belated diagnosis. Although treatment with levothyroxine in early childhood can restore normal growth, the potential for optimal intellectual development may never be realized if treatment for CH is not started in the newborn period.

The irreversibility of the damaged CNS in untreated CH is probably related to the unique pattern of active brain growth in the neonate. The bulk of the growth spurt of the developing

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brain occurs during the first 6 mo of life and then declines in the second and possibly also in the third year *(1)*. The growth spurt, which corresponds to the period of active neurogenesis, is when the brain is most vulnerable to insults, such as a lack of thyroxine or deprivation of essential nutrients. Consequently, it is at the critical period of peak brain growth that thyroid insufficiency leads to failure of brain maturation and hence to irreversible mental retardation.

Clinical experience had led physicians to believe that the neuropsychological abnormalities resulting from CH could be prevented or minimized by starting treatment within a few weeks after birth and before overt clinical signs of the condition were evident. The opportunity to intervene early in development required a reliable, expeditious means of screening all newborns for hypothyroidism.

Although procedures for total T_4 and thyroid-stimulating hormone (TSH) were generally available before the mid-1970s, screening for CH was not considered feasible because of the relatively large volume of serum required and the logistical nightmare inherent in implementing such testing. Such was the state of affairs until fate intervened through the action of a young French–Canadian physician, Dr. Jean Dussault. He had recently received an appointment at the University of Laval Medical School, where he was given a laboratory for thyroid research. By coincidence, his laboratory was located close to the provincial laboratory that screened neonatal blood specimens for phenylketonuria (PKU). Dussault noted that the laboratory received filter papers impregnated with dried capillary blood from which small paper blood spots were punched for testing. This serendipitous observation gave birth to the notion that screening for CH might be done by using the same network of filter-paper blood specimens. This approach, if successful, would eliminate many of the logistical problems associated with specimen collection and delivery when starting a screening program *de novo*.

After a period of trial and error, a radioimmunoassay (RIA) was developed for the measurement of T_4 on a minute amount of dried blood eluted from filter paper *(2)*. This, plus the subsequent application of RIA procedures for the estimation of TSH in dried blood eluates *(3,4)*, opened the way for widespread newborn screening for CH, and for its early diagnosis and treatment.

THE EARLY YEARS

Neither automation nor commercial kits for measuring thyroid hormones were readily available when screening for CH began. The instability and expense of tracer TSH, coupled with the labor intensity of the RIA for this hormone, prompted North American programs to screen using the less expensive and more stable total T_4 as the primary marker. Specimens with T_4 values below an established reference range for normal newborn T_4 values were then assayed for their TSH concentrations. In contrast, most European programs, with smaller workloads, as well as regions of iodine deficiency, opted for the inverse approach of using TSH as the primary marker and measuring T_4 only in specimens with elevated levels of TSH.

With the emergence of screening programs for CH, new questions were raised that were impossible to answer until sufficient data became available from prospective studies. Among the more pressing issues whose resolution had important clinical application were some of the following: How soon after birth should treatment with levothyroxine be initiated in order to prevent irreversible brain damage? Does the severity of CH at the time of diagnosis influence developmental outcome? Does the size of the replacement dose of thyroxine influence intellectual outcome? What biochemical markers should be used to monitor response to treatment? Will all infants with CH require lifelong thyroxine replacement?

Will treatment with thyroxine prevent all neuropsychological deficits? What is the true incidence of CH? Does it vary with race or gender? What are the underlying causes of CH? Answers to some of these questions have been elucidated and will be addressed in the remainder of the chapter.

EVOLUTION

The Screening Process

Although arguments can be mustered in favor of using either T_4 or TSH as the primary marker for CH, neither is as ideal as would be the combination of both in a single assay. The major disadvantages of the primary TSH approach are: (1) failure to detect secondary and tertiary hypothyroidism; (2) failure to detect thyroxine-binding globulin (TBG) deficiency; (3) failure to recognize delayed increases in TSH levels; and (4) increased false-positive values in neonates less than 3 d old. The major disadvantages of T_4 as the primary marker for CH are: (1) failure to detect hypothyroid infants who have elevated TSH but normal T_4 concentrations, and (2) increased need for repeat blood sampling because of low T_4 but normal TSH concentrations.

T_4 as the Primary Marker

As mentioned earlier, the strategy of North American screening programs for CH in newborns was to assay TSH in those specimens whose T_4 concentrations were below an established cutoff value. The defined value was usually 2 or 2.5 standard deviations (SD) below the mean of the normal distribution curve of T_4 values obtained for infants between 3 and 5 d of age. With the passage of years, as the trend toward earlier discharge took hold, the mean T_4 value for newborns increased as the interval between birth and specimen collection became shorter. This added another dimension of difficulty to the interpretation of screening results, and led to frequent adjustment of the T_4 reference range. Rather than relying on a specific T_4 cutoff value as the sole prompt for TSH assay, many programs chose to measure TSH in those specimens whose T_4 concentrations were in the lowest 10th percentile of the assays for the day. Such a strategy, currently in wide use, has reduced the margin of error associated with a specific T_4 cutoff value, thereby minimizing the risk of overlooking a potentially positive specimen because of a "normal" T_4 *(5)*.

Implications of a Low Total T_4 and Normal TSH. Although a low total T_4 accompanied by an increased TSH indicates primary hypothyroidism, a low T_4 by itself rarely reflects thyroid insufficiency. Most low T_4 values are associated with one of the following conditions: (1) TBG deficiency (approx 1 in 4000 births) or other protein binding deficiency; (2) prematurity; (3) perinatal distress and disease; (4) hypothalamic or pituitary disorders (approx 1 in 75,000 births); and (5) delayed elevation of TSH.

There is a surprising lack of uniformity among screening programs in their guidelines for managing infants with low T_4 and normal TSH values. Some programs request additional blood specimens for repeat testing, whereas others simply report the results on an initial specimen, rely on the primary-care provider to interpret the results, and then decide whether any further testing is warranted. Of the five potential conditions associated with a low T_4 and nonelevated TSH, only hypothalamic or pituitary disorders and delayed elevation of TSH require thyroxine treatment. Strategies to evaluate other instances of a low T_4 nonelevated TSH include: (1) obtaining subsequent specimens for T_4/TSH assay; (2) measurement of serum free T_4, (FT_4); and (3) dynamic testing of the hypothalamic–pituitary axis.

Hypothyroxinemia is especially common in premature and acutely ill neonates. The significance of the hypothyroxinemia, and the implications for developmental outcome, are

not yet fully understood. Premature infants are at increased risk for delayed elevations of TSH *(6,7)* and transient hypothyroidism *(8)*. Because of the observed incidence (1 in 150) of delayed TSH elevations in neonates whose birthweight was <1500 g, the Massachusetts screening program for CH presently recommends that dried blood specimens be obtained at regular intervals (2–4 wk) for all very-low-birth-weight infants until the child's weight is approximately 1,500 g or the infant is discharged to its home. The possibility that the administration of dopamine *(9)*, glucocorticoids *(10)*, and iodine and/or transfusions, or simply recovery of the thyroid axis following acute illness, may have contributed to the delayed detection of elevations in TSH has yet to be examined.

TSH as the Primary Marker

When TSH is the primary marker, T_4 measurement is of limited value in the diagnosis of primary hypothyroidism; however, the T_4 level can help estimate the degree of residual thyroid function and guide treatment. A low T_4 concentration (e.g., below 4.0 μg/dL) in a full-term infant, and which then declines significantly within a few days, usually indicates thyroid agenesis or hypoplasia. Thyroid imaging or ultrasound provides a more reliable means of defining underlying thyroid defects, which is relevant for counseling in terms of duration of TH-replacement therapy and the risk of occurrence of hypothyroidism in siblings of the affected individual.

Although TSH is unquestionably the most specific and sensitive biochemical marker for the diagnosis of primary hypothyroidism, the time of specimen collection influences its utility for screening. The peripartum period is characterized by dynamic changes in the thyroid axis. The neonatal physiologic surge of TSH after parturition complicates thyroid screening in the first days after birth. The use of age-specific TSH cutoff values can improve the specificity of the screening process and thereby reduce the labor and expense of repeated testing, which can overburden programs, alienate physicians, and impose anxiety on families.

Measurement of both TSH and FT_4 in the same specimen would be the ideal strategy for all screening programs for CH. This approach would allow more rapid identification of positive cases while minimizing the number of requests for repeat blood specimens. Unfortunately, a reliable and simple method for the determination of FT_4 in dried blood on filter paper has yet to be devised.

ETIOLOGY AND EPIDEMIOLOGY

Although the fundamental cause or causes of the various thyroid anomalies in CH have not been completely elucidated, data obtained from screening programs have shed considerable light on the types and frequencies of these pathologic defects. Despite some disagreement with regard to precise numbers, extrapolated figures suggest that approx 30% of infants have ectopic thyroid glands and 50% have aplastic or hypoplastic thyroid glands, and that the remainder have impaired thyroid hormone (TH) synthesis and/or secretion. These thyroid abnormalities account for the majority of cases of primary hypothyroidism in the neonate, and are the ones that fall within the purview of screening programs.

A molecular basis of thyroid-gland dysgenesis has been identified in the case of a few patients with such dysgenesis. In these cases, mutations of the genes that encode the TSH receptor and transcription factors PAX-8, TTF-1, and TTF-2 have been implicated *(11)*. Defects in thyroid-gland hormonogenesis have also been identified at the molecular level, and tend to have recessive inheritance. Altogether, genetic mutations account for only a small fraction of hypothyroid infants identified by screening programs.

Secondary or tertiary hypothyroidism, caused by pituitary or hypothalamic disease, has a much lower incidence than does primary (1 in 75,000 vs 1 in 4000 newborns), and will not be detected by programs that use TSH as the primary marker. Another relatively uncommon cause of hypothyroidism that has attracted considerable attention among investigators is the syndrome of generalized resistance to TH *(12)*. Identification of this disorder is also beyond the capabilities of screening programs because analytical methods for T_4 are designed for maximum sensitivity at the lower end of the standard curve. Consequently, because of the insensitive high end of the standard curve, an increased T_4 characteristic of TH resistance cannot be distinguished from high T_4 values in routine blood specimens Thus, the lack of specificity and relatively low prevalence of the syndrome of generalized resistance to TH have discouraged any serious efforts on the part of programs to incorporate it into routine, universal newborn screening.

Of the possible etiologic factors in CH, maternal autoimmune mechanisms have received considerable attention, but the inability to substantiate most reports has cast doubt on their importance *(13)*.

The overall incidence of primary CH is approx 1 in 4000 live births in North America, with a female-to-male ratio of 2.5 : 1. Ethnic and demographic factors also affect rates of hypothyroidism in certain populations; the lowest incidence of CH is found in black Americans (approx 1 in 30,000), whereas the highest incidence is found among Native and Hispanic Americans (approx 1 in 2000). The incidence of CH is also affected by the nutritional iodine status of a population *(14)*.

TRANSIENT HYPOTHYROIDISM

For purposes of discussion, transient hypothyroidism can be defined as an abnormally increased TSH found on initial screening (unrelated to the TSH surge) that falls to normal within days or weeks after birth. Although accurate figures are difficult to come by, transient hypothyroidism probably accounts for 10–20% of the cases of hypothyroidism in infants identified by North American screening programs. Potential causes include passive transfer of thyroid-blocking antibodies (from the mother, transfusion, or other source), antithyroid medication (such as propylthiouracil) or iodine exposure in the fetal/newborn period, and possibly zinc deficiency. Transient hypothyroidism may be difficult to distinguish from permanent hypothyroidism in the neonatal period, and for that reason treatment should be initiated if the TSH does not decline. Treatment should be continued until brain maturation is complete, at about 3 yr of age. Lack of the need for increased thyroxine doses as an affected child grows suggests the resolution of transient hypothyroidism.

Transient hypothyroidism is especially prevalent among premature infants, for reasons that may have to do with their exposure to large amounts of iodine either before birth or in the neonatal intensive-care unit. Interestingly, transient hypothyroidism is relatively common in premature infants in countries with borderline or deficient iodine intake, indicating that the premature newborn may be especially sensitive to either iodine excess or deficiency. Other factors known to be associated with transient hypothyroidism are passive transfer of TSH-receptor-blocking antibodies and transplacental passage of maternally ingested antithyroid medications.

There is no evidence that intellectual impairment is a consequence of transient hypothyroidism unless maternal and fetal hypothyroidism occur simultaneously. Such a situation, though uncommon, can occur in pregnant women with autoimmune hypothyroidism who remain untreated or undertreated while fetal thyroid function is inhibited by TSH receptor-

Table 1
Positive Predictive Value (PPV) and Percent of Confirmed Hypothyroid Infants Categorized by Initial TSH[a] (Years 2000 – 2001)

TSH range, mU/L	*PPV*	*Patients, %*
0–19	< 1	12
20–29	1	9
30–39	10	12
40–49	13	3
50–59	20	4
60–69	30	4
>70	100	56

[a]NENSP utilizes a primary T_4 screening strategy.

blocking antibodies. In this scenario, as well as in endemic cretinism, the amount of thyroxine transferred from the maternal to the fetal circulation is negligible and thus unable to protect the fetal brain against damage.

Whether or not TH treatment for premature infants with transient hypothyroidism affects later developmental outcome is not known.

DIAGNOSIS

Each screening program for CH establishes reference ranges for analytes tested. In the New England Newborn Screening Program, age-specific TSH ranges are utilized, in recognition of the normal, physiologic neonatal TSH surge. In Massachusetts, all out-of-range T_4 and TSH screening results are reported and tracked for appropriate follow-up of the out-of-range results. Because the degree of elevation of TSH correlates with the risk of hypothyroidism, initiation of treatment at the time of evaluation and confirmatory serum testing should be considered if: (1) TSH > 40 mIU/L for a specimen collected >24 h after birth and no maternal history of antithyroid medication, or (2) the TSH is above the newborn reference range on more than one occasion, especially in conjuction with a lower T_4.

Although the majority of cases of primary hypothyroidism show significant and easily recognizable elevations in TSH on initial screening (*see* Table 1), there is still an increased risk of hypothyroidism even with modest initial elevations in TSH, establishing the need for appropriate follow-up testing. A point worth remembering is that the range of normal TSH values for babies up to one year of age is broader than the normal adult TSH reference range *(15)*. Clinicians unaware of this might be misled into making the diagnosis of primary hypothyroidism in an infant with a TSH value above the upper limit of an adult reference range. Instances such as these can usually be resolved by measuring the serum FT_4 or FT_4 index.

MANAGEMENT

Depending on geographic location and type of practice, some physicians enlist the service of a pediatric endocrinologist whereas others assume full responsibility for the management of presumptive cases of CH. In either instance, the infant with out-of-range results on thyroid screening should be seen without delay and evaluated according to established guidelines, such as those formulated by the American Academy of Pediatrics *(16)*

or recommended by others with expertise in the field. One such group, The New England Congenital Hypothyroidism Collaborative, has developed a protocol for the management of CH that is based on outcome data from a large cohort of patients followed since 1976 *(17)*. Highlights culled from these data and from guidelines provided by the American Academy of Pediatrics have laid the groundwork for the outline shown below.

A. Immediate appointment.
1. Complete history, including detailed family history of thyroid disease, and physical examination.
2. Confirmatory testing
   a. If TSH elevations are modest, take repeat dried blood specimen for T_4 and TSH and/or serum T_4 /TSH.
   b. If TSH elevations are significant, assay serum FT_4 , total T_4 , TSH, and thyroglobulin.
3. Ancillary diagnostic studies. Depending on the individual circumstances consider ultrasound and/or scan of thyroid, testing for antithyroid antibodies in mother and/or baby, maternal thyroid testing, bone age.
4. Counsel family regarding:
   a. Good outcome of CH with appropriate and timely treatment.
   b. Recurrence risk (depends on type of CH).
   c. Signs and symptoms of hypothyroidism and hyperthyroidism.
   d. Avoidance of soy formula, which may impair absorption of medication.

B. Levothyroxine replacement: Aim to rapidly restore euthyroxinemia.
1. For significant TSH elevations, initiate thyroxine at time of confirmatory testing.
2. Starting dose of L-thyroxine (levothyroxine) should be 10–15 μg/kg/d (higher-range doses for cases with low T_4).

C. Patient monitoring.
1. T_4 and TSH determinations at 2 and 4 wk from start of treatment.
2. T_4 and TSH determinations every 1–2 mo during the first year of life, every 2–3 mo between 1 and 3 yr of age, and every 3 mo until growth is complete.
3. T_4 and TSH determinations 2–4 wk after any change in thyroxine dosage.
4. Maintain total T_4 and/or FT_4 in the upper half of the normal range, and TSH within the mid-normal range.
5. If permanency of the disorder is at question, discontinue treatment after age of 3 yr and observe for a significant increase in serum TSH and a decrease in FT_4.

The foregoing outline should not be construed as a complete document, but should serve solely as a reminder of some necessary steps to be taken when caring for an infant with CH. Foremost should be the goal of normalizing the serum T_4 (10–16 μg/dL) as rapidly as possible. This applies especially to athyreotic infants, in whom failure to attain the targeted T_4 concentration in a timely fashion can result in a poor developmental outcome *(18)*. Most infants with circulating T_4 levels of 10–16 μg/dL will have TSH values below 20 mU/L within 2 wk after treatment is begun. If at this T_4 range the TSH remains elevated, the physician should consider increasing the dose of levothyroxine. A modest increment in the circulating T_4 concentration will usually suffice to normalize the TSH.

Occasionally, physicians are faced with the situation in which the circulating T_4 remains persistently low and the TSH remains high despite progressively larger replacement doses of levothyroxine. Beyond the possibility of poor compliance, the most frequent reason for failure to respond in such cases has been interference by soy-based formula with adsorption of levothyroxine. Parents should be cautioned never to administer thyroxine in combination with any soy-based substance or with medications containing iron.

A question frequently posed by the family relates to whether treatment for CH needs to be lifelong. The answer can be summarized as follows: Children confirmed to have ectopic or athyreotic hypothyroidism (no detectable thyroglobulin in athyreotic infants) and children whose TSH levels have risen significantly at various times during thyroxine replacement therapy can be assumed to have permanent hypothyroidism. With regard to the remaining group of children with CH, the dose of thyroxine can either be discontinued or halved after the third birthday. Serum TSH and FT_4 levels should be measured shortly before discontinuing or reducing the thyroxine dose and every 2 wk thereafter. The child should be followed for 30 d or until there is a significant change in the TSH (and FT_4) concentration. It is important to advise families to be alert to possible signs and symptoms of hypothyroidism.

DEVELOPMENTAL OUTCOME

After being told that their infant has CH, and after the shock has worn off, parents invariably ask, "Will my baby be normal?" Fortunately, the fears and anxieties of most families can be allayed by data culled from several long-term prospective studies done in North America. Although all groups reported favorable neuropsychological outcomes for hypothyroid children treated early in life, the Canadians maintained that infants with the most severe disease at diagnosis had varying degrees of intellectual impairment *(19,20)*. Such findings differed from the results in New England, where the severity of hypothyroidism at diagnosis (reflected by retardation of bone age and magnitude of abnormality of T_4 and TSH) bore no relationship to outcome. The only variable that influenced intellectual behavior was adequacy of treatment during the first year of life *(21)*. The New England Collaborative hypothesized that the inferior outcome in the Toronto and Quebec cohorts was the result of inadequate replacement of levothyroxine during the critical first year of life, and not a product of the severity of the disorder *(22)*. In an effort to resolve the controversy, one of the Canadian groups designed a second study in which replacement doses of levothyroxine were larger and administered earlier than those in the first study. Data from the new study were recently analyzed, and the authors concluded that there was no difference in developmental outcome between infants with severe and moderate CH *(23)*. That these results are in concert with those from New England and others *(24,25)* further supports the assertion that, with few exceptions, all hypothyroid children, when treated early and adequately, are capable of fulfilling their optimal intellectual potential.

CH *per se* is not a barrier to achievement as long as physicians and patients adhere to the protocol for treating it. The New England Collaborative data on teenagers point out the importance of continued medication. In that particular study, unannounced TSH testing revealed significant hypothyroidism despite treatment *(17)*. After a period of tight supervision, during which the thyroxine deficiency was corrected, cognitive-test scores improved and the mean intelligence quotient (IQ) increased from 106 to 112. Although it is impossible to prove cause and effect, the evidence suggests that the diminished intellectual performance found by the New England Collaborative was associated with thyroxine insufficiency, and that the appropriate treatment was capable of reversing this phenomenon. The admonition to parents and physicians should be clear: growing children with thyroid disorders require constant monitoring.

PITFALLS

Although universal newborn screening has led to enormous strides in the early treatment of CH, physicians cannot afford to become complacent about its detection. The advent of

screening should not tempt physicians to ignore their clinical judgment when confronted with laboratory values that seem inconsistent with physical findings for a patient. An essential component of newborn screening is retesting when: (1) out-of-range results are found, (2) the presentation suggests a disorder, even if screening results at one point in time are within the normal range.

It is somewhat ironic that some of the challenges in newborn screening are in part a consequence of improved medical care. The introduction of pulmonary surfactants, slightly more than a decade ago, plus advances in neonatal medicine, achieved striking declines in the mortality of low- and very-low-birthweight infants. The increase in the rate of survival of even the tiniest of very-low-birthweight infants has been a major reason for some of the more disturbing issues with which physicians have had to struggle. In order to interpret appropriately the results of newborn thyroid-function tests, clinicians must have a clear understanding of all factors that bear on the outcome of those tests. It is for these reasons that physicians should be familiar with the advantages and disadvantages of the initial screening tests for CH and the influence of birthweight on test results. Perhaps in decades to come more will be known about this special population and how to more easily distinguish "normal" preterm physiology from preterm physiology with pathologic consequences.

As noted earlier, all programs that use TSH as the primary marker for CH will miss secondary or tertiary hypothyroidism caused by pituitary or hypothalamic disease. Programs that test for T_4 first are also likely to overlook the diagnosis if there is no process for following through on specimens with very low T_4 values. One final reminder is that infants can have completely normal T_4 and TSH values at birth, yet can at any point thereafter develop "acquired hypothyroidism."

CONCLUSION

Early treatment of CH, as a consequence of screening, has virtually eliminated the sequelae characteristic of the untreated disorder that were seen all too frequently in the era before screening. Follow-up data collected over a 20-yr period have shown that hypothyroid children who were treated early and adequately have IQs that are indistinguishable from those of normal controls. Intellectual outcome was found to bear no relationship to the severity of CH, but depended solely on adequacy of treatment during the first year of life (adequacy of treatment is defined as the circulating level of thyroxine in the mid-upper half of the normal reference range). Frequent monitoring of circulating hormone levels is the only means by which adequacy of treatment for CH can be documented and compliance assured throughout childhood and adolescence. Regardless of the unqualified success of newborn screening, no program is perfect; for that reason, physicians must not hesitate to question screening values reported to be normal when their clinical judgment dictates otherwise.

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5 Thyroid Disease in Infancy, Childhood, and Adolescence

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Contents

INTRODUCTION

Unlike that in the adult, thyroid disease in the child may be associated with important effects on brain development and/or physical growth, depending on the age at which the disease occurs. Although thyroid disorders are often subtle in presentation, the deleterious consequences of their late or inappropriate diagnosis and treatment may be irreversible. In general, severe congenital abnormalities of the thyroid gland present in infancy, whereas less severe congenital defects or acquired abnormalities, particularly autoimmune thyroid disease, develop later in childhood and in adolescence. In the newborn infant, thyroid disease may result not only from an abnormality in the child, but also as a result of transplacental passage from the mother of factors that affect the fetal thyroid gland.

THYROID DISEASE IN INFANCY

Hypothyroidism

Permanent Neonatal Hypothyroidism

Thyroid Dysgenesis. Neonatal hypothyroidism occurs in 1 in 3000 to 1 in 4000 live births worldwide, and is one of the most frequent preventable causes of mental retardation *(1)*. Because of the subtle signs and symptoms of this condition at birth, and the demonstration that only early, adequate treatment will prevent mental retardation, newborn screening for hypothyroidism is now done on all infants both in the United States and in most other industrialized nations. The causes of neonatal hypothyroidism and their relative frequencies are listed in Table 1. In contrast to iodine-deficient areas of the world, where endemic cretinism continues to be a major health hazard, most permanent cases of congenital hypothyroidism (CH) in North America (85–90%) result from thyroid dysgenesis, a sporadic

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Table 1
Causes of Neonatal Hypothyroidism and Their Approximate Incidences

Causes	*Incidence*
Permanent (90%)	
Thyroid dysgenesis	1 in 4500
Inborn errors of thyroid hormonogenesis	1 in 30,000
Hypothalamic/pituitary	1 in 100,000
Thyroid hormone (TH) resistance	Rare
Transient (10%)	
Prenatal or postnatal iodine exposure	Unknown
Maternal TSH-receptor-blocking antibodies	1 in 180,000
Maternal antithyroid drugs	Unknown

disease. Thyroid dysgenesis may result in the complete absence of thyroid tissue (agenesis) or it may be partial; the latter condition is often accompanied by a failure of the thyroid gland to descend into the neck (ectopy). Females are affected twice as often as males. Thyroid dysgenesis is less frequent among black Americans and more frequent among Hispanics and Asians. An increased incidence has been noted in infants with Down's syndrome *(2)*.

Both genetic and environmental factors have been implicated in the etiology of thyroid dysgenesis. Abnormalities in the genes encoding the transcription factors (*TTF-1*, *TTF-2*, and *Pax-8*), which are important in thyroid organogenesis and thyroid-specific gene expression, have been found in a few patients *(3–5)*, but in most cases *(6,7)* the molecular basis is unknown. The possible role of maternal autoimmunity is controversial. There is no increased prevalence in the maternal circulation of antibodies to thyroid peroxidase (TPO) formerly called microsomal antibodies), and often used as a marker of autoimmunity *(8)*. Although both thyroid growth-blocking immunoglobulins *(9)* and cytotoxic antibodies *(10)* have been reported to be present in some mothers of infants with thyroid dysgenesis, evidence is lacking that these have an etiologic role in the disorder. Furthermore, these original reports have not been confirmed *(11)*.

Inborn Errors of Thyroid Hormonogenesis. Inborn errors of thyroid hormonogenesis are responsible for most remaining cases of permanent neonatal hypothyroidism (10–15%). A number of different defects have been characterized (Table 2). Unlike thyroid dysgenesis, a sporadic condition, these disorders tend to be autosomal recessive. The molecular basis for many of these abnormalities of thyroid hormonogenesis has now been identified *(12)*.

Secondary and Tertiary Hypothyroidism. Rarely, neonatal hypothyroidism results from a deficiency in thyroid-stimulating hormone (TSH) owing to either a pituitary or hypothalamic abnormality. Secondary or tertiary hypothyroidism is often associated with evidence of other trophic-hormone deficiencies (e.g., hypoglycemia and microphallus, a consequence of associated growth hormone and/or gonadotropin deficiency) *(13)*. A number of different syndromes, some of which are associated with abnormal midline facial and brain structures, have been identified. The most common of these syndromes is septooptic dysplasia (DeMorsier's syndrome), caused in some cases by a genetic mutation in the *HES*X1 gene *(14)*. Congenital hypopituitarism also may be the result of a molecular defect in the pituitary transcription factors *PIT1* (now called *POU1F1*) *(15)* or *PROP1 (16)*.

Table 2
Inborn Errors of Thyroid Hormonogenesis

Functional abnormality	*Gene involved*
Decreased thyrotropin (TSH) responsiveness	TSH receptor
Failure to concentrate iodide	Na^+/I^+ symporter
Defective organification of iodide	
Abnormal peroxidase enzyme	Peroxidase enzyme
Abnormal H_2O_2-generating system	Not yet identified
Associated with sensorineural deafness (Pendredis syndrome)	Pendrin
Abnormal iodotyrosine deiodinase	Not yet identified
Defective thyroglobulin (Tg) synthesis or transport	Tg

On the other hand, secondary hypothyroidism may be unassociated with other pituitary-hormone defects. Causes have included decreased TSH secretion, the secretion of an abnormal TSH β-subunit *(17)*, and resistance to the action of thyrotropin-releasing hormone (TRH) *(18)*.

Thyroid-Hormone Resistance. Generalized resistance to thyroid hormone (GRTH), although usually diagnosed later in life, may be identified in the newborn period by neonatal screening programs that primarily assay TSH *(19)*. Affected infants are usually not symptomatic. Most cases of GRTH result from a mutation in the thyroid-hormone receptor (TR) β gene and follow an autosomal dominant pattern of inheritance *(20)*.

Transient Neonatal Hypothyroidism

Prenatal and Postnatal Iodine Exposure. Both the fetus and newborn infant are particularly sensitive to the thyroid-suppressive effects of excess iodine, whether administered to the mother during pregnancy or directly to the infant. Iodine-induced transient hypothyroidism is most common in premature infants *(21)* and in infants weighing less than 1500 g *(22)*. Transient hypothyroidism owing to both iodine deficiency and iodine excess appears to be more common in relatively iodine-deficient areas of Europe than in North America, an iodine-sufficient region *(23)*. Reported sources of iodine have included drugs (e.g., potassium iodide, amiodarone), radiocontrast agents (e.g., for intravenous pyelography, oral cholecystography, or amniofetography), and antiseptic solutions (e.g., povidone–iodine) used for skin cleansing or vaginal douching.

Antibodies to Maternal Thyrotropin Receptor. Maternal TSH receptor-blocking antibodies, a population of antibodies closely related to the TSH receptor-stimulating antibodies in Graves' disease (GD), may rarely be transmitted to the fetus in sufficient titer to cause transient neonatal hypothyroidism *(24)*. These antibodies usually are found in mothers who have been treated previously for GD or who have the nongoitrous form of chronic lymphocytic thyroiditis (also called primary myxedema). Unlike TSH-receptor stimulating antibodies, which mimic the action of TSH, TSH receptor-blocking antibodies inhibit both the binding and action of TSH *(11)*. Because TSH-induced growth is blocked, infants with such antibodies do not have a goiter. Similarly, inhibition of TSH-induced radioactive iodine (RAI) uptake may result in a misdiagnosis of thyroid agenesis.

Infants with TSH receptor-blocking-antibody-induced hypothyroidism are difficult to distinguish at birth from those with the more common thyroid dysgenesis, but they differ from the latter in a number of important ways (Table 3). They do not require lifelong therapy,

Table 3
Thyroid Dysgenesis and Blocking Antibody-Induced Neonatal Hypothyroidism: *Comparison of Clinical Features*

Feature	*Thyroid dysgenesis*	*Blocking antibody*
Severity of hypothyroidism	+ to + + + +	+ to + + + +
Palpable thyroid	No	No
^{123}I uptake	None to low	None to normal
Clinical course	Permanent	Transient
Familial risk	No	Yes
Anti-TPO antibodies	Variable	Variable
Anti-TSH-receptor antibodies	Absent	Potent
Cognitive outcome	Normal	May be delayed

and there is a high recurrence rate in subsequent offspring owing to the tendency of these antibodies to persist for many years in the maternal circulation. It has been suggested that if maternal hypothyroidism was present *in utero*, some infants with maternal blocking-antibody-induced hypothyroidism may have a permanent deficit in intellectual development despite early and appropriate postnatal treatment *(25)*.

Maternal Antithyroid Medication. Transient neonatal hypothyroidism may develop in infants whose mothers required as little as 200 mg propylthiouracil (PTU) per day for the treatment of GD. Such infants usually develop a prominent goiter. Both the hypothyroidism and goiter resolve spontaneously with clearance of the drug from the infant's circulation. Usually, replacement therapy is not required.

Other Abnormalities of Thyroid Function

Isolated Hyperthyrotropinemia. Isolated hyperthyrotropinemia has been described in screening programs that utilize TSH testing as a primary screening method. Although some of these infants represent cases of "compensated" hypothyroidism, in other instances the etiology is not clear. In infants whose blood specimen is obtained within the first day or two of life because of early discharge from the hospital, isolated hyperthyrotropinemia may result from the cold-induced surge in TSH observed postnatally. In other cases, a maternal heterophile antibody that cross-reacted in the TSH radioimmunoassay has been implicated *(26)*. Isolated hyperthyrotropinemia of unknown etiology has been reported in infants in Japan. In these cases, the level of TSH normalized without treatment within the first year of life *(27)*.

Hypothyroxinemia. Hypothyroxinemia in the presence of a "normal" TSH is not uncommon, particularly in premature infants, in whom it is found in as many as 50% of those of less than 30 wk gestation *(28)*. In many premature neonates, hypothyroxinemia is aggravated by the existence of severe systemic illness (sick euthyroid syndrome). It is very difficult to determine whether this hypothyroxinemia is associated independently with adverse effects on the fetal brain, since most affected infants also are very premature and have other systemic illnesses (e.g., intraventricular hemorrhage) that may affect prognosis. Thus, although in one retrospective study transient neonatal hypothyroxinemia was associated with subsequent problems in motor and cognitive development *(29)*, it is not known whether this association was causal or coincidental. Other causes of hypothyroxinemia without associated hyperthyrotropinemia include thyroxine-binding-globulin deficiency (TBG), drugs (steroids, dopamine), and secondary and tertiary hypothyroidism.

Clinical Manifestations

Clinical evidence of hypothyroidism in the neonate is difficult to appreciate. Many of the classic features (large tongue, coarse cry, umbilical hernia, hypotonia, mottling, cold hands and feet, and lethargy), when present, are subtle. In one study, these features were present in fewer than one-third of infants in whom hypothyroidism was diagnosed in the newborn period *(30)*. Other findings that should suggest the diagnosis of neonatal hypothyroidism include large fontanelles, gestation longer than 42 wk, feeding difficulties, delayed passage of stools, prolonged unexplained hyperbilirubinemia, and respiratory distress in an infant weighing more than 2.5 kg. In contrast to those with acquired hypothyroidism, infants with CH are of normal size. The finding of palpable thyroid tissue suggests hypothyroidism caused by an abnormality in thyroid hormonogenesis or action, or hypothyroidism that will be transient.

Laboratory Evaluation

The diagnosis of neonatal hypothyroidism is usually suspected because of abnormal values in newborn thyroid screening tests, and is confirmed by the demonstration of a decreased concentration of T_4 (thyroxine) (< 6.5 μg/dL (3.7 nmol/L) and an increased TSH (>20 mIU/L) in serum. Most infants with permanent abnormalities of thyroid function have a serum TSH concentration >50 mIU/L. Physicians should be alert to the much higher serum T_4 concentration in the first 2 mo of life (6.5–16.3 μg/dL; 3.7–210 nmol/L) than in adults, for whom reference values are given in most laboratories *(28)*. Thyroid function also varies with gestational age *(31)*. A bone-age determination is usually done as a reflection of the duration and severity of hypothyroidism *in utero*. In the author's opinion, all infants with confirmed CH should have a radionuclide scan (preferably ^{123}I) in order to verify that a permanent abnormality is present and to distinguish thyroid dysgenesis, a sporadic condition, from abnormalities in thyroid hormonogenesis, which are autosomal recessive disorders. Alternatively, an ultrasound study may be done to confirm the presence of a eutopic thyroid gland if a transient abnormality is suspected. Hypothyroid infants of mothers with autoimmune thyroid disease or with previously affected offspring should be checked for TSH receptor-blocking antibodies (*see* "Graves' Disease" later in this chapter). Urinary iodine should be quantitated if a diagnosis of iodine-induced hypothyroidism is being considered. The detailed evaluation of infants suspected of having an abnormality in thyroid hormonogenesis is described elsewhere *(32)*.

In infants in whom hypothyroxinemia unaccompanied by TSH elevation is found, the free-T_4 (FT_4) concentration should be evaluated and the level of TBG should be measured. If pituitary or hypothalamic hypothyroidism is suspected, pituitary-function testing and brain imaging should be performed. In premature, low-birth-weight or sick babies with a low T_4 and a "normal" TSH, testing should be repeated every 2 wk because of the rare occurrence of delayed onset of congenital hypothyroidism *(33)*. Similarly, any infant suspected of being hypothyroid clinically should have repeat thyroid-function testing.

Treatment

Replacement therapy with levothyroxine sodium should be begun as soon as a diagnosis of CH is confirmed. This need not be delayed in anticipation of performing thyroid imaging studies, as long as the latter are done within 5 d of initiating treatment (before suppression of the serum TSH). An initial dosage of 10–15 μg/kg is recommended so as to normalize the serum T_4 concentration as soon as possible, preferably within 2 wk. Subsequent adjustments are made according to the clinical picture and the results of thyroid-function tests (T_4 and TSH). The goal is to maintain the T_4 above 10 μg/dL (128.7 nmol/L) and the TSH at less

than 10 mIU/L. In some infants, normalization of TSH may be delayed because of relative pituitary resistance. In such cases, characterized by a normal or increased serum T_4 and an inappropriately high TSH level, the T_4 value is used, but noncompliance should be excluded. Detailed recommendations for therapy and follow-up in CH have been published *(34)*. Although still controversial, an increasing number of experts now believe that early, appropriate postnatal treatment will normalize development even in infants with *in utero* hypothyroidism as evidenced by a finding of delayed bone age at birth *(35)*, and that treatment failures are most likely to result from noncompliance, inadequate therapy, or, rarely, coincident use of soy formula, which reduces the absorption of thyroid hormone (TH) *(36)*. The most compelling evidence to date for the benefit of early treatment was the recent finding by Bongers-Shokking et al. that both early treatment (before 13 d of life) and high initial dose of levothyroxine (>9.5 μg/kg/d) were independent predictors of subsequent intellectual development *(37)*. When both of these treatment measures were taken, normal psychomotor development was found at 10–30 mo of age irrespective of the severity of CH.

Whether or not premature infants with hypothyroxinemia should be treated remains controversial. Van Wassanaer et al. conducted a placebo-controlled, double-blind trial of T_4 treatment at 8 μg/kg/d for 6 wk in 200 infants of less than 30 wk gestation *(38)*. Although no overall difference was found in cognitive outcome, there was an 18-point increase in the Bayley Mental Development Index score in the subgroup of levothyroxine-treated infants of < 27 wk gestation. Of some concern was the additional finding that treatment with levothyroxine was associated with a 10-point decrease in mental score in infants of >27 wk gestation. Although further studies are needed, it would seem reasonable to treat any premature infant with a low T_4 and increased TSH concentration, and to consider treatment of any infant <27 wk gestation, with a low T_4, whether or not the TSH is elevated. A dose of levothyroxine of 8 μg/kg/d for these infants has been recommended. Whether or not older premature infants with hypothyroxinemia should be treated, and what dosage of levothyroxine to use in such cases, remain uncertain.

In hypothyroid infants in whom an organic basis for the condition was not established at birth and in whom transient disease is suspected, a trial of replacement therapy can be initiated after the age of 3 yr, when most thyroxine-dependent brain maturation has occurred.

Euthyroid Goiter

Goiter in the newborn is rare, but may be large enough to cause respiratory embarrassment. It is most frequently associated with maternal treatment with high-dose thionamides (PTU or methimazole [MMI]) for GD, and is self-limited. Other causes include neonatal GD (*see* "Graves Disease" following), GRTH, an error of thyroid hormonogenesis, or iodine deficiency or excess, although goiter is rarely prominent at birth in these latter conditions.

Hyperthyroidism

Transient Neonatal Hyperthyroidism

Unlike neonatal hypothyroidism, which usually results from a permanent abnormality, neonatal hyperthyroidism is almost always transient and results from the transplacental passage of maternal TSH receptor-stimulating antibodies. Hyperthyroidism develops only in infants born to mothers with the most potent stimulatory antibody activity in their serum *(39)*. This corresponds to 1% of mothers with GD, or 1 in 50,000 newborns *(40)*, an incidence that is approximately fourfold greater than that for transient neonatal hypothyroidism caused by maternal TSH receptor-blocking antibodies *(24)*. Some mothers have mixtures of stimulating and blocking antibodies in their circulation. In such cases, the clinical picture results from

the relative proportion of activity of each type of antibody, which may change over time. For example, one affected mother gave birth in turn to a normal infant, an infant with transient hyperthyroidism, and an infant with transient hypothyroidism *(41)*.

Clinical Manifestations

Neonatal thyrotoxicosis usually is not evident at birth but develops several days later. This is owing both to the clearance from the infant's circulation of PTU administered to the mother and to the increased conversion of T_4 to the more metabolically active T_3 after birth. Characteristic signs and symptoms of neonatal thyrotoxicosis include tachycardia, irritability, poor weight gain, and prominent eyes. Goiter, when present, may be related to maternal antithyroid drug treatment as well as to the neonatal GD itself. Occasionally, thrombocytopenia, hepatosplenomegaly, jaundice, and hypoprothrombinemia have been reported, a picture that initially may be confused with a chronic infection, such as toxoplasmosis *(32)*. Rarely, arrhythmias and cardiac failure develop and may cause death, particularly if treatment is delayed or inadequate. In addition to a significant mortality rate, which approximates 20% in some older series, untreated neonatal hyperthyroidism is associated with deleterious long-term effects, including premature closure of the cranial sutures (cranial synostosis), failure to thrive, and developmental delay *(42)*.

The half-life of antibodies to the TSH receptor is 1–2 wk *(43)*. The duration of neonatal hyperthyroidism, a function of antibody potency and the rate of metabolic clearance, is usually 2–3 mo, but may be longer. In one reported case, delayed onset of neonatal hyperthyroidism, occurring 1 or 2 mo after birth, was caused by the coexistence of blocking and stimulating antibodies derived from the mother *(44)*. In this instance, the higher-affinity blocking antibodies initially masked the stimulatory effect, which became clinically evident only after the titer of blocking antibodies was diminished.

Laboratory Evaluation

Because of the importance of early diagnosis and treatment, infants at risk for neonatal hyperthyroidism should undergo both clinical and biochemical assessment soon after birth. Situations that should prompt consideration of neonatal hyperthyroidism are listed in Table 4. A high index of suspicion is necessary in infants born to women who have had thyroid ablation, because a high titer of anti-TSH-receptor antibodies in such infants would not be evident clinically.

The diagnosis of neonatal hyperthyroidism is confirmed by the demonstration of an increased concentration of circulating free T_4 (and T_3) accompanied by a suppressed TSH level as determined by a supersensitive assay. Demonstration in the infant or mother of anti-TSH-receptor antibodies will confirm the etiology of the hyperthyroidism and, in infants whose thyroid-function results are normal initially, will indicate the degree to which the infant is at risk. Although the finding of a suppressed TSH level is most frequently the result of neonatal hyperthyroidism, it may rarely be associated with central hypothyroidism, a result of prolonged hyperthyroidism *in utero (45)*. An increased TSH level is most often the result of maternal antithyroid medication, and does not require treatment unless hypothyroidism persists beyond 2 wk. Rarely, an increased TSH may result from coexistent blocking antibodies.

Treatment

Treatment of neonatal hyperthyroidism is expectant. Either PTU (5–10 mg/kg/d) or MMI (0.5–1.0 mg/kg/d) is usually used initially, in three divided daily doses. If necessary, a strong iodine solution (Lugol's solution or SSKI, 1 drop every 8 h) can be added for a more

Table 4
Situations That Should Prompt Consideration of Neonatal Hyperthyroidism

Unexplained tachycardia, goiter, petechiae, hyperbilirubinemia, hepatosplenomegaly in newborn infant
History of persistently high TRAb-titer in mother during pregnancy
History of persistently high requirement for antithyroid medication in mother during pregnancy
History of thyroid ablation for hyperthyroidism in mother
History of previously affected sibling

TRAb, anti-TSH-receptor antibody

immediate effect. Therapy with both PTU and iodine is adjusted subsequently, depending on the response. Propranolol (2 mg/kg/d in two or three divided doses) is added if sympathetic overstimulation is severe, particularly in the presence of pronounced tachycardia. If cardiac failure develops, treatment with digoxin should be initiated and propranolol should be discontinued. Rarely, prednisone (2 mg/kg/d) is added for immediate inhibition of TH secretion. Alternately, sodium ipodate (0.5 g every 3 d) has been used successfully as a sole treatment for neonatal hyperthyroidism *(46)*.

Permanent Neonatal Hyperthyroidism

Rarely, neonatal hyperthyroidism is permanent. Most cases have occurred in infants with a strong family history of hyperthyroidism *(47)*, and are caused by a mutation in the TSH receptor resulting in its constitutive activation *(48)*. An autosomal dominant inheritance of this condition has been described. It is important to distinguish permanent neonatal hyperthyroidism from transient neonatal GD because permanent neonatal hyperthyroidism is often difficult to treat medically, and early, permanent thyroid ablation is important to prevent delay in cognitive development.

THYROID DISEASE IN CHILDHOOD AND ADOLESCENCE

Hypothyroidism

Chronic Lymphocytic Thyroiditis

The most frequent cause of hypothyroidism after the neonatal period is chronic lymphocytic thyroiditis (CLT), an autoimmune disease that is closely related to GD. Although in CLT lymphocyte- and cytokine-mediated thyroid destruction predominates, whereas in GD antibody-mediated thyroid stimulation occurs, overlap of the two conditions may occur in some patients. Both a goitrous (Hashimoto's thyroiditis [HT]) and a nongoitrous (primary myxedema) variant of thyroiditis have been distinguished. The disease has a striking predilection for females, and a family history of autoimmune thyroid disease (both CLT and GD) is found in 30–40% of patients. During childhood, the most common age at presentation is adolescence, but the disease may occur at any age, even in infancy *(49)*. There is an increased prevalence of CLT in patients with insulin-dependent diabetes mellitus, 20% of whom have positive antithyroid antibodies and 5% of whom have an increased serum TSH level *(50)*. CLT may also occur as part of an autoimmune polyglandular syndrome (APS) *(51)*. In APS-1, also called autoimmune polyendocrinopathy candidiasis ectodermal dystrophy (APECED) syndrome, CLT is found in approximately 10% of patients *(52)*. APS-1, a disorder associated with defective cell-mediated immunity that tends to present in

childhood, has recently been shown to result from a mutation in the autoimmune regulator *(AIRE)* gene *(53)*. CLT and diabetes mellitus with or without adrenal insufficiency (APS-2), also referred to as Schmidt's syndrome, tends to occur later in childhood or in adulthood, *(51)*. In addition to its occurrence in these polyglandular syndromes, CLT occurs with an increased incidence in patients with certain chromosomal abnormalities (Down's syndrome, Turner's syndrome, Klinefelter's syndrome), as well as in patients with Noonan's syndrome. Rarely, CLT may be associated with chronic urticaria *(54)* and with immune-complex glomerulonephritis *(55)*.

Antibodies to thyroglobulin (Tg) and TPO, the antithyroid antibodies measured in routine clinical practice, are detectable in over 95% of patients with CLT. Therefore, they are useful as markers of underlying autoimmune damage to the thyroid, TPO antibodies being more sensitive and specific. Antibodies to the TSH receptor are also found in a small proportion of patients with CLT. When stimulatory anti-TSH-receptor antibodies are present, they may give rise to a clinical picture of hyperthyroidism, the coexistence of CLT and GD being known as "Hashitoxicosis." Blocking antibodies, on the other hand, have been postulated to underlie both the hypothyroidism and the absence of goiter in some patients with primary myxedema, but are detectable in only a minority of children *(56)*. In rare instances, the disappearance of blocking antibodies has been associated with a normalization of thyroid function in previously hypothyroid patients *(57)*.

Goiter, present in approximately two thirds of children with CLT, results primarily from lymphocytic infiltration of the thyroid and, in some patients, from a compensatory increase in TSH. The role of antibodies in goitrogenesis is controversial *(58)*. Contrary to previous beliefs, accumulating evidence now suggests that primary myxedema arises as a result of independent immune mechanisms and does not represent the "burned out" phase of CLT *(56)*.

Children with CLT may be euthyroid, or may have compensated or overt hypothyroidism. Rarely, children with CLT may experience an initial thyrotoxic phase owing to the discharge of preformed T_4 and T_3 from the damaged gland. Alternatively, as indicated above, thyrotoxicosis may result from concomitant thyroid stimulation by stimulatory anti-TSH-receptor antibodies (Hashitoxicosis).

Long-term follow-up studies of children with CLT have suggested that, although most who are hypothyroid initially remain hypothyroid, spontaneous recovery of thyroid function may occur, particularly in those with initial compensated hypothyroidism *(59,60)*. On the other hand, some initially euthyroid patients will become hypothyroid during observation. Therefore, close follow-up is necessary for all children and adolescents with CLT, whatever their thyroid status at presentation.

Other Causes of Acquired Hypothyroidism

Thyroid Dysgenesis and Inborn Errors of Thyroid Hormonogenesis. Occasionally, patients with thyroid dysgenesis will escape detection by newborn screening and present later in childhood with nongoitrous hypothyroidism or with an enlarging mass at the base of the tongue or along the course of the thyroglossal duct. Similarly, children with inborn errors of thyroid hormonogenesis may be recognized only later in childhood by detection of a goiter.

Drugs or Goitrogens. A number of drugs used in childhood may affect thyroid function, including certain anticonvulsants, and lithium *(61)*. Similarly, a large number of naturally occurring goitrogens have been identified *(62)*. Worldwide, iodine deficiency continues to be an important cause of hypothyroidism, but this is not the case in North America, an iodine-sufficient area.

Secondary or Tertiary Hypothyroidism. Secondary or tertiary hypothyroidism in children with less severe congenital abnormalities may be recognized later in childhood. Alternatively, secondary or tertiary hypothyroidism may develop as a result of acquired damage to the pituitary or hypothalamus (e.g., by tumors [particularly craniopharyngioma], granulomatous disease, head irradiation, infection [meningitis], or trauma). Usually, other trophic hormones and particularly growth hormone are affected in such cases.

Thyroid-Hormone Resistance. In contrast to neonates, children with GRTH usually come to attention when thyroid-function tests are performed because of poor growth, hyperactivity, a learning disability, or other nonspecific signs or symptoms. A small goiter may be appreciated. There is a high incidence of attention deficit–hyperactivity disorder in children with GRTH *(63)*. Rarely, GRTH may be found in patients with cystinosis and in patients with Albright's hereditary osteodystrophy.

Miscellaneous Causes of Acquired Hypothyroidism. Rarely, the thyroid gland may be involved in generalized infiltrative (cystinosis), granulomatous (histiocytosis X), or infectious disease processes that are of sufficient severity to result in a disturbance in thyroid function. Alternatively, hypothyroidism may be a long-term complication of mantle irradiation for Hodgkins' disease or lymphoma.

Clinical Manifestations

The onset of hypothyroidism in childhood is insidious. Affected children are usually recognized either because of the detection of a goiter on routine examination or because of a poor interval growth rate for several years before diagnosis. Because the deceleration in linear growth tends to be more affected than weight gain, these children are relatively overweight for their height, although they rarely are significantly obese (Fig. 1). If the hypothyroidism is severe and longstanding, immature facies with an underdeveloped nasal bridge and immature body proportions (increased upper-to-lower body ratio) may be noted. Dental and skeletal maturation are delayed, the latter often significantly.

Causes of hypothyroidism associated with a goiter (CLT, inborn errors of thyroid hormonogenesis, GRTH) should be distinguished from nongoitrous causes (primary myxedema, thyroid dysgenesis, secondary or tertiary hypothyroidism). The typical thyroid gland in CLT is diffusely enlarged and has a rubbery consistency. Although the surface is classically described as "pebbly" or bosselated, asymmetric enlargement occasionally occurs and must be distinguished from thyroid neoplasia. A pyramidal lobe superior to the isthmus may be present and may be confused with a thyroid nodule. On the other hand, the thyroid gland in TH-synthetic defects tends to be softer and diffusely enlarged.

The classical clinical manifestations of hypothyroidism can be elicited on careful evaluation, though they often are not the presenting complaints. These include lethargy, cold intolerance, constipation, dry skin or hair, and periorbital edema. A delayed relaxation time of the deep tendon reflexes may be appreciated in more severe cases. In patients with severe hypothyroidism of longstanding duration, the sella turcica may be enlarged owing to thyrotrope hyperplasia. There is an increased incidence of slipped femoral capital epiphyses in hypothyroid children. The combination of severe hypothyroidism and muscular hypertrophy, which gives the child a "Herculean" appearance, is known as the Kocher–Debré–Sémélaign syndrome.

Puberty tends to be delayed in hypothyroid children in proportion to the retardation in their bone age, although in longstanding severe hypothyroidism sexual precocity has been described. Females with sexual precocity have menstruation, breast development, and galactorrhea but relatively little pubic hair. Multicystic ovaries, the etiology of which is unknown, may be demonstrated on ultrasonography. An increased concentration in serum

gonadotropins and prolactin is found. It has been postulated that this syndrome results from an increase in the secretion of TRH, which is known to stimulate prolactin as well as TSH. The stimulation of gonadotropins may result from paracrine effects of TRH-stimulated second messenger.

Laboratory Evaluation

Measurement of TSH with a third-generation ultrasensitive assay is the best initial screening test for primary hypothyroidism. If the TSH is elevated, then evaluation of the free T_4 (FT_4) (or free-T_4 index) will distinguish whether the child has compensated (normal FT_4) or overt (low FT_4) hypothyroidism. Measurement of TSH, on the other hand, is not helpful in secondary or tertiary hypothyroidism. In these cases, hypothyroidism is demonstrated by the presence of a low FT_4. GRTH is characterized by elevated levels of T_4 and T_3 and an inappropriately normal or elevated TSH concentration.

A diagnosis of CLT is made by the demonstration of elevated titers of anti-Tg and/or anti-TPO antibodies. Ancillary investigations (thyroid ultrasonography and/or thyroid scintigraphy) may be performed if thyroid antibody tests are negative, but are rarely necessary. In fact, the typical picture of spotty uptake of radioactive iodine that is seen in adults with CLT is rare in children *(64)*.

If no goiter is present, thyroid ultrasonography and/or scanning are helpful in identifying the presence and location of thyroid tissue and, therefore, in distinguishing primary myxedema from thyroid dysgenesis. Inborn errors of thyroid hormonogenesis beyond a trapping defect are usually suspected when there is an increased radioiodine uptake and a large gland on scanning. Other etiologies of hypothyroidism are usually evident from the history.

Treatment

In contrast to treatment of neonatal hypothyroidism, rapid replacement of T_4 is not essential in the older child. This is particularly true in children with longstanding, severe thyroid underactivity, in whom rapid normalization of thyroid hormone levels may result in unwanted side effects (deterioration in school performance, short attention span, hyperactivity, insomnia, and behavioral difficulties) *(65)*. In these children it is preferable to increase the replacement dose slowly over several weeks to months. Severely hypothyroid children should also be observed closely for complaints of severe headache when therapy is initiated, because of the rare development of pseudotumor cerebri *(66)*. In contrast, full replacement of T_4 can be initiated at once without much risk of adverse consequences in children with mild hypothyroidism. In patients with compensated hypothyroidism, thyroid function should be reassessed in 3–6 mo, prior to initiating therapy, because of the possibility that the thyroid abnormality will be transient.

The typical replacement dose of levothyroxine in childhood is approx 100 $\mu g/m^2$, or 4–6 μg/kg for children 1–5 yr of age, 3–4 μg/kg for those aged 6–10 yr, and 2–3 μg/kg for those 11 yr of age and older. In patients with a goiter, a somewhat higher levothyroxine dosage is used so as to keep the TSH in the low normal range (0.3–1.0 mIU/L in an ultrasensitive assay), and thereby minimize its goitrogenic effect.

Serum levels of T_4 and TSH should be measured after the child has received the recommended dosage for at least 8 wk. Once a euthyroid state has been achieved, patients should be monitored every 6–12 mo. Close attention is paid to interval growth and bone age, as well as to the maintenance of a euthyroid state. Some children with severe, longstanding hypothyroidism at diagnosis may not achieve their adult height potential even with optimal therapy, emphasizing the importance of early diagnosis and treatment *(67)*. Treatment is usually continued indefinitely.

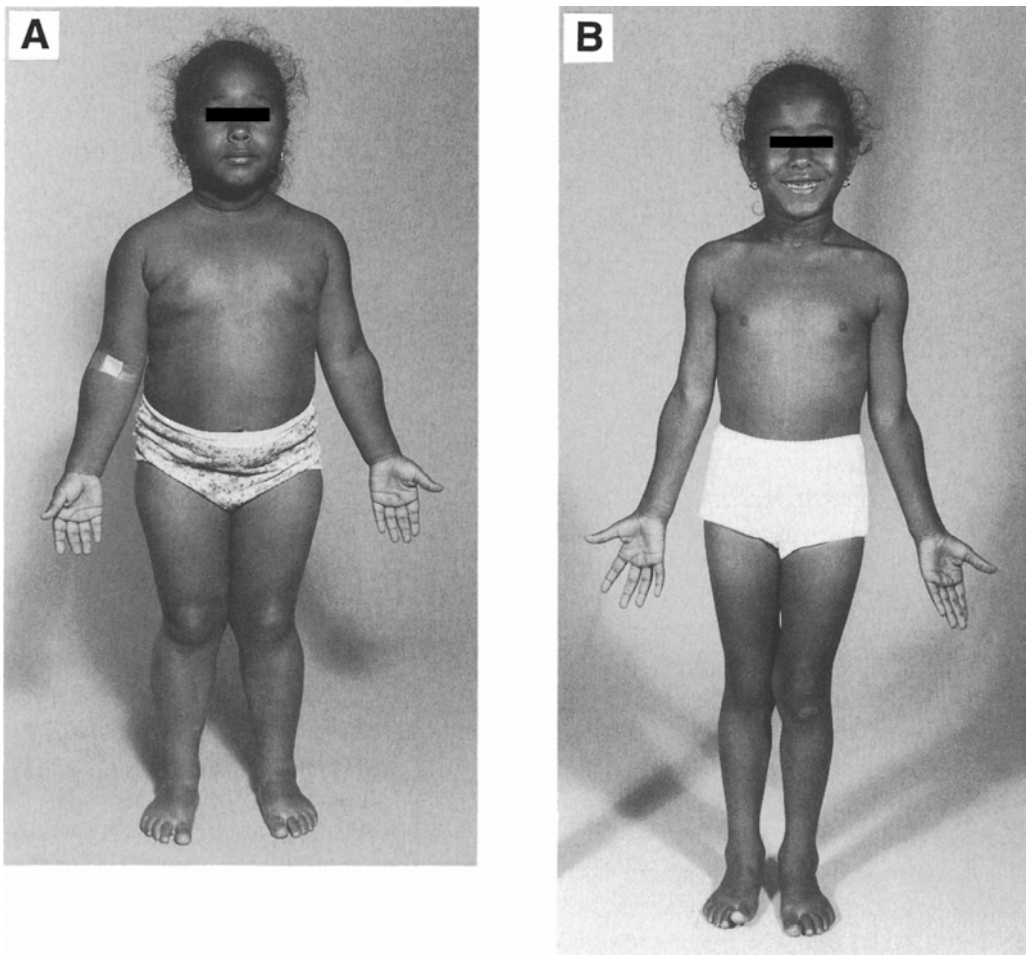

Fig. 1. Ten-year-old female with severe primary hypothyroidism attributable to primary myxedema before **(A)** and after **(B)** treatment. The presenting complaint was poor growth. Note the dull facies, relative obesity, and immature body proportions prior to treatment. At age 10 yr, the patient had not lost a deciduous tooth.

Euthyroid Goiter

Goiter, the most common thyroid disorder in pediatrics, occurs in 4– 6% of schoolchildren in North America *(59)*. Like thyroid disease in general, there is a female preponderance, the female-to-male ratio being 2–3 to 1. Patients with goiter may be euthyroid, hypothyroid, or hyperthyroid, euthyroid goiters being by far the most common. The most frequent cause of asymptomatic goiter in North America is CLT (*see* "Hypothyroidism" earlier). Causes of goiter that are associated with abnormal thyroid function are discussed elsewhere in this chapter.

Colloid or Simple (Nontoxic) Goiter

Colloid goiter is the second most common cause of euthyroid thyroid-gland enlargement in childhood. Not infrequently there is a family history of goiter, CLT, and GD, leading to the suggestion that colloid goiter, too, might be an autoimmune disease. Thyroid-growth immunoglobulins have been identified in a proportion of patients with simple goiter *(68)*, but their etiologic role is controversial *(58)*. It is important to distinguish patients with colloid

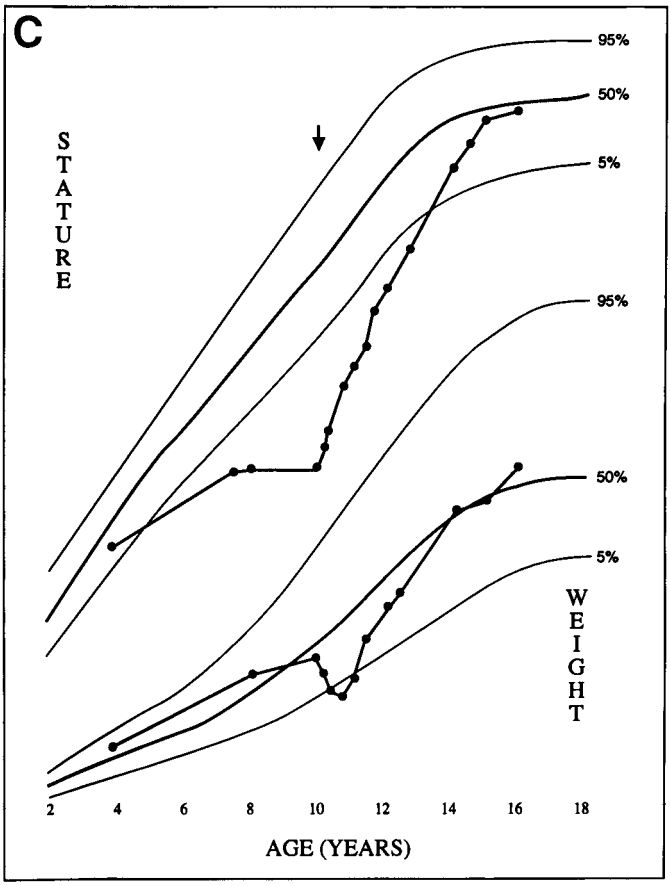

Fig. 1. (*cont'd*). **(C)** After treatment was initiated (arrow), the patient lost six teeth in 10 mo and had striking catch-up growth. Her bone age was 5 yr at a chronologic age of 10 yr. Assays for TSH-receptor-blocking antibodies were negative.

goiter from those with CLT because of the risk of hypothyroidism developing in patients with CLT but not those with colloid goiter. Whereas many colloid goiters regress spontaneously, others appear to undergo periods of growth and regression, resulting ultimately in large, nodular thyroid glands later in life.

Clinical Manifestations and Laboratory Investigation

Evaluation of thyroid function with an ultrasensitive TSH assay is the initial approach to the diagnosis of asymptomatic goiter. In euthyroid patients, who represent the most common situation, CLT should be distinguished from colloid goiter. Clinical examination in both instances reveals a diffusely enlarged thyroid gland. Therefore, the distinction depends on the presence of increased titers of anti-TPO and anti-Tg antibodies in CLT but not in colloid goiter. All patients with negative thyroid antibody titers initially should have repeat examinations, because some children with CLT will develop positive titers with time.

Therapy

Thyroid suppression in children with a euthyroid goiter is controversial. There is no evidence of its efficacy in CLT *(60,69)*, and no long-term studies of its use are available in children with colloid goiter. A therapeutic trial may be undertaken when the goiter is large.

Painful Thyroid

Acute (Suppurative) Thyroiditis

Painful enlargement of the thyroid is rare in pediatrics and suggests the probability of either acute (suppurative) or subacute (nonsuppurative) thyroiditis. Acute (suppurative) thyroiditis is characterized by high fever and cervical adenopathy with severe pain, redness, and swelling in the region of the thyroid gland. Progression to abscess formation may occur rapidly. Acute thyroiditis frequently is preceded by an upper respiratory infection. Recurrent attacks and involvement of the left lobe suggest a piriform sinus fistula between the oropharynx and the thyroid as the route of infection. Gram-positive organisms (hemolytic streptococcus, *Staphylococcus aureus*, and pneumococcus) are the most frequent pathogens, although mixed cultures of anaerobic and aerobic organisms have also been reported. Thyroid function is not affected.

Subacute (Nonsuppurative) Thyroiditis

Subacute (nonsuppurative) thyroiditis, most likely a viral disease, is characterized by a less fulminant course than acute thyroiditis. Low-grade fever, malaise, mild thyromegaly, and pain and tenderness in the region of the thyroid gland are seen initially and, as in the acute variety of thyroiditis, often follow an upper respiratory illness. Mild thyrotoxicosis, owing to the release of preformed TH from the damaged gland, is often present for the first week. This is followed by a recovery phase during which mild hypothyroidism may occur for 2–9 mo. In contrast to GD, the thyrotoxicosis in subacute thyroiditis is associated with a low RAI uptake. Complete recovery is the rule.

Laboratory Investigation

Acute suppurative thyroiditis can usually be readily distinguished from the subacute variety. A bacterial infection, acute thyroiditis is characterized by a moderate-to-marked leukocytosis with a shift to the left in the differential count. The sedimentation rate is mildly elevated. Aspiration of the affected area and determination of antimicrobial sensitivity by culture in both aerobic and anaerobic media should be performed. Ultrasonography may help in evaluating possible abscess formation. Barium ingestion and imaging should be done if a piriform sinus is suspected. On the other hand, in subacute thyroiditis, a viral disease, the white blood count is normal or slightly elevated. Thyroid-function tests may reveal transient hyperthyroidism or hypothyroidism, or may be normal. The erythrocyte sedimentation rate is markedly increased.

Treatment

Treatment of acute thyroiditis involves the use of high-dose parenteral antibiotics, which should be initiated as soon as possible to prevent abscess formation. Surgical drainage and/or lobectomy is necessary if abscess formation occurs. In patients with repeated attacks and in whom a piriform sinus is found, complete extirpation of the fistula is necessary to prevent recurrence.

Treatment of subacute thyroiditis is supportive. Antiinflammatory drugs (e.g., acetylsalicylic acid) are used when symptoms are mild. Glucocorticoids are reserved for severe cases. If necessary, propranolol can be used to control thyrotoxic symptoms. The hypothyroid phase is usually mild and self-limited, and requires no therapy. If it persists, transient levothyroxine therapy may be needed.

Table 5
Nomenclature of TSH Receptor Antibodies

Method of detection	*Name*	*Abbreviation*
Radioreceptor assay	TSH-receptor antibodies	TRAbs
	TSH-binding-inhibitory immunoglobulins	TBII
Bioassay		
Stimulation of adenyl cyclase	Thyroid-stimulating antibodies	TSAbs
	Thyroid-stimulating immunoglobulins	TSI
Inhibition of TSH-induced adenyl-cyclase stimulation	TSH receptor-blocking antibodies	TRBAbs
	TSH stimulation-blocking immunoglobulins	TSI-block

Hyperthyroidism

Graves' Disease

More than 95% of cases of hyperthyroidism in children and adolescents result from GD, an autoimmune disorder that, like CLT, occurs in a genetically predisposed population *(32)*. There is a strong female predisposition for Graves' disease, the female-to-male ratio being 6–8 to 1. GD is much less common in childhood than in the adult. Although it can occur at any age, it is most common in adolescence.

Unlike CLT, in which thyrocyte damage is predominant, the major clinical manifestations of GD are hyperthyroidism and goiter. GD is caused by anti-TSH-receptor antibodies, which mimic the action of TSH. Binding of both types of ligand to the receptor results in stimulation of adenyl cyclase and thyroid hormonogenesis and growth. As noted earlier, TSH receptor-blocking antibodies, in contrast, inhibit TSH-induced stimulation of adenyl cyclase. Both stimulatory and blocking antibodies to the TSH receptor bind to the extracellular domain of the receptor, but the specific epitope with which they interact is different *(70)*.

Clinical assessment for the presence of anti-TSH-receptor antibodies takes advantage of the ability of these antibodies to compete with radiolabeled TSH for binding to thyroid membranes (radioreceptor assay) or to stimulate (or inhibit) TSH-induced stimulation of adenyl cyclase (bioassay) (Table 5). Since both stimulatory and blocking antibodies inhibit TSH binding, the radioreceptor assay is an excellent screening method for the presence of anti-TSH-receptor antibodies, but does not provide information about function. A reasonable strategy is to initially test for the presence of anti-TSH-receptor antibodies by radioreceptor assay, reserving bioassay for subsequent elucidation of the biologic activity of these antibodies.

A confusing number of terms have been applied to anti-TSH receptor antibodies, depending on the assay used for their detection (Table 5). When measured by radioreceptor assay, these antibodies are referred to as TSH-receptor antibodies (TRAbs) or TSH-binding-inhibitory immunoglobulins (TBIIs). When measured by bioassay, the stimulatory antibodies have been termed thyroid-stimulating antibodies (TSAbs) or thyroid-stimulating immunoglobulins (TSIs). In contrast, the blocking antibodies are called TSH-receptor-blocking antibodies (TRBAbs) or TSH-stimulation-blocking immunoglobulins (TSI-block). It is important to appreciate that the incidence of anti-TSH-receptor antibodies depends on the method used for their detection and on its sensitivity. Furthermore, when FRTL-5 cell lines are used for bioassay of antibodies to the TSH receptor, sensitivity is lost after

repeated passage *(71)*, a problem not encountered with newer cell lines that employ Chinese hamster ovary cells stably transfected with recombinant TSH receptor. Whether measured by radioreceptor assay or by bioassay, anti-TSH-receptor antibodies can be detected in approx 90% of children and adolescents with active GD when a sensitive assay is used *(72)*. Recently a second-generation TSH radioreceptor assay has been reported in which 99% of patients with GD were positive *(73)*. In contrast, only 70% of children with GD have anti-TPO and anti-Tg antibodies (present in >95% of children with CLT) in their sera. Therefore, measurement of antibodies to the TSH receptor is more sensitive and specific than measurement of anti-Tg or anti-TPO antibodies for the diagnosis of GD in childhood.

Rarer Causes of Hyperthyroidism

Rarely, hyperthyroidism may be caused by a functioning thyroid adenoma or by constitutive activation of the TSH receptor, or it may be seen as part of the McCune–Albright syndrome. Hyperthyroidism also may result from inappropriately increased TSH secretion, the result either of a TSH-secreting pituitary adenoma or selective pituitary resistance to TH. Miscellaneous causes of thyrotoxicosis (TH excess) without hyperthyroidism (thyroid overactivity) include the toxic phase of CLT (*see* "Chronic Lymphocytic Thyroiditis" earlier in this chapter), and TH ingestion (thyrotoxicosis factitia).

Clinical Manifestations

All but a few children with GD present with thyroid enlargement, and most have symptoms and signs of excessive thyroid activity, such as tremors, inability to fall asleep, weight loss despite an increased appetite, proximal muscle weakness, and tachycardia. Shortened attention span and emotional lability may lead to behavioral and school difficulties. Some patients complain of polyuria and nocturia, the result of an increased glomerular filtration rate.

Physical examination reveals a diffusively enlarged, soft or "fleshy" thyroid gland, smooth skin and fine hair texture, excessive activity, and a fine tremor of the tongue and fingers. A thyroid bruit may be audible. In contrast, the finding of a thyroid nodule suggests the possibility of a toxic adenoma. On the other hand, café au lait spots, particularly in association with precocious puberty, suggest a possible diagnosis of McCune–Albright syndrome. If a goiter is absent, thyrotoxicosis factitia should be considered. Infiltrative ophthalmopathy is considerably less common in children than in adults, although a stare and mild proptosis are frequently observed.

Laboratory Evaluation

The clinical diagnosis of hyperthyroidism is confirmed by the finding of increased concentrations of circulating FT_4 and T_3, the T3 to T4 ratio (ng/dL to μg/dL) characteristically being >20 to 1. Demonstration of a suppressed TSH by an ultrasensitive method excludes the rare causes of thyrotoxicosis, such as TSH-induced hyperthyroidism and pituitary resistance to TH, in which the TSH is inappropriately "normal" or slightly elevated. If the latter diseases are suspected, free α-subunit of TSH should be measured and a TRH test performed. Alternatively, an increased total T_4 in association with an inappropriately "normal" TSH may result from an excess of TBG (either familial or acquired, such as from oral contraceptive use) or rarer protein binding abnormalities (e.g., familial dysalbuminemic hyperthyroxinemia). In the latter cases, by contrast to hyperthyroidism, the free T_4 concentration is normal. The diagnosis of GD is confirmed by the demonstration of anti-TSH-receptor antibodies in serum. In contrast to the case with adults, radioactive iodine uptake and scan are used to confirm the diagnosis of GD only in atypical cases in children and adolescents (e.g., if

measurement of anti-TSH-receptor antibodies is negative or if the thyrotoxic phase of CLT or a functioning thyroid nodule is suspected).

Treatment

Medical therapy with one of the thiouracil derivates (PTU or MMI) usually is the first approach to treating hyperthyroidism in children and adolescents. In severe cases, an β-adrenergic antagonist drug such as propranolol can be added to control the signs and symptoms of hyperthyroidism until a euthyroid state is obtained. Although PTU but not MMI inhibits to some extent the conversion of T_4 to the more active isomer T3, this effect is not pronounced in vivo. On the other hand, MMI has a longer half-life, an advantage in adolescents, in whom compliance is often an issue. The initial dosage of PTU is 5 mg/kg/d given in three divided daily doses, and that of MMI is 0.5 mg/kg/d given in two daily doses. Patients should be followed every 4–6 wk until the serum concentration of T_4 (and total T_3) normalizes. At this point, one can either decrease the dosage of thionamide drug by 30–50% or, alternatively, wait until the TSH begins to rise and add a small, supplementary dose of levothyroxine (1 μg/kg/d). Maintenance doses of PTU may be given twice daily. MMI may be administered once daily. The optimum duration of therapy is unknown. Approximately 50% of children will go into long-term remission within 4 yr, with a continuing remission rate of 25% every 2 yr for up to 6 yr of treatment *(73)*. In patients treated with antithyroid drugs alone, a small drug requirement, diminution in goiter size, lack of orbitopathy, and lower initial degree of hyperthyroxinemia (T_4 < 20 μg/dL [257.4 nmol/L]; T3 to T4 ratio <20) are favorable indicators that drug therapy can be tapered gradually and withdrawn. Persistence of antibodies to the TSH receptor, on the other hand, indicates a high likelihood of relapse. Initial studies suggesting that combined therapy (i.e., antithyroid drug plus levothyroxine) might be associated with an improved rate of remission *(74)* have not been confirmed *(75)*.

Toxic drug reactions (erythematous rashes, urticaria, fever, transient leukopenia, and arthralgias) have been reported in 1–9% of children treated medically for hyperthyroidism *(76)*. Rarely, more severe sequelae, such as hepatitis, a lupus-like syndrome, thrombocytopenia, and agranulocytosis, occur. Most mild reactions disappear in a few days with or without antihistamine therapy; if this does not occur, substitution of an alternative thioamide drug is usually effective. In more severe cases, antithyroid medication should be discontinued immediately. The risk of hepatitis and agranulocytosis appear to be greater within the first 3 mo of therapy, when a higher dosage of medication (particularly MMI) is used, and in older individuals. Hepatitis may be more frequent in children and after PTU use. Routine monitoring of the white blood cell count, or liver function testing, is not generally recommended, although this has recently been questioned. However, it is important to caution all patients to stop their medication immediately and consult their physician should they develop an unexplained fever, sore throat, gingival sores, or jaundice.

Definitive therapy with either medical (RAI) or surgical thyroid ablation is usually reserved for patients in whom drug therapy has failed or who have developed a toxic reaction, or for noncompliant patients. In recent years, however, RAI is being favored increasingly, even as the initial approach to treating hyperthyroidism. This is particularly so in adolescents with behavioral problems, in children who are mentally retarded, and in those about to leave home (e.g., to go to college). The advantages are the relative ease of administration of RAI, the reduced need for medical follow-up, and the lack of demonstrable long-term adverse effects *(77,78)*. The major concern about the use of RAI in children relates to the

possibly increased risk of papillary thyroid cancer. Following the Chernobyl nuclear disaster, a 62-fold increase in the incidence of papillary thyroid cancer was observed in children who were <10 yr of age when the nuclear emission occurred *(79)*. This frequency was even higher in those <6 yr of age and those *in utero*. Therefore, if RAI therapy is used in children and adolescents, an ablative dose should be used. There is no evidence of an increased risk of thyroid or of other solid-tissue neoplasia, nor of leukemia or birth defects following RAI therapy. However, long-term safety data are still limited to fewer than 1000 individuals.

Subtotal thyroidectomy, the third therapeutic modality for hyperthyroidism, is performed less frequently now than in the past. Surgery is usually reserved for patients in whom medical management has failed, who have a markedly enlarged thyroid, or who refuse RAI therapy, and for the rare patient with significant orbitopathy in whom RAI therapy is contraindicated. Because of the potential complications of surgery (recurrent laryngeal nerve paralysis, hypoparathyroidism, and, even, very rarely, death), it should be performed only by an experienced pediatric thyroid surgeon. Occasionally, unsightly keloid formation occurs at the site of the scar. Following both medical and surgical thyroid ablation, most patients become hypothyroid and require lifelong TH-replacement therapy. On the other hand, with antithyroid medication the major problem is recurrence of the hyperthyroidism.

Thyroid Nodules

Thyroid nodules are rare in childhood, but there is some evidence that they are more likely to be carcinomatous than are similar masses in adults *(80)*. Follicular adenomas and colloid cysts account for the majority of benign nodules. Children exposed to high levels of radiation as a result of the Chernobyl disaster *(78)*, or who have received therapeutic mantle irradiation *(81)*, constitute particularly high-risk groups. The most common form of thyroid cancer is papillary thyroid carcinoma, but other histologic types found in adults may also occur in children. *(80,82,83)*. The possibility of a rare medullary thyroid carcinoma should be considered if there is a family history of thyroid cancer or pheochromocytoma, or if the child has multiple mucosal neuromas and a marfanoid habitus. Thyroid cancer is discussed elsewhere in this volume. Some excellent reviews of childhood cancer have been published recently *(81,82)*.

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6 Thyroid Disease in Older Persons

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Contents

INTRODUCTION

For over 100 years, physicians have thought that aging and the thyroid gland must somehow be connected; older persons simply looked too much like those with hypothyroidism for their appearance to be coincidental. In 1886 this thinking by analogy led Victor Horsley, then the experimental surgeon for the now-famous Myxoedema Committee, to attribute aging itself to a thyroid defect *(1)*. We now know that this idea is clearly not correct on its face, but there is enough truth to it that good medical practice requires that we look closely at older persons for the presence of thyroid disease.

Thyroid dysfunction, both hypothyroidism and hyperthyroidism, shows a different face in older persons (>60 yr) than in younger ones; the signs and symptoms are more vague and less specific, and the disorders are harder to detect clinically. Thyroid nodularity is probably more common in older persons. In considering and managing thyroid diseases in older persons, physicians need to abandon the idea that the "typical" presentations of thyroid disease in younger adults also typify these disorders in older adults. What is typical about thyroid dysfunction in an older person is not the same as what is typical in a younger one. A thorough review of the aging thyroid, including discussions of thyroid disease, appeared a few years ago *(2)*.

THYROID CHANGES WITH NORMAL AGING

Does thyroid function change with increasing age? To some extent it probably does, but the changes are slight and do not affect the ability of the now standard thyroid tests to detect thyroid disease. One should keep in mind the difficulty of defining whether a change is accounted for by aging or by disease; the distinction is basically an arbitrary one. A change

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that occurs in everyone is a "normal" process (unless one wishes to label all older persons as diseased), whereas one that occurs in only a few persons is a disorder.

The serum thyroxine (T_4) concentration does not change with increasing age, whether measured as the total T_4 or the free T_4 (FT_4) *(3,4)*. This is curious in a way, because the uptake of radioiodine and the secretion of T4 by the thyroid glands of older persons is lower than in younger persons *(5–7)*. The explanation lies in the fact that with age there is a slower degradation and clearance rate of T_4, which matches the lower secretion of this hormone *(6,7)*; the result is a normal serum T_4 level. On the other hand, although data originally suggested that there was a markedly lower serum concentration of triiodothyronine (T_3) in the elderly *(8)* (which might have explained some of the changes of aging), this finding was confounded by concomitant disease. In older persons without significant illness, the level of serum T_3 is only slightly lower than in younger adults, and in most remains within the reference range *(3,9–13)*. The serum T_3 level can show a significant decrease by the age of 100 yr *(14–17)*, although even at this age other data suggest that the decrease may not be significant *(18)*. There may be selection bias in these data on centenarians, in that the well-known increase in antithyroid antibodies in older persons is not present in those who reach 100 yr of age *(19)*. If there is a true decrease in serum T_3 levels, it may be attributable to a relatively slow conversion of circulating T_4 to T_3. Whether or not the decrease is real, there seem to be no adverse consequences.

These modest changes in thyroid hormone (TH) physiology do not cause the serum concentration of thyroid-stimulating hormone (TSH; thyrotropin) to rise above normal *(11)*, although there is a slight increase in the mean serum level of TSH within the reference range *(20)*. One can reasonably conclude that the thyrotrope does not sense a deficiency of TH whatever the changes might be in the serum T_3 level. There may actually be a slight decrease in the secretion of TSH with age *(21)*, but the mean decrease is well within the reference range for younger adults, and does not interfere with the ability to diagnose hyperthyroidism. For practical purposes, the thyroid tests used to diagnose thyroid disease in younger adults are equally useful in older ones, provided one keeps in mind that some older persons have a low serum level of TSH without ever becoming hyperthyroid (*see* "Diagnosis" under "Hyperthyroidism" following).

That thyroid function shows only slight changes with age does not mean that the likelihood of thyroid disease is the same at all ages; as discussed in the following section, some disorders, such as hypothyroidism, are clearly more common in older persons.

HYPOTHYROIDISM

Primary hypothyroidism is much more common (>99% of all hypothyroidism) than secondary hypothyroidism *(22)*; in adults, the secondary form is usually seen only when there is clinically recognized hypothalamic or pituitary disease.

Diagnosis

If signs and symptoms were specific, the diagnosis of primary hypothyroidism would be simple and would be made on clinical grounds, but they are not *(23,24)*. The signs may be few and may seem no different to either the patient or the examiner than those of growing older. The symptoms may be vague, if recognized as symptoms at all, and attributed to age, arthritis, or any of the other illnesses so common in older persons. Even if vague, the symptoms can benefit from treatment when actually caused by hypothyroidism, and hypothyroidism should therefore be uncovered if it does exist. A direct test of thyroid function is needed to make the diagnosis.

Of the two circulating thyroid hormones, T_4 and T_3, one would expect the serum T_4 concentration to be a more direct reflection of thyroid function, because most T_3 production occurs outside the thyroid gland, whereas all circulating T_4 comes from thyroid secretion. A low serum T_4 concentration should then be a good test for thyroid failure. Although this is true for moderate to advanced hypothyroidism, the serum T_4 concentration is often within the reference range in persons with mild to moderate hypothyroidism *(25)*, whether measured as the total T_4 or the FT_4 concentration *(26)*. This is so mainly because the population range of the serum T_4 concentration is wide (about 5–12 μg/dL); a given patient could have a significant decrease in circulating T_4 and still have a serum T_4 concentration within the "normal" reference range. The thyrotroph, however, is not fooled; it easily senses even a slight fall in circulating T_4, and responds by secreting more TSH *(27)*. For practical purposes, nothing else is observed to raise the serum TSH concentration in an ambulatory patient except primary hypothyroidism as long as one uses a reliable assay. The principal marker for primary hypothyroidism at any age, including older age, is an increased serum TSH concentration. A good diagnostic approach to thyroid dysfunction is a "TSH-first" strategy; one measures the serum T_4 concentration only if the serum TSH level is abnormal, unless there is a clinical reason to do otherwise *(28,29)*.

Although the preferred test for thyroid failure is measurement of the serum TSH concentration, the test can be costly and the charge varies widely throughout the United States. Although the actual cost of doing the TSH assay is generally about the same as that for the serum T_4 assay, the charge for the TSH assay may be several times higher. The best solution is to find a reliable laboratory that charges reasonably for the serum TSH assay.

Prevalence and Incidence

Certainly not all older persons are hypothyroid. But many more older persons have thyroid failure than do younger adults, and although the percentage affected is relatively small, the actual number affected is well over a million persons in the United States.

Older surveys that did not use the serum TSH concentration as a marker found that about 2% of older persons were hypothyroid *(30)*. Community population studies in the United States and the United Kingdom showed that 4–7% of those over age 60 yr had thyroid failure when the serum TSH was used to detect it; as expected, more women than men were affected *(3,23,31,32)*. Iodine-related geography plays a role in hypothyroidism because among older women who live in relatively iodine-depleted areas the prevalence is only about 1% *(33–35)*; in a sense, therefore, iodine deficiency is protective against the development of hypothyroidism, however inadvisable it might be for thyroid function in general.

There are few incidence data for hypothyroidism in older persons, but our unpublished data show that the incidence of new thyroid failure, defined as a serum TSH concentration >10 mIU/L, is only 0.5%/yr in persons over the age of 60 if one starts with a normal serum TSH concentration, but that the incidence rises 10-fold, to 5%/yr, if the initial serum TSH concentration is slightly increased (5–10 mIU/L), findings that are roughly consistent with the follow-up data for a younger population in the United Kingdom *(36)*. Thus, a slightly increased serum TSH concentration is a clear indicator of future thyroid failure, although in some patients it may point to already symptomatic hypothyroidism.

Etiology

Almost all primary hypothyroidism in older persons is related to an autoimmune process that slowly destroys functioning thyroid tissue to the point at which the gland fails to produce enough hormone to keep TSH secretion within normal bounds. This autoimmune destruction

is associated with a lymphocytic thyroiditis and with abnormal levels of antithyroid antibodies (anti-TAbs), especially antibodies directed against thyroid peroxidase (TPO) (anti-TPO antibodies). In younger persons the disease may lead to thyroid enlargement (Hashimoto's disease), but this is much less likely in older persons, in whom the disease usually leads to an atrophic gland. The speculation is that the immune system changes with age to allow the production of abnormal anti-TAbs, although there may also be a genetic component to their production.

While it is etiologically important, measurement of these anti-TABs in serum is not a good test for thyroid failure because they may be absent in proven hypothyroidism *(37)* and, more importantly, because many older persons have abnormal anti-TAbs yet are not hypothyroid *(37–39)*. Still, a patient who has an abnormal titer of anti-TPO antibodies along with a slightly increased serum TSH concentration is more likely ultimately to develop hypothyroidism than one with normal antibodies.

In some instances, direct damage to the thyroid gland contributes to hypothyroidism in older persons. Examples are the thyroid failure that follows treatment of head-and-neck cancer with surgery and/or radiation (a common cause of hypothyroidism in older men), and hypothyroidism in anyone who has been treated for hyperthyroidism.

Risk Factors

There are a number of risk factors for thyroid failure, the presence of any one of which should trigger testing for the disorder at any age (Table 1). It is probably cost-effective to test all persons over the age of 60 yr for thyroid failure by measuring their serum TSH level (*see* "Screening and Case Finding" following), although this approach remains somewhat in dispute because of differences of opinion about what cost should be borne for this. If all persons >60 yr of age are tested, no other risk factor need be considered.

"Subclinical" Hypothyroidism

Logically, one would expect the term, "subclinical" hypothyroidism to refer to patients who are hypothyroid but who have no findings apparent to the clinician. This would mean simply that the metabolic abnormality sensed by the pituitary thyrotroph is not detected at a routine clinical encounter, and that the thyrotroph is simply more sensitive than the clinician. However, and probably because it is hard to quantify clinical sensitivity, the term, subclinical hypothyroidism, has come to be defined by laboratory tests: it now means a person with an increased serum TSH concentration and a serum T4 concentration within the reference range. This use of the term removes the word "clinical" from the definition and leaves it to the laboratory. In fact, there is no consensus on the precise meaning of the term "subclinical hypothyroidism."

Strictly speaking, "subclinical" should refer to an abnormality that has no clinical correlate. Yet a routine examination taking only a few minutes can hardly be expected to detect the subtle symptoms so commonly found on close questioning of patients with subclinical hypothyroidism. One variable, then, in the definition of "subclinical" might well be the intensity of questioning the patient. In one report symptoms were in fact found in a minority of patients *(32)*. Whether or not there are symptoms in a particular patient is important, since some of the variables to be followed during treatment are the signs and symptoms uncovered by a detailed history.

Other ill-defined aspects of subclinical hypothyroidism are: (1) whether any increase in the serum TSH concentration applies, or only those levels that are clearly raised (e.g., >10 mIU/L); (2) whether the serum T_4 concentration can be below the reference range and

Table 1
Risk Factors for Hypothyroidism

History
Age >60 yr
Hyperthyroidism
Other autoimmune disease
Addison's disease
Pernicious anemia
Diabetes mellitus (type 1)
Subacute thyroiditis (overt or silent)
Head/neck cancer (treated)
Family member with thyroid disease
Medication use
Lithium carbonate
Amiodarone
Iodine (any form)
Routine tests (if previously done)
Hypercholesterolemia
Thyroid tests (if previously done)
Slightly raised serum TSH[a] concentration
Abnormal anti-TPO[b] antibodies

[a]TSH, thyroid-stimulating hormone; TPO, thyroid peroxidase.

the patient still fit the definition if there are no apparent clinical abnormalities; and (3) whether there must be abnormal levels of anti-TAbs. Because the principal issue facing the clinician caring for the older patient is whether or not to treat, I use the term "subclinical" hypothyroidism more as a spur to take a more careful history in a patient with an increased serum TSH concentration, with an eye toward potential treatment, rather than as a diagnostic category.

Consequences of Thyroid Failure

The raised serum TSH concentration and the signs and symptoms, however vague, are some of the biologic consequences of thyroid failure in older persons, but there are others as well.

Overt thyroid deficiency has long been associated with an increased serum cholesterol level, but there is still controversy about whether or not the consequence is more cardiovascular disease. The less overt forms of thyroid failure do seem to lead to abnormal serum lipid concentrations. In younger adults with milder hypothyroidism the serum total cholesterol concentration is probably only slightly raised *(31)*, if at all *(40)*, but the serum low-density lipoptrotein (LDL)-cholesterol level can be slightly higher *(41)*, and the serum (HDL)-cholesterol level can be slightly lower, than expected in these patients *(42,43)*. In older women, one finds essentially the same pattern, with a modestly higher level of serum LDL-cholesterol and a slightly lower level of HDL-cholesterol *(44)*; there are no data on older men.

Probably more important is the question of whether or not treatment of the thyroid deficiency affects serum cholesterol levels in these patients. A recent metaanalysis indicates that treatment significantly lowers the concentration of serum LDL-cholesterol without

much effect on HDL-cholesterol; the effect was greater the higher the initial level of serum cholesterol *(45)*. One should note, however, that the average age in this metaanalysis was only 54 yr, and that in another study, although there was a significant beneficial effect of treatment on serum cholesterol, most patients still had increased levels of serum cholesterol *(46)*.

Another risk factor for coronary heart disease, the serum level of homocysteine, is also increased in younger women with hypothyroidism and fails to return entirely to normal with thyroid therapy *(47)*. Whether this is so for older persons, and whether treatment with folic acid would be of benefit, are unknown.

Whatever the effect of overt or mild hypothyroidism on serum lipids, does the hypothyroidism actually lead to coronary heart disease (CHD)? Some investigators have found an association between subclinical hypothyroidism and CHD in older men *(48)* and in older women *(49,50)* even when the data were adjusted for the level of serum cholesterol *(50)*. However, long-term follow-up of younger adults with abnormal anti-TAbs did not show an increase in heart disease or death *(51)*; there are no similar prospective data in older persons. Perhaps one must be both >60 yr of age and have persistently untreated thyroid failure to be more likely to later develop cardiovascular disease.

There is a clear neurologic deficit in overt hypothyroidism; sometimes one can even see "myxedematous madness" *(52)*. In addition to the expected mental slowness and cognitive defects *(53)*, one can see poor ventilatory responses and sleep apnea. In younger adults with subclinical hypothyroidism there are both a significant impairment in memory skills *(54)* and significant neuromuscular symptoms, such as cramps, fatigue, weakness, and paresthesias *(55)*; in older persons, our unpublished data show a significant association of mild thyroid failure with diminished responses on multiscale cognitive function testing.

The issue of an association between hypothyroidism and either dementia or depression in older persons is a difficult one, since there are no good population data to answer these questions. However, the limited data available suggest that the prevalence of thyroid failure in older persons with dementia may be no higher *(56)*, and in those with depression only slightly higher *(56,57)*, than in those without these psychologic difficulties.

One of the difficulties in assessing the consequences of mild thyroid failure is that, as one might expect, and despite the foregoing data, many patients may have no difference in clinical symptoms from euthyroid persons *(58)*. Any adverse effects may be apparent only in retrospect (i.e., after treatment), when comparisons can be made in individual patients. As noted above, however, levels of serum LDL-cholesterol improve with therapy, at least in younger patients. Also, in at least some older persons with mild hypothyroidism, treatment brings an improvement in overall symptoms *(59,60)*, in psychometric test results *(60)*, and in constipation, that bane of the older person *(61)*. No one yet knows whether the cardiovascular outcome is changed with treatment of thyroid failure in these older patients, although it is a reasonable presumption.

On balance, then, when there is a lipid or neurologic abnormality in an older person, it seems sensible to test for thyroid failure because treatment is specific and it is at least possible that other treatment for the lipid *(62,63)* or neurologic disorder *(64–66)* may be obviated.

Treatment

Several recent reviews nicely summarize the treatment of thyroid failure *(67–69)*. Once the diagnosis is made in a patient with a clearly increased serum TSH concentration (>10 mIU/L), treatment is straightforward. Oral T_4 (sodium L-thyroxine; levothyroxine) is the treatment of choice; any branded preparation that meets modern standards of the United

States Pharmacopoiea is suitable. Failure to meet these standards will lead to poor treatment *(70)*. The aims are to reverse any symptoms, to bring the increased serum TSH level down to within the reference range, and to prevent any presumed untoward outcomes of untreated hypothyroidism. The measurement of serum TSH is therefore not only the diagnostic test par excellence, but also serves as the monitor of successful therapy.

The decision to treat thyroid failure is not so simple when the serum TSH concentration is only slightly increased (>5 <10 mIU/L), particularly in older persons in whom such treatment might have more adverse consequences than in younger persons. Only some of these patients are in fact hypothyroid; those who are not would not be expected to benefit from treatment. About 40% are simply normal persons at the upper end of the TSH spectrum (remember that a reference range by definition excludes the upper 2.5% of the normal population). In the remainder, the serum TSH concentration either stays slightly elevated or progresses into the clearly increased level over time.

With a single slightly increased value of the serum TSH concentration, the clinician cannot tell whether the patient is normal or has thyroid failure. There are no studies that help to decide whether or not to treat in such cases, and clinical judgment must prevail. Symptomatic of this lack of data are recent "point–counterpoint" articles on why one should or should not treat a mild degree of hypothyroidism *(71,72)*. One approach is to undertake the detailed questioning of the patient mentioned earlier; if reasonable symptoms turn up, one can treat and follow the patient closely, looking for improvement. Because some patients do not recognize symptoms except in retrospect ("Yes, I feel better, although I didn't have a problem before"), one can also decide not to treat and to repeat the TSH measurement later; if it is still raised, particularly if there are abnormal levels of anti-TPO antibodies, one can then treat. A third method is to treat all of these patients on the assumption that by so doing one will prevent the future development of overt hypothyroidism. I do not favor this approach. If one decides not to treat a particular patient, there should be continued follow-up, with periodic measurement of the serum TSH concentration, perhaps yearly, in order to detect the development of symptoms or a clearly increased serum TSH level.

The average older hypothyroid patient needs less oral levothyroxine than does a younger patient *(73,74)*. In older persons with hypothyroidism the initial dose should be no greater than 25 μg/d, both to minimize the risk of cardiac symptoms and because the required replacement dose in older hypothyroid patients may be only 50 μg/d or slightly less *(74)*. Common sense mandates caution in treating patients with active angina or recent myocardial infarction. There is usually no hurry to completely correct thyroid failure in such cases, and one can optimize the cardiac therapy, including angioplasty *(75,76)* or bypass surgery *(77)*, even while a patient is overtly hypothyroid, before normalizing the patient's serum TSH level (in fact, the outcome of anesthesia and surgery in general seems unaffected by the presence of untreated hypothyroidism *(78)*).

After the initial dose of levothyroxine has been taken for at least 4–6 wk, and preferably for 2–4 mo, another measurement of the serum TSH concentration will show whether or not its value has returned to normal. Treatment of shorter duration may not allow the serum TSH level to reach equilibrium, and there is no need to hurry. If the serum TSH value has not come down to within the reference range with the initial dose of levothyroxine, which is the usual case, the daily dose is increased to 50 μg/d for another 2–4 mo and another measurement is made of the serum TSH concentration. This "titration" of the oral dose of levothyroxine against the serum TSH value, with the daily dose of levothyroxine being increased by 25 μg/d at the interval visits, then continues until the value falls to within the reference range. If cardiac symptoms occur or persist, one may have to compromise

and limit the dose of oral levothyroxine to one that leaves the serum TSH level somewhat increased.

Once a stable dose of oral levothyroxine is established, treatment should be continued for life; hypothyroidism remits spontaneously in a few patients (e.g., occasionally in those with Hashimoto's thyroiditis), but this is uncommon and unpredictable. Moreover, patients are not entirely reliable; a substantial minority of older treated patients have either too high or too low a serum TSH concentration *(79–81)*. Thus, monitoring the serum TSH level also needs to be continued for life; the usual practice is to measure it one to three times per year (more often if there is an abnormality), but there are no data on the optimum frequency of measurement. If the TSH value remains within the reference range for a year or two, one can judge that the patient is stable and decrease the frequency of TSH measurement to once every year, or even every two years.

An example of diffident compliance that shows the need for periodic monitoring is the fairly common combination of high levels of both serum TSH and serum T_4 as a result of the patient's having "made up for" missed doses in the several days before a visit. In such cases the serum TSH concentration is high because of the missed doses, and does not come down as quickly as the serum T_4 rises. If this erratic dosing persists, one can consider giving a single weekly dose of levothyroxine equal to seven times the required daily dose *(82)*. This regimen would be needed only for an unusual patient; although safe and effective in younger adults, it has not been shown to be so in older persons. An abnormal serum TSH level in a patient taking a constant dose of levothyroxine, not explained by poor or erratic compliance, is reason enough to change the dose.

During the course of thyroid therapy, one should watch for the effect of concurrent medications that might alter the efficacy of oral levothyroxine. The absorption of oral levothyroxine, which is already somewhat less in older persons *(83)*, can be decreased by several medications, including aluminum hydroxide antacids, sucralfate, ferrous sulfate, large amounts of dietary fiber or soy-based foods, or any of the exchange resins used as drugs, including cholestyramine, colestipol, and Kayexalate™. Further, androgen therapy for breast cancer in older women can increase the effect of a given dose of oral levothyroxine, necessitating a reduction in dose *(84)*. The opposite is also true, that is, the institution of estrogen therapy in an older woman already being treated for hypothyroidism can raise the required dose of oral levothyroxine *(85)*. These effects are not quite predictable and are best assessed by more frequent measurements of serum TSH.

One concern with regard to an untoward outcome of oral levothyroxine therapy is the induction of bone loss, which might predispose to fractures in older persons. However, bone mineral density seems unaffected by levothyroxine therapy provided one does not "overshoot" and the serum TSH level stays within the reference range. Similarly, one might avoid the increased risk of atrial fibrillation associated with a low serum TSH concentration induced by excessive levothyroxine therapy *(86)* by careful monitoring of the serum TSH.

Screening and Case-Finding

Physicians rarely do true "screening," which is the testing of an entire community or population; they usually do "case-finding," which is testing for a disease in patients who come to them with a complaint of one kind or another. In the case of thyroid failure, the presence of a risk factor (Table 1) is sufficient reason to test for hypothyroidism at any age, and age itself is also a risk factor for hypothyroidism. Because: (1) the clinical findings of thyroid failure in older persons are vague and nonspecific, (2) the only reliable way to make the specific diagnosis is to measure the serum TSH concentration, (3) the number

of older persons with thyroid failure is reasonably high, and (4) there is an effective and easily given treatment, one can argue that on these grounds alone all older persons should be tested for thyroid failure *(87–89)*.

Others prefer to be more cautious because the benefits and cost-effectiveness of testing for and treating thyroid failure have not been shown in a prospective, controlled trial. They would not measure the serum TSH concentration in every older person, but would focus on women rather than on men or on measurement of the serum T_4 rather than the TSH concentration *(90)*. Whether to use the measurement of serum T_4 or TSH is largely a matter of the predictive value and the charge for the test; the serum TSH assay is more predictive of hypothyroidism, and its cost should soon more closely approach that of the serum T_4 assay, since the actual costs are not much different. To focus only on women rather than on men does a disservice to older men, 2.5% of whom have subclinical hypothyroidism *(23)*.

It is true that there is no prospective treatment trial but that is also true for the treatment of overt hypothyroidism itself. Furthermore, using decision analysis and reasonable assumptions, one can show that the measurement of serum TSH for detecting and treating thyroid failure in women as young as 35 yr of age is as cost effective (in terms of quality-adjusted life-years) as is the use of mammography to detect breast cancer *(91)*. Although the benefits of treating mild thyroid failure are not as clear as one might like, the data discussed previously indicate that a substantial group of patients do improve with such treatment, and that the only way to identify improvement is for the physician to treat and assess the outcome in the individual patient. The only way to do this is to identify the patient in the first place.

In sum, thyroid failure in older persons, whether mild or overt, is common and can be detected reliably only with measurement of the serum TSH concentration. A strong case can be made for testing all persons over the age of 60 yr. Treatment with oral levothyroxine is straightforward, although it needs reasonable caution. Its benefits are likely to be real, but require individual assessment.

HYPERTHYROIDISM

As does thyroid failure, hyperthyroidism in older persons often does not produce the overt signs and symptoms seen in younger adults *(92,93)*. Many have only weight loss, fatigue, or irritability as complaints, and there may be no goiter, tachycardia, tremor, or exophthalmos *(94–96)*. They can appear placid or depressed, may have no increase in appetite, and actually have constipation rather than frequent stools. This so-called "masked" or "apathetic" hyperthyroidism *(97)* is more the norm in the older patient than is the hyperactive, nervous, tremulous, and sweaty state of the younger hyperthyroid patient.

Diagnosis

Because the clinical findings are not particularly specific, there must be a high index of suspicion in order to detect hyperthyroidism in older persons. Measurement of the serum T_4 concentration is not good enough, because it is sometimes increased in older persons without hyperthyroidism *(98)*, particularly if they are otherwise ill *(99–101)*; conversely, the serum T_4 concentration may only be in the upper end of the reference range even in those who actually do have hyperthyroidism *(102)*.

The development of highly sensitive assays for serum TSH, with a functional sensitivity of <0.05 mIU/L, has completely changed our ability to diagnose hyperthyroidism accurately. Few if any patients have hyperthyroidism if the serum TSH concentration is >0.1 mIU/L; with one of the new assays, a value >0.1 mIU/L nicely eliminates the diagnosis *(103)*. On the

other hand, a clearly low value in a highly sensitive assay (<0.1 or even <0.05 mIU/L) does not of itself make the diagnosis. There should be a clearly increased serum T_4 concentration, or if necessary an increased serum T_3 concentration, and at least some clinical clue before the diagnosis is made. The laboratory cannot supply the complete answer.

Prevalence

The prevalence of hyperthyroidism in older persons is about 0.2–0.3% *(104–106)*, and is less than one-tenth the prevalence of thyroid failure; older data suggesting that hyperthyroidism is more common than this did not have the advantage of modern TSH assays. Further, although some authors have thought that the disorder was more common in older persons *(107)*, that seems not to be so in iodine-replete areas such as parts of Sweden *(108)* or the northeastern United States (Sawin, unpublished data). The data that suggest an increased incidence of hyperthyroidism with age largely arise from areas of previous iodine deficiency. The consequent growth of autonomous thyroid nodules raises the susceptibility of older persons living in these areas to the induction of hyperthyroidism as toxic nodular goiter *(109)*. Correction of the endemic iodine deficiency eventually removes this problem *(110,111)*.

Etiology

Most hyperthyroidism in older persons in the United States is caused by the same autoimmune process that causes Graves' disease in younger patients *(95)*, although as just noted, toxic nodular hyperthyroidism accounts for a larger but still minor fraction in areas of prior iodine deficiency, and as a result there is a certain degree of geographic variation in the relative incidence of the two types. Older persons are also subject to less common causes of hyperthyroidism, such as thyroiditis *(112)* or lithium carbonate therapy *(113)*.

Risk Factors

There are some clinical findings that should raise the suspicion of hyperthyroidism. Most of these (Table 2) are obvious to the clinician, but are sometimes forgotten when the focus of attention lies elsewhere. Cigarette smoking has recently been proposed as another risk factor *(114)* but epidemiologic data suggest that this may not be so *(105)*.

"Subclinical" Hyperthyroidism

The term "subclinical" hyperthyroidism is often used to describe persons who have a clearly low serum TSH concentration (<0.1 mIU/L) in the absence of increased concentrations of either serum T_4 or T_3. In general, one excludes from the definition those patients who have a reasonable explanation for the low value of serum TSH (e.g., the administration of dopamine or large doses of glucocorticoids), in whom the serum T_4 level is usually low-normal or low, and confusion with possible hyperthyroidism is unlikely. These patients are usually quite ill and in the hospital; note that serious illness itself can occasionally induce a low value for serum TSH.

One can include in the definition of subclinical hyperthyroidism hypothyroid patients who take too much oral T_4. Sometimes such doses are intentional, as in the treatment of thyroid cancer. Sometimes, however, they are inadvertent and occur when patients with primary hypothyroidism take too much oral T_4. Once recognized, the management of such patients is a matter of clinical judgment about whether or not the patient's dose should be adjusted and by how much.

Table 2
Risk Factors for Hyperthyroidism

History
Family member with thyroid disease
Known goiter
Cigarette smoker (?)
Current findings
Atrial fibrillation
Goiter
Osteopenia
Congestive heart failure, unexplained
Medication
Amiodarone
Iodine (any form)
Lithium
Routine tests
Radioopaque dye
Thyroid tests
Low serum TSH[a] concentration

[a]TSH, thyroid-stimulating hormone.

A quandary arises with ambulatory patients who have a spontaneously low serum TSH concentration and a serum T_4 level within the reference range, in the absence of symptoms of hyperthyroidism or of a recognized cause for the low serum TSH level. Some of these patients are actually hyperthyroid, as noted earlier; a clearly increased level of serum T_3 may provide the answer in such cases, although an older person is unlikely to have an increased serum T_3 level without a high concentration of serum T_4. Another group of these patients has autonomous thyroid function, often associated with a nodular gland (the "hot" nodule or multinodular goiter), but are still euthyroid *(103)*; production of T_4 is approximately adequate to maintain the euthyroid state, but is not under the control of pituitary TSH, and therefore the serum TSH concentration falls. There are no firm data on the natural history of spontaneous subclinical hyperthyroidism, but certainly some older persons with this condition go on to overt hyperthyroidism *(86,106,115,116)*, probably more often than do those with a normal serum TSH level. It is equally certain that in many the serum TSH concentration returns to the easily detectable or even normal range *(86,106,115)*; one would not want to take action without repeating the test.

Whether or not to treat spontaneous subclinical hyperthyroidism is not an easy decision, but the problem is even more difficult because some older persons (about 1%) seem perfectly normal and just happen to have a serum TSH concentration well below the reference range (<0.1 mIU/L) *(106,117)*; there are only a few such patients but they outnumber the ones who are actually hyperthyroid.

Consequences of Clinical or Subclinical Hyperthyroidism

The main consequences of concern in both clinical and subclinical hyperthyroidism are those affecting the brain, the bones, and the heart. One is always worried that abnormal behavior might be attributable to excess TH, but no one has shown that hyperthyroidism is more common in older persons with psychiatric disease than in others. However, patients

with subclinical hyperthyroidism do have an increased risk of becoming demented, a factor that might be taken into account in whether or not to treat the condition *(see* below*) (118)*.

There has been much concern and many publications about the effect of hyperthyroidism on the bones of older persons, because any resulting decrease in bone density could predispose to fractures. In fact, a history of hyperthyroidism is one of many risk factors for hip fractures in older white women *(119,120)*, and these fractures occur at an earlier age than otherwise *(121)*.

It now seems that in levothyroxine-treated older women, the two main thyroid factors that lead to lower bone density are a past history of overt hyperthyroidism *(122–124)* and the use of suppressive doses (serum TSH concentration <0.1 mIU/L) *(125–128)*. Of the two, past hyperthyroidism is more important in older women than is the use of oral levothyroxine *per se (123,124,129)*, unless the suppressive dose of T_4 is continued for a decade or more, as in the treatment of thyroid cancer *(125–127)*. Yet even then there may be no significant bone loss *(130)*. It is important to note that one can avoid thyroid-related bone loss in older women through the use of estrogen therapy *(131,132)*. One can, as expected, also avoid it by adjusting the dose of oral levothyroxine so as to maintain the concentration of serum TSH within the reference range *(133)*, although this may be difficult to do in patients who have had thyroid cancer.

Almost no data exist on thyroid-related bone density in older men but one modest-sized study failed to find decreased bone density in men treated with oral levothyroxine for an average of 15 yr *(134)*.

What about spontaneous subclinical hyperthyroidism? Older women with autonomously functioning nodular goiter have a lower bone density *(135,136)*, and our unpublished data show a significantly lower bone density in older women with a low serum TSH level, but only in the distal radius. Perhaps more importantly, the risk of fracture is significant (about threefold) even when hyperthyroidism is only subclinical *(137)*. Fortunately, the bone loss is stopped, at least in women with nodular goiter, if the subclinical hyperthyroidism is successfully treated *(138)*.

The cardiac effects of hyperthyroidism are of real concern because of the high rate of cardiovascular disease in older persons. Only anecdotal stories exist of hyperthyroidism in association with myocardial infarction, worsening angina, or precipitation of congestive heart failure, but the wise clinician will be on the lookout for these.

There is a clear and well-known association of hyperthyroidism with atrial fibrillation (AF), a common arrhythmia in older persons. Of interest is that the association also holds for those older persons with subclinical hypothyroidism; a low serum TSH concentration triples the risk of later AF *(86)*. Because AF is now often treated with amiodarone, clinicians need to be aware that this drug can itself cause hyperthyroidism (as well as hypothyroidism) *(139)*; amiodarone can also prolong the action of warfarin and cause bleeding if the patient is not appropriately monitored.

Treatment

In general, treatment of the older person with proven hyperthyroidism is no different than for the younger person. The modalities have not changed in 40 yr: antithyroid drugs (ATDs), radioiodine (RAI), and surgery. The wide international differences in the use of the ATDs and RAI *(140)* show that neither modality is better than the other; few clinicians use surgery as the initial approach. If for some reason these therapies cannot be used, stable iodine in one form or another is an option *(141)*. Most clinicians in the United States lean toward

the use of RAI in older hyperthyroid patients, but informed patient preference should be the determining factor.

What to do with a patient who has subclinical hyperthyroidism is not so clear. Unless there is a clinical finding such as weight loss or AF, one would hesitate to offer an ablative therapy such as RAI even when a low serum TSH concentration has been confirmed as persistently low. One could search for an autonomous nodule which, if found, would usually lead to treatment with RAI. One could treat with an ATD, even when an autonomous area exists, with the idea of avoiding further bone loss *(142)*. Because of the increased risk of later AF, some clinicians would empirically treat anyone with persistently low values of serum TSH. I am ambivalent about this last option. My preference is careful follow-up with attention to monitoring not only the serum TSH level to see if it remains low, but also the serum T_4 level, and perhaps the serum T_3 level. If the serum level of T_4 rises by more than 2 μg/dL, or if the serum T_3 concentration clearly rises out of the reference range, then I would lean toward treatment with an ATD, since this degree of change is unusual in a normal person *(143)*. I have learned that some practitioners now make the diagnosis of subclinical hyperthyroidism when the serum TSH level is low but not diagnostic (i.e., between 0.1 and 0.5 mIU/L), and then treat with antithyroid medication to see if the serum TSH level reenters the reference range; this approach should be strongly discouraged.

Whether any of these approaches changes the overall outcome, or whether treatment of subclinical hyperthyroidism avoids the presumed increased long-term mortality of overt hyperthyroidism *(144)*, is unclear. The sad fact is that no one knows what approach is best.

In sum, hyperthyroidism in older persons, like hypothyroidism, presents in vague and nonspecific ways. The diagnosis requires the use of a highly sensitive TSH assay; the TSH concentration must be low (<0.1 or <0.05 mIU/L), but this is not sufficient for diagnosis because a low serum TSH concentration is not entirely specific for hyperthyroidism. Other markers, such as the serum T_4 concentration, the serum T_3 concentration, or clinical findings are necessary to make a diagnosis.

Subclinical hyperthyroidism has definite associative risks, but the decision to treat should not be automatic, and requires careful thought.

GOITER, THYROID NODULES, AND THYROID CANCER

The evidence regarding changes in thyroid size with age is conflicting. Some data show an increase in thyroid size with age *(145)*, which may lead to a palpably nodular goiter *(146)*, whereas other data show no change *(147)* or a decrease in size with age *(148)*. Because the distribution of these data is widespread, local variation in iodine supply may account for these differences.

There does not seem to be a systematic increase in palpable goiter in older persons. This impression is supported by data from the Framingham Study; the cross-sectional prevalence of palpably single or multiply nodular goiter was 4.2% in the early 1950s *(149)* and only 0.9% 28 yr later in the same population (Sawin, data unpublished). This low prevalence of goiter in older persons is consistent with U.S. National Health Interview Survey data, based on patient interviews, which showed a goiter prevalence of 0.4% without a change with age above the age 45 yr *(150)*.

The advent of thyroid ultrasonography has permitted confirmation of older autopsy data showing that 18% of clinically normal thyroid glands had nodules of >2 cm at autopsy, and that many glands with a single palpable nodule were actually multinodular *(151)*. The

use of ultrasound to detect thyroid nodules reveal a slight but not statistically significant trend toward increasing thyroid nodularity with age *(152)*. Nevertheless, the prevalence of ultrasound-detected thyroid nodules is so high (>50%) in admittedly selected populations that include older persons *(152–154)*, as is also the likelihood of multinodularity in apparently singly nodular glands *(155)*, that most nodules cannot represent cancer in the sense of a lethal disorder; they are "incidentalomas," and can be left alone unless they grow *(155,156)*.

Sometimes, a nontoxic goiter, usually multinodular, will become quite large even in iodine-replete areas. Most of the patients in such cases are older women; in many the goiter becomes obstructively symptomatic or cosmetically distressing. A reasonable treatment option, particularly for those who do not care for surgery or are poor surgical risks, is a large dose of RAI that is effective at reducing goiter size and relieving symptoms *(157–159)* without worsening the degree of obstruction *(158,160)*.

When an older person has a "cold" palpable thyroid nodule, it is more likely to harbor histologic malignancy than that in a younger person *(161)*, but even so, the percentage of older persons with malignancy is still only about 10%. When an older person gets overt thyroid cancer, survival is poorer than in younger persons *(162–164)*. Few patients with anaplastic cancer—a condition more common in older persons—live more than a few months no matter what is done; a few may survive up to several years if the tumor is resectable *(165)*.

THYROID DISEASE AND SERIOUS ILLNESS

Not only are some thyroid disorders more common in older persons, but so also are other serious illnesses. Serious illness of itself, as well as some medications, can distort thyroid tests, and can sometimes disturb thyroid function, so that it can be difficult, when one suspects thyroid dysfunction in an older sick person, to tell whether the patient has thyroid disease or not. These changes in thyroid tests, and possibly in thyroid status, are often called "nonthyroid illness" (NTI), an awkward term that implies a thyrocentric view of medicine, or are alternatively called the "euthyroid sick syndrome," which suggests that there is a specific constellation of findings and that the patients have normal thyroid function *(166)*. Either of these terms is reasonable, since there is no good term for such conditions. Suffice it to say that thyroid tests can give strange results in a seriously ill patient, which reverse spontaneously if the patient survives *(167)*.

Two difficulties arise: (1) How does one make a diagnosis of thyroid disease in an older sick person? (2) Should illness-induced test abnormalties lead to TH treatment?

The effect of illness on the conversion of T_4 to T_3 is well-known *(168)*; the resulting lower levels of serum T_3, however, do not usually affect the diagnosis of thyroid disease. As mentioned above, illness in older persons can raise the level of serum T4, whether measured as total or FT_4 *(169)*, but illness can also lower the serum T_4 concentration, whether total *(170,171)* or free *(169,171–173)*, thus suggesting hypothyroidism. One serious problem is that the detection of a low serum FT_4 concentration is highly method-dependent, which may point to the method rather than to the patient as the source of an abnormality *(169,172)*. With some methods, only a few (<10%) patients have a low FT_4 value *(169,172)*.

None of these changes in serum T_4 test results would matter in efforts to diagnose thyroid dysfunction in sick persons if the serum TSH concentration were always reliable. Fortunately, most of the time the serum TSH level is quite reliable and stays within the reference range *(172)*. In this case, the difficulty is that a normal serum TSH level can occur in combination with a low level of FT4, with the implication that there is an illness-induced form of secondary hypothyroidism *(170,173)*. In fact, it is highly likely that there

is a decrease in hypothalamic TRH secretion in seriously ill patients *(174)*. The issue is further confounded by the suppression of the serum TSH concentration by the dopamine and glucocorticoids often given to these sick persons in the hospital; these medications can cause the serum TSH level to fall to <0.1 mIU/L, and may account for most of the very low levels of serum TSH seen in sick patients *(171,175)*. Even with a good modern assay for TSH, there remains some overlap of the values found in seriously ill patients with those in hyperthyroid patients *(176)*.

Probably the best tack to take in seriously ill persons is to defer the assessment of thyroid dysfunction unless there is reason to think that the outcome of the serious illness would be benefited thereby. The classic findings in overt hypothyroidism (a clearly increased TSH and low T_4 concentration) and overt hyperthyroidism (a clearly increased T_4 and low TSH concentration) are likely to remain definitely abnormal in the presence of serious illness. But if there is only mild thyroid dysfunction, confusion may reign; fortunately, a lesser degree of thyroid disease is less likely to affect the outcome of the serious illness. In any case, one needs to use caution in coming to a therapeutic conclusion.

If there is a low serum T_4 concentration and a probable hypometabolic state, does it help to give the patient T_4? Briefly, the answer is no *(170)*. It should also be noted that when patients recover from an episode of serious illness the serum TSH concentration can rebound and rise to within the clearly "hypothyroid" range (>20 mIU/L) *(177)*. This is not true hypothyroidism, but is part of the recovery phase; it is associated with an increase in the reduced serum T_4 level toward normal, and lasts only a few days.

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7 Autoimmune Thyroid Diseases

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Contents

THE IMMUNE SYSTEM AND AUTOIMMUNITY: *A BRIEF OVERVIEW*

The immune system represents the body's main defense against substances derived from external sources (e.g., bacteria and viruses) and from abnormal processes within the body (e.g., tumor cells). These substances are collectively termed "non-self." In order to keep the immune system in the correct range of activity, the process of non-self recognition must be highly specific and possess a memory. The specificity is conferred by peculiar receptors on the cells of the immune system able to bind foreign substances (antigens), and by the production of soluble effector proteins (antibodies) with specific antigen-recognition capacity.

The basic constituent of the immune system is the lymphocyte, which may be further subdivided in two major independent but interrelated populations: T cells (which mature within and are functionally dependent on the thymus) and B cells *(1)*. T and B lymphocytes have cell-surface receptors that can interact only with a small number of structurally related antigenic molecules, and the specificity of these receptors for a given antigen exists before the first contact of the antigen with the receptor. The other constituents of the immune system are the mononuclear phagocytic cells, consisting of the two different functional cell types of tissue macrophages, whose principal role is to remove particulate antigens, and monocytes and other antigen-presenting cells (APCs), whose role is to present antigen to specifically sensitized lymphocytes *(1)*.

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Table 1
List of Select CD Molecules Characterizing Lymphocyte Subsets and Leukocytes

Antigen (synonyms)	*Main cellular reactivity*	*Function*
CD2 (LFA-2)	T cells	Adhesion molecule
CD3 (T3, Leu 4)	T cells	Part of T-cell-receptor complex
CD4 (T4, Leu 3)	Class II-restricted T-helper subset	MHC class II receptor
CD8 (T8, Leu2)	Class I-restricted T suppressor-cytotoxic subset	MHC class I receptor
CD11a (LFA-1)	Leukocytes	Adhesion molecule (cognate to ICAM-1)
CD16 (FcR III)	NK cells, monocytes, granulocytes	Fc receptor (low affinity)
CD 19	Pre-B and B cells	Part of B-cell antigen-receptor complex
CD 25 (TAC)	T cells, B cells (activated); monocytes	α-Chain of IL-2 receptor
CD 28 (Tp44)	T cells	Receptor for B7-1 and B7-2
CD 40	B cells	Marker of B-cell activation
CD45RO	Subset of peripheral T cells	Marker of naive T cells
CD54 (ICAM-1)	T and B cells	Cognate to LFA-1
CD56 (NKH1)	NK cells, activated T cells	NK marker

ICAM-1, intracellular adhesion molecule-1; LFA-1, leukocyte function-associated antigen-2; MHC, major histocompatibility complex; NK, natural killer.

Besides antigen receptors, lymphocytes and other leukocytes express several molecules on their surface that can be used as markers to distinguish populations and subsets of these cells. A systematic nomenclature (the cluster determinant [CD] system), has been developed for these markers, the term "cluster determinant" deriving from the cluster analysis of a large number of monoclonal-antibody reactivities of these markers. The ontogenesis and the functions of T and B lymphocytes can be operationally defined by functional and phenotypic (based on CD expression) criteria *(2)*, as summarized in Table 1.

T lymphocytes are mainly involved in cell-mediated immunity reactions and in the regulation of the immune response itself. As shown in Fig. 1, T-cell receptors (TCRs) for an antigen are expressed on the membrane surface. In most T cells (95%), the antigen-specific binding portion consists of 2 disulfide-linked α and β chain heterodimers, while in about 5% are present different chains (γ and δ) *(3)*. TCR proteins derive from very large genes containing 40–100 different variable (V) segments, diversity (D) segments, many junctional (J) segments, and one or two constant (C) segments *(4)*. During development these gene segments are differently rearranged in each T cell, resulting in a random contribution of different V, D, J, and D segments and a unique gene sequence. This mechanism provides the wide diversity of TCRs required to recognize any potential antigen, and implies that the development of specific TCRs is a consequence of genetic instruction rather than a response to antigen exposure. Thus, before the induction of tolerance, T lymphocytes are able to recognize not only foreign antigens, but also autologous structures, including thyroid autoantigens. The individual DNA sequence characteristic of rearranged TCR genes can be analyzed by restriction-enzyme digestion or by reverse transcription–polymerase chain reaction (RT–PCR) to detect the individual V-gene repertoire of T cells *(5)*. Using this

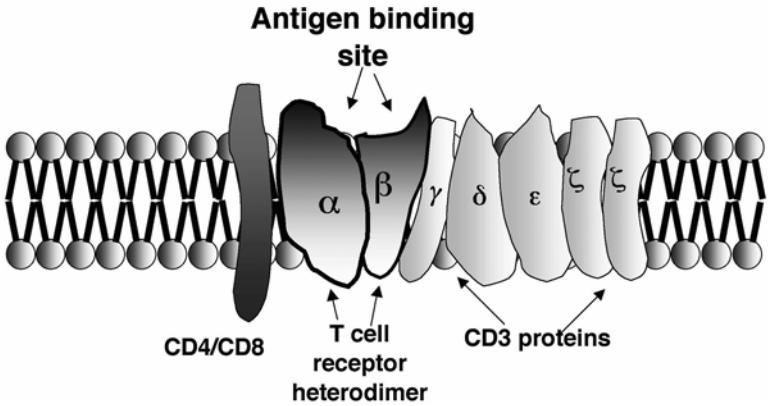

Fig. 1. T-cell receptor (TCR) and its subunits. The TCR subunits are anchored in the plasma membrane and held together by S–S bonds. (*See* text for details.)

approach on cloned, antigen-specific T lymphocytes, it has been possible to show that specific V-gene segments are preferentially used in response to certain antigens *(5)*.

As shown in Fig. 1, TCR molecules are strictly associated with five other transmembrane polypeptides (γ, δ, ε, η, and θ) comprising the subunit presently termed CD3 in humans *(6)*. The TCR is responsible for the specific recognition of antigen, while the five components of the CD3 complex are involved in the signal transduction that initiates cellular events *(6)*. For a proper recognition, the antigen must be associated with a class I or class II major histocompatibility-complex (MHC) molecule *(1)*; furthermore, accessory molecules recognizing MHC determinants and adhesion molecules interacting with their ligands on the surface of APC are needed for full stimulation of T lymphocytes (*see* following in this Subheading and Fig. 2). The epitope recognized by each TCR consists of one specific short peptide sequence of eight or nine amino acids (aa) for MHC class I-restricted, and 13–17 aa for MHC class II-restricted T cells *(7)*.

The T lymphocytes recognizing antigens associated with class I [human leukocyte antigen [HLA]-A,B.C) and class II (HLA-DR, DP, DQ) MHC molecules are phenotypically and functionally distinct. Typically, $CD8^+$ T cells are class I MHC-restricted and $CD4^+$ T cells recognize antigens in association with class II MHC molecules. This phenomenon underscores the pivotal role of the MHC in the immune response. The genes for the HLA molecules are located on chromosome 6 in a large complex involved in the control of the immune response *(8)*. HLA molecules are surface dimeric molecules (α-chain and β_2-microglobulin for class II, and α and β chains for class II) (Fig. 3).

The HLA genes are highly polymorphic (many alleles for each gene), and display a strong evolutionary drive. Although the precise reason for such a drive toward diversity is unknown, it is conceivable that each HLA allele leads to the selection of a unique T-cell reportoire in the individual, thus providing a wide diversity of repertoires in the general population, and this may be beneficial for the species.

The presentation of antigen to T-lymphocytes is mediated preferentially by "professional" antigen-presenting cells (APCs) (i.e., monocytes, macrophages, dendritic cells, and Langerhans cells) *(9)*. B cells (which express class II MHC molecules) are also able to present antigens. A variety of other cells (fibroblasts, glial cells, epithelial cells [including thyroid cells]) that normally do not express class II MHC molecules on their surface may present antigens to T cells after abnormal expression of HLA-DR. These "nonclassical" APCs,

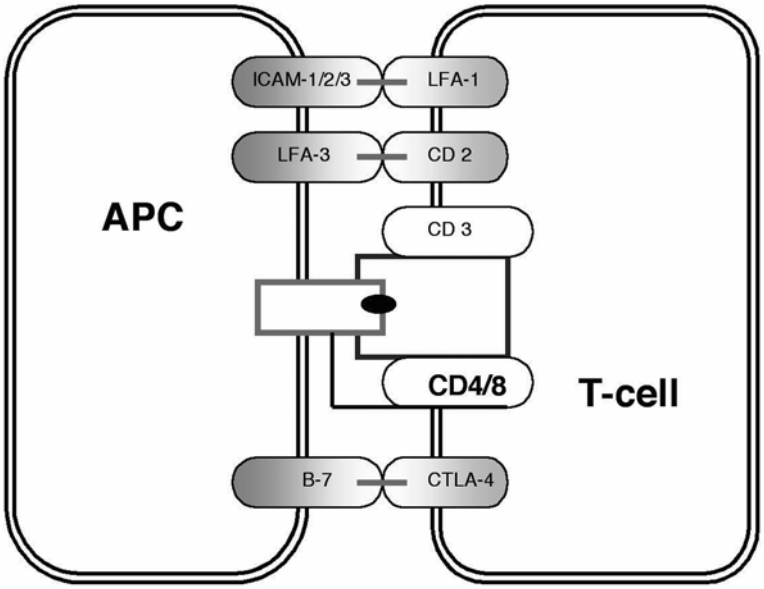

Fig. 2. Cartoon representing the molecules involved in antigen recognition by T lymphocytes. The antigenic epitope is presented to the TCR in strict association with MHC molecules. Other costimulatory and adhesion molecules are also depicted. (*See* text for details.)

however, are less effective in antigen presentation because they do not provide costimulatory signals (*see* "Cellular Mechanisms" following). The process of antigen presentation by professional APCs includes endocytosis of the antigen (e.g., a foreign protein), followed by internalization and digestion of the protein into short linear peptides *(1)*. Some of these peptides become associated in the Golgi apparatus with HLA molecules and are then transported to the cell surface and presented as epitopes to T-lymphocytes.

In addition to TCRs and HLA molecules, other T-cell membrane proteins also participate in the immune response. These peptides specifically bind some ligands on the APC surface and transduce an additional or "second" signal needed to activate T lymphocytes *(10)*. Some of these peptide molecules have been identified, as shown in Fig. 2. CD4 and CD8, which bind MHC molecules, class I and class II respectively, are the most widely studied. It is also well documented that stimulation of T cells requires the presence of costimulatory molecules such as B7-1 (also known as CD80) and B7-2 (also known as CD86), expressed on professional APCs. B7-1 reacts with its receptor (CD28), located on $CD4^+$ cells, to provide stimulatory signals needed for T-cell differentiation and cytokine production. B7-2 reacts with a second receptor, called cytotoxic T-lymphocyte A-4 (CTLA-4), which mediates inhibitory signals to T lymphocytes *(11)*. Adhesion molecules (surface molecules involved in cell–cell interactions) are also involved in the immune response. The main function of these molecules is to facilitate processes in which close contact of cells is required *(1)*. On the basis of structure and function, there are three main families of adhesion molecules, known respectively as integrins, selectins, and cadherins (calcium-dependent adherins) *(1)*. There are also tissue-specific adhesion molecules called addressins, which are mainly involved in targeting lymphocytes to particular groups of peripheral tissues *(1)*. Integrins mediate the interaction between APCs and T cells; the binding of leukocyte function-associated antigen-1 (LFA-1), expressed on T cells, thymocytes, and other mononuclear cells including APCs, to intercellular adhesion molecule-1 (ICAM-1, a glycoprotein present on endothelial

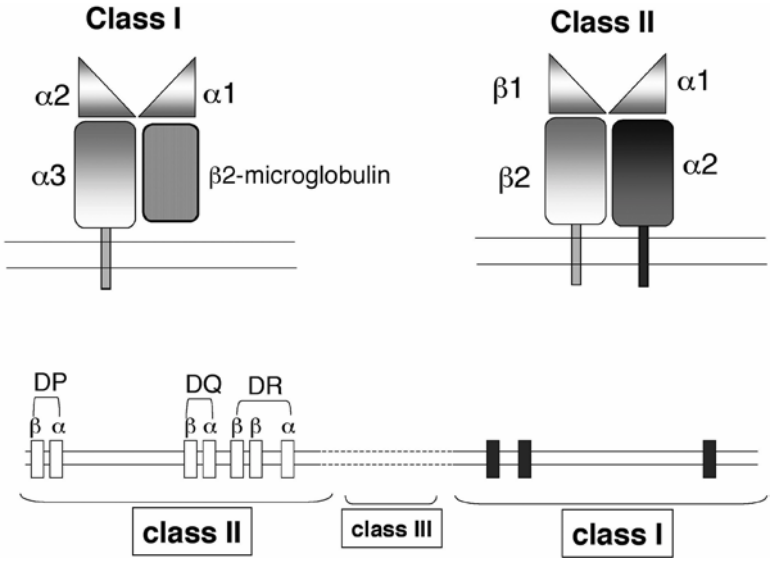

Fig. 3. The structure of class I and class II MHC molecules (above) and a schematic representation of the genes encoding the human MHC molecules (HLA) on chromosome 6 (below). The gene encoding β_2 microglobulin is located in a different chromosome (chromosome 15).

cells and extracellular matrix) is important for the initial interaction of these immune cells with surrounding tissues. Both antigen presentation and costimulatory signals are required to activate a naive T-lymphocyte, whereas previously activated T cells are less dependent on costimulation *(10)*.

The function of B-lymphocytes is to produce antibodies, a family of glycoproteins called immunoglobulins (Igs), which bind specifically to the antigen that induces their formation. Ig molecules have a common basic structure consisting of two light (L) and two heavy (H) polypeptide chains linked together by disulfide bonds. Igs may be differentiated into five different types (IgG, IgM, IgA, IgD and IgE), which have different molecular weights, amino-acid composition, carbohydrate content, electric charges, and effector functions.

The specificity of the Igs for an antigen is conferred by the extremely polymorphic amino-acid sequence of the amino-terminal regions of both the H and L chains, which are known as variable (V) domains. Variation in the V domain, especially within the highly variable segments known as hypervariable regions, produces more than 10^7 different antibody molecules, each having an individual structure (idiotype) that specifically fits the conformation of an antigen molecule (called an epitope). Each B cell produces a unique Ig molecule that results from rearrangements of the Ig genes, which share the same segment organization (V, D, J, and C) as TCR genes *(12)*. Further diversity is provided by antigen-driven somatic mutations occurring during B-cell clonal expansion. In contrast to T-cells, B-lymphocytes secrete their unique Igs into the surrounding fluids, where they represent circulating antibodies. Some Igs remain on the B-cell surface, behaving as antigen receptors. This recognition process normally involves the "conformational" shape of antigen epitopes, and requires (in contrast to the case for T cells) unprocessed or "native" antigen.

After antigen presentation, T-lymphocytes undergo proliferation and functional differentiation into several subsets, including helper, suppressor, and cytotoxic cells. To some extent, these types of lymphocytes may be phenotypically distinguished on the basis of CD markers (Table 1). Helper ($CD4^+$) T lymphocytes are typically activated by antigens

presented in strict association with MHC class II proteins. Cooperation of helper T cells with B lymphocytes is the prerequisite for B-cell proliferation and differentiation into antibody-secreting plasma cells. This cooperation is effected through a direct interaction of helper T cells with B lymphocytes having antigen bound to the cell surface, and by the production of antigen-nonspecific cytokines. Some $CD4^+$ and some $CD8^+$ T lymphocytes provide suppressor signals (suppressor T cells) that govern several immune responses: they control the number of helper T lymphocytes, directly inhibit B-cell activation and Ig secretion by plasma cells, and may also suppress the activity of cytotoxic T cells. The precise pathway of T-suppressor activation is still unknown: it probably involves feedback circuits to limit responses to antigens *(10)*. A similar feedback has been identified in B cells, where the specific Ig idiotype behaves as an antigen able to stimulate other B cells to secrete antiidiotype antibodies *(13)*. The main function of cytotoxic T lymphocytes is to specifically recognize and destroy cells containing non-self components. Unlike helper T cells, this activity is exerted after recognition of the antigen in strict association with autologous MHC class I molecules, or class I antigens alone on allogeneic and xenogeneic cells. The majority of cytotoxic T cells are $CD8^+$, although approx 10% are $CD4^+$ and may recognize antigens associated with class II MHC molecules. Cytotoxic T cells are particularly involved in protection against viral diseases, and possibly some types of tumors.

It should be noted that T cell function is much more complex than briefly summarized before. In particular, the very concept of "suppression"—particularly the possibility of identifying T-suppressor cells on the basis of specific CD expression—has been often contested. This is because T lymphocytes can function as suppressor or helper cells at different times in their life cycle, and because the CD4 antigen, although typically associated with helper cells, may be expressed on "suppressor" or "cytotoxic" T lymphocytes. Nevertheless, a suppressor activity may be operationally defined under certain experimental conditions, and this activity is often associated with expression of specific CDs. If the inherent simplifications of this approach are recognized, the large number of studies carried out in autoimmune thyroid disease (AITD) and other autoimmune conditions on T-cell subsets defined by their surface antigen and/or function can be better understood.

Several other, partly overlapping T and non-T lymphoid-cell populations may exert antigen-nonspecific, MHC non-restricted cytotoxicity. These include natural killer (NK) cells, which largely recognize determinants expressed on neoplastic cells; killer (K) cells, which recognize the Fc moiety of IgG antibody bound to cell-surface antigen; and other, mixed-cell populations with nonspecific killing capacity *(1)*.

The function and the activity of T lymphocytes are regulated by a complex network of soluble polypeptide factors called cytokines *(14)*. Cytokines are released by the immune system in response to antigenic challenge, but, in contrast with antibodies, their structure is unrelated to the antigen. The most important cytokines, their current denominations, main sources and biologic activities, are listed in Table 2.

When a specifically sensitized T-helper lymphocyte recognizes an antigen (in physical association with an MHC class II molecule and in the presence of interleukin-1 (IL)-1 produced by APCs), it becomes activated and synthesizes a variety of proteins *(1)*. Some of these proteins are secreted, whereas others become integral components of the cell membrane, as in the case of IL-2 receptors. Activated T cells produce interferon- (IFN)-γ, IL-2, IL-3, IL-4, and IL-6. High-affinity receptors for IL-2, which are absent on resting T cells, also appear within a few hours after antigen challenge. Binding of IL-2 to its receptors stimulates, through an autocrine loop, the clonal expansion of activated T lymphocytes. B cells are activated by antigen binding to cell-surface antibodies, and differentiate to

Table 2
List of Selected Cytokines

Cytokine	*Sources*	*Targets*	*Biologic activities*
IL-1 (α and β)	Activated macrophages and other APCs	T, B, NK cells, thymocytes	T- and B-cell costimulation
	Astrocytes, microglia	Several other tissue cells	Enhancement of leukocyte/endothelial adhesion
	Fibroblasts, endothelial and some epithelial cells		Hyperpyrexia, cachexia
			Acute-phase reactants
Il-2	Activated T cells	T, B, NK cells	T-cell growth stimulation
			Enhancement of cytokine production
			Enhancement of antibody synthesis and cytotoxic reactions
IL-3	Activated T cells	Bone-marrow stem cells	Stimulation of multilineage colonies
	Fibroblasts, Endothelial cells		
IL-4	Activated $CD4^+$ cells; mast cells	B and T cells, monuclear phagocytes	B- and T-cell growth stimulation
			Isotype Ig switching
			Activation of phagocytosis
IL-5	T cells	B cells, eosinophils	B-cell growth and activation
			Eosinophils activation
IL-6	Mononuclear phagocytes, T cells, B cells	Thymocites, mature B cells	Costimulator of growth
	Endothelial cells	Liver cells	Acute-phase reactants
IL-7	Bone marrow stromal cells	Thymocytes, T and B cells	Stimulation of pre-B cell differentiation
			Stimulation of thymocyte and T-cell growth
IL-8	Leukocytes	Granulocytes	Granulocyte recruitment and activation
IL-10	T cells	Mononuclear phagocytes	Inhibition of phagocytosis
		B cells	Activation of B cells
IL-12	Macrophages	T cells, NK cells	Activation of T and NK cells
Type 1 IFN (IFN-α, β)	Mononuclear phagocytes	All cells	Antiviral and antiprolipherative activity
	Fibroblasts		Increased MHC class I expression
IFN-γ	Activated T cells, NK cells	All cells	Activation of phagocytosis, NK-cell activity, increased class I and class II expression
TGF-β	T cells, mononuclear phagocytes	T cells, mononuclear phagocytes	Inhibition of activation and growth
	Other cells	Other cells	
TNF-α	Macrophages, activated T cells	Granulocytes, monocytes	Activation of cytotoxic lymphocytes, Macrophages and granulocytes
	Mast cells	Several tissue cells	
TNF-β			Increased leukocyte/ endothelial adhesion
			Induction of cachexia, acute phase reactants, fever

IL, interleukin; IFN, interferon; NK, natural killer; MHC, major histocompatibility complex; TGF, transforming growth factor.

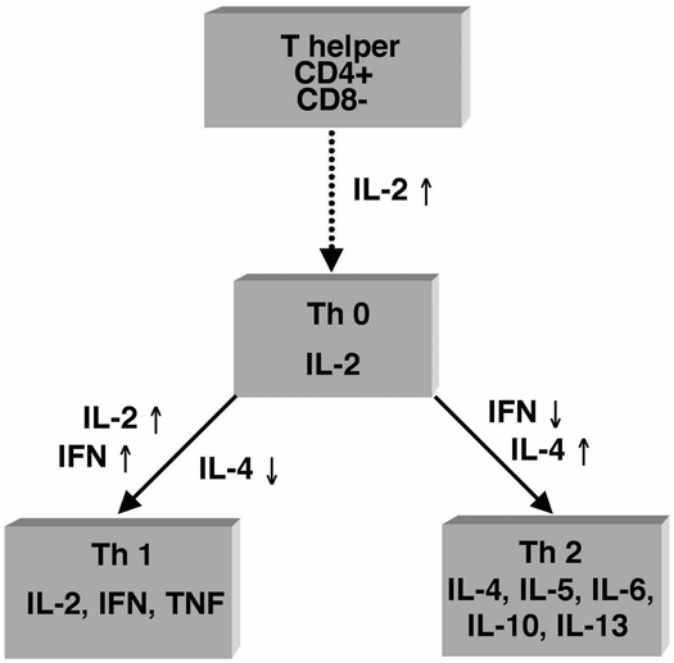

Fig. 4. Schematic representation of T-helper-cell subset (TH0, TH1, and TH2). The main cytokine produced by each subset is indicated within the box. The effects (↑, stimulation; ↓, inhibition) of cytokines on the cell subset are shown outside the boxes.

mature, antibody-secreting plasma cells under the influence of IFN-γ, IL-1, IL-2, and IL-6. Other cytokines produced by activated T lymphocytes include tumor necrosis factor (TNF)-α, IL-10, and IL-13. In mice there is a clear functional separation of $CD4^+$ helper T cells based on preferential cytokine secretion (Fig. 4). After antigenic stimulation, helper T lymphocytes, which are initially able to secrete only IL-2 (TH0 cells), may differentiate into TH1 cells (mainly producing IL-2, IFN-γ, and TNF-α) or TH2 cells (mainly producing IL-4, IL-5, IL-6, IL-10, and IL-13) *(15)*. TH1 lymphocytes function as mediators of delayed-type hypersensitivity (DTH) reactions, whereas TH2 cells are mainly helper cells for B-cell Ig synthesis *(16)*. Although less well defined, this functional separation is also considered valid in humans *(16)*. TCR affinity, ligand density, and different amounts of tissue chemoattractant factors (chemokines) or non-T-cell-derived cytokines (such as IL-4 and IL-12) appear to be involved in the generation of a predominantly TH1 or TH2 immune response *(17)*.

The immune system may lose its selective recognition properties for foreign constituents and react toward self components of the body itself (autoimmunity). Autoimmunity is characterized by an abnormal or excessive activity of the immune system, including autoantibody production by B lymphocytes and tissue infiltration by T lymphocytes and macrophages. This is said to reflect a loss of immunologic tolerance to self-antigens, but the mechanisms by which self-tolerance is normally maintained are not well defined.

According to the "forbidden clone" theory, lymphocytes responding to autoantigens (and other antigens as well) during ontogeny would be eliminated before maturation. More recent evidence indicates, however, that lymphocytes capable of reacting to self-antigens are normally present, suggesting that tolerance may be also achieved through suppression or inactivation rather than elimination of sensitized cells. An important series of events leading

to the immunologic tolerance is observed within the fetal thymus, where self-antigens are presented together with HLA molecules to developing T lymphocytes. Within the fetal thymus, autoreactive T cells (i.e., T lymphocytes bearing TCRs that react strongly with self MHC molecules in the absence of foreign antigen) are selectively destroyed, whereas T cells recognizing foreign antigens associated with MHC molecules survive *(18)*. Most, but not all, T lymphocytes carrying TCRs can bind self-antigens associated with class I and class II MHC molecules are also clonally deleted *(19)*. A limited number of autoreactive and self-antigen-reactive T cells survive, providing the basis for the development of autoimmune diseases. The foregoing mechanism is called "central tolerance," and implies that the antigens involved must be present in the thymus or in the circulation during fetal life. A different mechanism, not involving the thymus ("peripheral tolerance") *(20)*, is involved in deleting clones of T lymphocytes that recognize antigens not present in the thymus or in the circulation of the fetus (e.g., antigens expressed only in the mature individual). Blockade of TCR sites by soluble antigen or by immune complexes, and inhibition by "suppressor" circuits, may be involved in peripheral tolerance *(20)*. The negative selection within the thymus of autoreactive and self-antigen-reactive T lymphocytes leads to the elimination of more than 95% of the entire pool of lymphocytes through apoptosis (see "Apoptosis" following) *(21)*; in a similar way, any naive T cell stimulated by a specific antigen is deleted by apoptosis in the absence of a costimulatory second signal. Interestingly, defects in genes involved in apoptosis may preserve autoreactive T cells in some models of animal autoimmune diseases *(22)*.

A similar selection is observed during fetal life for B lymphocytes present in bone marrow and in the liver *(23)*. Exposure to antigen during the early stage of development leads to permanent inactivation of B lymphocytes. This process does not involve MHC molecules and, resembling what is observed with T-cells, is not complete, leaving in adult individuals some B lymphocytes able to produce antibodies to self-antigens, provided that the appropriate T-cell help is present.

Whatever their relative importance, these mechanisms may be overcome by a number of maneuvers. In general, injection of unmodified homologous or autologous antigen does not elicit an autoimmune response in the experimental animal unless an immunopotentiator such as Freund's complete adjuvant is used. Other means by which autoimmunity can be induced include an abnormal presentation or manipulation of autoantigens (alterations of the molecule, association with some new carrier), exposure to cross-reacting foreign antigens, binding of foreign haptens (such as drugs) to tissue components, nonspecific stimulation of helper T cells by infectious or other agents, loss of suppressor cell activity, or whole-body radiation *(24)*. Maintainance of self-tolerance may also be related to the inability of T-helper lymphocytes to recognize potentially autoantigenic molecules on cells that do not normally express MHC class II molecules. It has therefore been hypothesized that abnormal expression of DR antigens could play an important role in the induction and/or maintainance of thyroid and other organ-specific autoimmune diseases.

Viral infections are frequently implicated in human and animal autoimmune disorders. Viruses can act through several mechanisms: they may contain cross-reacting antigens, or produce carrier effects, or render host antigens autoimmunogenic by alterating their molecules, or by inducing inappropriate expression of HLA class II antigens. In addition, viruses may interfere with the function of the immune system. It has also been postulated that self-antigens may become immunogenic because of genetic or environmentally induced abnormalities in their enzymatic degradation. Considerable evidence has also accumulated indicating that autoimmunity is genetically controlled. It is well known that autoimmune

phenomena are much more common in women than in men, and that they frequently show familial aggregation. It appears that genes associated with the major histocompatibily locus in humans (HLA) are important in the pathogenesis of autoimmunity, since several autoimmune diseases have been found to be linked with certain HLA haplotypes. Other genes, located outside the HLA locus, such as Ig and TCR genes, may be involved in the development of autoimmune disorders. The relationship between genetic factors and autoimmunity is best demonstrated by animal models, in which experimentally induced as well as spontaneously occurring autoimmune diseases, including thyroiditis, are clearly under genetic control. The relevance of several of the above factors in triggering and/or modulating thyroid autoimmune reactions will be discussed in detail later in this chapter.

AUTOIMMUNE THYROID DISEASE

Introduction

The term "autoimmune thyroid disease" (AITD) encompasses several conditions with widely disparate clinical and laboratory expression *(25)*. Hashimoto's thyroiditis (HT), initially described as a goiter with atrophy of parenchymal cells, diffuse lymphocytic infiltration, fibrosis, and oxyphilic (Hürthle) cells, represents a classic example of an organ-specific autoimmune disorder. This concept was first postulated in 1956 when, independently, Roitt et al. demonstrated antithyroglobulin antibodies in the serum of patients with Hashimoto's thyroiditis, and Rose and Witebsky produced experimental lymphocytic thyroiditis in animals by immunization with homologous thyroid antigens. The concept that Graves' disease (GD) (also called Parry's disease, Basedow's disease, and autoimmune hyperthyroidism) should also be included in the group of AITDs, stems from the discovery in 1956, by Adams and Purves, of the long-acting thyroid stimulator (LATS), and has since gained universal support from the subsequent demonstration that LATS is a thyroid-stimulating autoantibody responsible for the hyperthyroidism of GD. Graves' ophthalmopathy, and the less common pretibial myxedema, (Graves' dermopathy) and thyroid acropathy, are also recognized autoimmune manifestations associated with GD *(25)*.

As listed in Table 3, several variants of autoimmune thyroiditis are now recognized in addition to the "classic" HT. In the "chronic fibrous thyroiditis" variant, fibrosis predominates, and lymphocytic infiltration is less marked. In lymphocytic thyroiditis of childhood and adolescence, Hürthle cells, fibrosis, and germinal centers are less evident than in the adult form, and anti-thyroid antibodies (anti-TABs) are often absent or present in lower titers. Postpartum thyroiditis is a transient (silent, painless) condition occurring after pregnancy; it is often associated with a thyrotoxic phase followed by a hypothyroid phase, or with a hypothyroid phase occurring *de novo*, and it usually resolves spontaneously thereafter. In silent (painless) thyroiditis (unrelated to pregnancy), as in postpartum thyroiditis, the thyroid gland is infiltrated with lymphocytes, but germinal centers and Hürthle cells are generally absent. In idiopathic myxedema (IM) or atrophic thyroiditis, the thyroid is atrophied either from total destruction of thyroid parenchyma or from the presence of thyroid-stimulating-hormone (TSH) receptor-blocking antibodies. In minimal or occult thyroiditis, the degree of lymphocytic infiltration is less marked, with either no evidence of clinical thyroid dysfunction or with subclinical (compensated) hypothyroidism. There appear to be subtle genetic and/or pathogenetic differences between these variants, as mentioned above, but they also share many similarities and thus deserve to be included under the generic term "autoimmune thyroiditis."

Table 3
Thyroid Disease and Thyroid Autoimmunity

Autoimmune thyroid diseases
Autoimmune hyperthyroidism (Graves' disease, Parry's disease, Basedow's disease, Flajani's disease)
Autoimmune thyroiditis
Hashimoto's thyroiditis (struma lymphomatosa)
Fibrous variant
Lymphocytic thyroiditis of childhood and adolescence
Silent (painless) thyroiditis (at least some cases)
Atrophic thyroiditis (idiopathic myxedema)
Asymptomatic (minimal, focal) autoimmune thyroiditis
Nonimmune thyroid diseases with secondary thyroid-autoimmune responses
Subacute (De Quervain's) thyroiditis
Papillary thyroid carcinoma
Nontoxic nodular goiter

HT, idiopathic myxedema, and GD almost completely fulfill the strict criteria proposed by Milgrom and Witebksy for establishing the autoimmune origin of an organ-specific disease (Table 4). However, in addition to those entities that may strictly be considered as primary autoimmune disorders, there are other thyroid conditions (e.g., subacute thyroiditis, nontoxic nodular goiter, and papillary thyroid carcinoma) that are not primary autoimmune diseases but may nevertheless manifest (secondary) immunologic disturbances (*see* Table 3) *(26)*.

Autoimmune thyroiditis has been diagnosed much more frequently in the past two generations than in earlier years. About 3% of the population is affected, with the prevalence increasing with age, and about 16% of elderly females have some degree of thyroidal lymphocytic infiltration (26). The apparent recent increase in prevalence may in part be owing to improved means of recognition and awareness, but may also be a result of an actually increased number of cases, probably caused by increased iodine consumption that has characterized the Western world in the past 60 yr *(25,26)*. About l% of the population has or has had GD *(26)*.

The overlap between GD and autoimmune thyroiditis has long been recognized. Both disorders frequently aggregate in the same family, and there are several reports of identical twins one of whom has GD and the other autoimmune thyroiditis. In some circumstances both conditions can coexist within the same thyroid gland, and the clinical expression will depend on which disease predominates *(26)*.

Histopathologic Changes

In HT the thyroid gland shows a diffuse infiltration by mononuclear cells, mainly consisting of lymphocytes. These may aggregate to form lymphoid follicles with germinal centers. Plasma cells and macrophages are also present. The normal architecture of the gland is disrupted, some follicular cells may show oxyphilic changes in their cytoplasm (Askenazy of Hürthle cells), and a variable degree of fibrosis is present in most cases. In idiopathic myxedema the gland is markedly reduced in size as a result of atrophy of the follicular epithelium. There is an extensive fibrosis associated with a variable lymphocytic infiltration

Table 4
Criteria for Organ-Specific Autoimmune Diseases

Lymphocytic infiltration of the target organ
Presence of circulating autoantibodies and/or cellular immunity against the target organ
Identification of specific antigen(s)
Production of humoral and/or cellular autoimmune responses in animals sensitized by autologous antigen
Presence of organ-specific lesions in autosensitized animals
Close association with other autoimmune disorders

similar to that in HT. In GD the thyroid is diffusely enlarged from parenchymal hypertrophy and hyperplasia, but lymphocytic and plasma-cell infiltration may often occur. Focal and occasionally diffuse lymphoid infiltration may also be encountered in other thyroid diseases, such toxic or nontoxic goiter or papillary thyroid carcinoma. Lymphocytic infiltration has been observed at autopsy even in subjects with no overt clinical manifestations of thyroid disease, especially in elderly women. The term "asymptomatic (or mild) atrophic thyroiditis" has been applied to this condition *(27)*.

Thyroid Autoantigens and Related Autoantibodies

Several thyroid antigen–antibody systems have been identified, as summarized in Table 5.

Antithyroglobulin Antibodies

Thyroglobulin (Tg) represents the first recognized thyroid autoantigen, and is the major component of the follicular colloid. Tg is a large glycoprotein of 670 kDa, in which the thyroid hormones thyroxine (T_4) and triiodothyronine (T_3) are produced. Tg is a normal constituent of the serum, and its concentration rises under physiologic or pathologic conditions leading to thyroid stimulation or damage. This finding implies that Tg by itself does not evoke an autoimmune response unless normal tolerance is altered.

Anti-Tg autoantibodies are most often of the IgG class, but IgA and IgM antibodies may also be found; they are normally not complement (C)-fixing, may produce precipitin reactions, and react with four to six large-conformation epitopes of the Tg molecule. In animal models, the antigenicity of Tg appears to be related to its iodine content, but this is not observed with human autoantibodies *(28)*. In contrast to anti-Tg autoantibodies, T-cells recognize small linear peptide segments of Tg molecules *(28)*. In the mouse, the induction of immunity to Tg is obtained by overcoming the tolerance to dominant T-cell epitopes, while the full expression of thyroiditis (including T-cell infiltration of the thyroid) results from the spreading of the immune response to "cryptic epitopes" *(28)*. Whether and to what extent this mechanism also applies to human AITD is unknown. A potential molecular mimicry with acetyl cholinesterase has been detected on the basis of Tg cDNA and, accordingly, a partial cross-reaction of anti-Tg autoantibodies with acetyl cholinesterase has been observed *(29)*. This could represent one of the pathogenetic links of Graves' ophthalmopathy to AITD, as discussed subsequently.

Anti-Thyroid Peroxidase Antibodies

Autoantibodies reacting with a structure formerly called thyroid microsomal antigen, present both in the cytoplasm and on the surface of thyroid follicular cells, have been known for a long time *(30)*. Evidence has subsequently been provided that thyroid microsomal antigen is antigenically related and possibly identical to human thyroid peroxidase (TPO).

Table 5
Main Thyroid Autoantigen-Autoantibody Systems

Autoantigen	*Molecular weight*	*Amino acid sequence*	*Main function*	*Detection methods*	*Remarks*
Thyroglobulin (Tg)	640 kD	Determined	Pro-thyroid hormone	Precipitin; hemagglutination; IFL; western blotting; RIA, ELISA and other immunometric techniques	Main colloid constituent
Thyroid peroxidase (TPO)	101-107 kD	Determined	Iodine oxidation and coupling of iodothyrosine residues	Complement fixation; IFL; cytotoxicity on TEC; hemoagglutination; western blotting; RIA, ELISA, and other immunometric techniques; TPO activity inhibition	Present in the cytoplasm and in the apical cell surface
TSH receptor	Extracellular (α) subunit: 53-kD Transmembrane and cytoplasmic (β) subunit: 30–42 kD	Determined	Transduction of TSH message	Stimulation assays: mouse LATS bioassay; cAMP production by thyroid membranes; stimulation of cAMP production, iodine uptake, growth (thymidine incorporation, cytochemical assays) in human or animal TEC or CHO cells transfected with hTSH-R Binding assays: LATS-p assay; radioreceptor assays with TSH-R Inhibition assays: TSH receptor blocking antibodies (TSHBAb)	Antibodies with different functional activities identified with different acronyms; see text for details
Sodium/iodide symporter (NIS)	70–90 kD	Determined	Specific cotransporter of 1 iodide ion with 2 sodium ions in the basolateral membrane of TEC	Western blotting; inhibition of iodide uptake in cultured TEC	Precise prevalence and clinical relevance unknown
Second colloid antigen (CA2)	Unknown	Unknown	Unknown	IFL (fixed thyroid sections)	Precise prevalence and clinical relevance unknown

CHO, Chinese hamster ovary; IFL, indirect immunofluorescence; ELISA, enzyme-linked immunosorbent assay; LATS, long-acting thyroid stimulator; RIA, radioimmunoassay; TEC, thyroid epithelial cells; TSH, thyroid-stimulating hormone; hTSH-R, human thyroid-stimulating hormone receptor.

The cDNA and amino-acid sequence of TPO have been determined: binding studies of human autoantibodies and mouse monoclonal antibodies with native and denatured TPO provide evidence that it has multiple B-cell-reactive conformational epitopes, whose three-dimensional structure has been modeled and located *(31,32)*. Epitopes of TPO, recognized by T cells from AITD patients, are linear peptides that have been partially characterized *(33,34)*. The fine epitopic specificity of anti-TPO autoantibodies is variable, but appears to be stable within a patient, and may be genetically determined *(35)*.

Antibodies to Thyroid Hormones

Antibodies to thyroid hormone (anti-T_4 and anti-T_3) may be found in patients with AITD *(36)*. These antibodies produce different interferences in serum T_4 and/or T_3 measurements, depending of the assay procedure, but do not interfere with the biologic action of the two hormones *(36)*. The antibodies may represent a particular subset of anti-Tg antibodies specifically recognizing Tg sequences containing iodothyronines *(37)*.

Antibodies to the TSH-receptor

Autoantibodies (anti-TSH-receptor antibodies [TRAbs]), able to bind to the TSH receptor are present in patients with GD and those with HT. As discussed later, TRAbs produce thyroid dysfunction either by stimulating the TSH receptor or by inhibiting TSH activity. The TSH receptor is a 764-amino-acid glycoprotein that has been fully cloned and sequenced. This protein is a member of a superfamily of membrane receptors sharing a common structure represented by an NH_2-terminal extracellular portion, seven transmembrane loops, and a COOH-terminal intracellular domain that binds the Gs subunit of adenyl cyclase *(38)*. The precise structure of the TSH receiver has been identified as a heterodimer comprising an extracellular α subunit (about 53 kDa) and a broad transmembrane and intracellular β subunit (30–42 kDa), which are held together by disulfide bridges *(39)*. Like Tg and TPO, B-cell epitopes of the TSH receptor are conformational, and appear to be related to the stimulating or inhibiting activity of TRAbs. Stimulating TRAbs (TSAbs) bind to amino-acid sequences located in the NH_2-terminal region of the extracellular portion of the receptor, some of which are also involved in binding of TSH. TRAbs that are able to block TSH activity (TSH-blocking antibodies, TRBAbs) bind preferentially to sequences nearer to the cell surface (amino acids 261–370 or 388–403). Linear fragments of TSH receptor are able to stimulate T cells from patients with AITD, but the precise characterization of the T cell epitopes is still lacking. The extracellular portion of the TSH receptor is found in the bloodstream *(40)*: this may be due to the production of abnormal TSH receptor (lacking a transmembrane domain) *(41)* or to shedding of the receptor *(42)*. mRNA for the TSH receptor has been reported in retroocular tissue, and this could represent a further pathogenetic link of Graves ophthalmopathy with AITD (*see* "Association of AITD with Other Autoimmune Disorders").

Other Antibodies

A noniodinated protein contained in the thyroid colloid and unrelated to Tg was detected a long time ago by immunofluorescence (IFL), and is called second colloid antigen (CA2). The nature of CA2 and the relevance of anti-CA2 autoantibodies are still unknown. Recently, autoantibodies to the sodium iodide symporter (NIS) have been found in variable percentages of patients with GD and HT *(43–45)*. The precise prevalence of anti-NIS antibodies in AITD remains to be defined, owing to the lack of a reliable and reproducible method for detecting these antibodies. Some anti-NIS antibodies may inhibit NIS-mediated iodine uptake in vitro,

but the relevance in vivo of this phenomenon is unknown. Other antibodies reacting with thyroid components such as tubulin, calmodulin, megalin, and DNA-associated proteins are occasionally present in sera of patients with AITD *(46,47)*.

Cell-Mediated Immunity in AITD

Cell-mediated reactions to human thyroid antigens have been documented in patients with AITD, through the use of unfractionated peripheral mononuclear cells or T-cell-enriched preparations *(25,26)*, by producing leukocyte migration inhibition factor (MIF) or with proliferation assays *(25,26)*. Accordingly, unfractionated T-lymphocytes and T-cell lines or clones isolated from intrathyroidal infiltrates of patients with either HT or GD proliferate when cocultured with autologous DR^+ thyroid cells. T-cell clones specific for several linear epitopes of Tg, TPO, and TSH receptor have subsequently been obtained and partially characterized. Indirect evidence for T-cell involvement in AITD is also provided by the increased number of circulating and intrathyroidal T cells bearing DR antigen, a marker of T-cell activation, on these cells' surfaces *(48)*. An increase in DR^+ cells has been observed in vitro by incubating peripheral blood lymphocytes from AITD patients with thyroid autoantigens *(48)*.

Further insight into T-cell activation in AITD derives from studies of the TCR V gene of thyroid-infiltrating lymphocytes. T-cell responses to an antigenic stimulus may use a wide variety or only a few (V) TCR gene segments. Restriction of autoreactive T cells to using one or more V gene segments has been observed in some experimental autoimmune models *(5)*, and in the whole intrathyroidal lymphocyte population as reported by some authors *(49)* but not confirmed by others *(50)*. This discrepancy may perhaps be explained by the inclusion of patients having AITD in different phases of its natural history. It is in fact conceivable that the T-cell response is clonally restricted, but as AITD advances, spreading of the immune response occurs, leading to an unrestricted response to a wider range of epitopes *(51)*. Autoreactive T lymphocytes must also be involved in humoral thyroid responses because autoreactive B lymphocytes need the help of T-helper lymphocytes to produce anti-TPO and anti-Tg antibodies *(52)*.

Association of AITD with Other Autoimmune Disorders

The occurrence of pernicious anemia in patients with HT, primary myxedema, or GD is well documented *(26)*. Other organ-specific autoimmune disorders known to be or probably associated with AITD include idiopathic Addison's disease, type I diabetes mellitus, myasthenia gravis, thrombocytopenic purpura, acquired hemolytic anemia, and vitiligo. All of these associations are much more frequently observed at the serologic than at the clinical level. AITD is also frequently found in patients with autoimmune Addison's disease and/or type 1 diabetes mellitus as a part of the autoimmune polyglandular syndrome (APS) type 2 *(53)*. In contrast, AITD is an infrequent feature of the much rarer APS type 1 (autoimmune Addison's disease, idiopathic hypoparathyroidism, and cutaneous mucocandidiasis) caused by mutation of the autoimmune-related (aire) gene *(54)*. Furthermore, there is no correlation between mutations in the aire gene and sporadic AITD *(55)*. Non-organ-specific autoimmune diseases such as rheumatoid arthritis, systemic lupus erythematosus, and particularly, Sjögren's syndrome, also show variable degrees of association with AITD *(26)*.

The association of AITD with other autoimmune diseases is one of the most convincing items of evidence supporting the existence of a shared immunoregulatory defect, at least partially genetically determined. The immunogenetics of AITD will be discussed later in this chapter, under the etiopathogenesis of these conditions.

Animal Models of Thyroid Autoimmunity

Several animal models of experimentally induced (EAT) or spontaneous (SAT) autoimmune thyroiditis are known *(25)*.

EAT may be induced by injection of homologous or autologous thyroid antigen in virtually all animal species, including rabbit, guinea pig, mouse, rat, dog, monkey, and chicken. The thyroid lesions are similar to those of human lymphocytic thyroiditis, although oxyphilic changes are not observed in the experimental animal. The principal antigen responsible for EAT induction is Tg *(56)*. Autologous Tg may be rendered immunogenic by the use of adjuvants, by several chemical modifications, or by incomplete proteolytic digestion, indicating that minor modifications of the protein or exposure of hidden epitopes is sufficient to break the self-tolerance. Interestingly, however, EAT may be induced in highly responsive mice by repeated injection of unaltered syngenic Tg *(25)*, providing good evidence for the importance of genetic control of this experimental disease (*see* following in this subheading). The appearance of serum anti-Tg antibodies is well documented in EAT, whereas in contrast to human disease, cytoplasmic antibodies are not observed, with the remarkable exception of the monkey *(57)*. T lymphocytes play a central role in the mechanisms responsible for EAT, and the disease can be induced in different animal species by passive transfer of lymphoid cells. In these experiments, the active cells are T lymphocytes, while no effect is produced by B cells alone *(25)*. Both $CD4^+$ and $CD8^+$ T lymphocytes are able to transfer EAT *(58)*. EAT may be induced in normal recipients by injection of immune serum, and the resulting thyroid lesion appears to be mediated mainly by the in situ formation of Tg–anti-Tg immune complexes *(59)*. EAT has also been induced by immunization with TPO, and a specific sequence (amino acids 774–778) appears to constitute the pathogenic epitope involved in the induction of the disease in C57/BL6 mice *(60)*. Injection of lymphoid cells sensitized in vitro to thyroid antigens or syngeneic thyroid cells in culture *(61,62)* is another way to induce EAT in mice.

T-cell hybridomas derived from animals with EAT allowed the identification of a thyroiditogenic epitope of Tg including a hormonogenic (T_4) site at amino-acid residue 2553 *(63)*. This finding suggested a key role for iodine atoms of T_4 in the immunogenicity of the epitope. However, comparison of other Tg peptides containing hormonogenic sites showed that the immunogenicity was mainly related to the amino-acid sequence, and that the number of iodine atoms, although able to enhance antigenicity and/or binding affinity, were not primarily required for a Tg hormonogenic site to be an autoepitope *(63)*.

The induction of EAT appears to be under genetic control, as exemplified by the mouse model. Briefly, the severity of this disease depends upon different genes localized in several regions of the MHC of the mouse (H2), but genes located outside the H2 complex are also involved *(25)*. Experimental animal models have also been developed to directly prove the molecular basis for a human genetic predisposition to AITD. Transgenic mice expressing the human HLA-DRB1*0301 antigen (known to be strongly associated with the predisposition to thyroid and other organ-specific autoimmune diseases [*see* "Genetic Predisposition to AITD" following] are susceptible to Tg-induced EAT, unlike mice transgenic for DR2 antigen (which is known to be protective against organ-specific autoimmunity) *(64)*. This is probably one of the most elegant example of experimental evidence of the importance of HLA polymorphism in the human susceptibility to AITD.

Quite recently, the effects of constitutive expression of cytokines by thyroid cells have been evaluated in transgenic mice models. Transgenic mice expressing IFN-γ at the thyroid cell level develop severe hypothyroidism with a profound inhibition of NIS-gene expression *(65)*. This finding indicates a direct role of IFN-γ in producing thyroid dysfunction. Given

the great amount of IFN-γ and other cytokines produced by thyroid-infiltrating lymphocytes, these data support the concept of a direct pathogenic role of cytokines in producing hypothyroidism in HT (*see* "Mechanisms Mediated by Cytokines" following).

SAT has been described in chickens, rats, dogs, and monkeys. The obese strain (OS) of chicken is the best animal model of human HT, being characterized by the spontaneous appearance of high titers of antibodies to Tg and to other thyroid and nonthyroid organ-specific antigens *(25,66)*. Evidence has been provided that SAT in the OS chicken is under strict genetic control. In particular, a three-locus model has been suggested: one gene located within the chicken MHC complex (B2), controls the immune response to Tg; a second gene is responsible for abnormal thymic development and a consequent T-suppressor-cell defect; and a third gene (or gene complex) is related to subtle target-organ abnormalities *(67)*. Exogenous factors like steroid hormones *(67)* and dietary iodine *(68)* are also involved in the development and severity of OS thyroiditis.

Other important animal models of SAT include BB rats *(69)* and non-obese diabetic (NOD) mice *(70)*. These animals also show high incidences of insulin-dependent diabetes mellitus and other autoimmune endocrinopathies mimicking the human conditions of polyglandular autoimmunity. The genetic predisposition to SAT in BB rats and NOD mice has been extensively studied and shown to be polygenic (including genes both outside and inside the MHC) *(71,72)* and related to complex abnormalities of the immune system. Similarly to the chicken model, iodine exerts positive modulation of the thyroiditis expression *(73,74)*, possibly by increasing Tg immunogenicity

So far, no clear counterpart of GD has been found in the experimental animal. TSAbs have been produced in rabbits by immunization with human thyroid homogenate, microsomes, or bovine plasma membranes, but there was no evidence of hyperthyroidism in the immunized animals *(75)*. Recently, Costagliola et al. *(76)* reported that genetic immunization of outbred NMRI mice with a cDNA encoding the human TSH receptor produced, beyond the presence of antibodies recognizing the extracellular portion of TSH receptor, hyperthyroidism and ocular changes suggestive of Graves' ophthalmopathy in a minority of immunized animals. This model represents the most convincing murine model of GD available to date.

EFFECTOR IMMUNE MECHANISMS IN AITD

Humoral Immune Reactions

Complement-Fixing Antibodies and Immune Complexes

It is well known that anti-TPO autoantibodies are C-fixing in vitro and are cytotoxic to human thyroid cells previously dispersed with trypsin *(75)*. The relevance of these autoantibodies in the cytotoxic processes involving thyroid follicular cells in vivo is still controversial. No clear relationship is observed in different autoimmune thyroid diseases between titers of circulating anti-TPO autoantibodies and thyroid hypofunction and atrophy. In contrast to TSH-receptor-blocking antibodies (*see* "Nature and Biological Activities of TSH-Blocking Antibodies" following), passive placental transfer of anti-TPO autoantibodies does not affect thyroid function in newborns *(77)*. Furthermore, it has been argued that TPO would not be easily available to the corresponding antibody in the presence of an integral plasma membrane *(75)*. On the other hand, the potential relevance of C-mediated cytotoxicity exerted by anti-TPO autoantibodies has been supported by the demonstration that TPO is expressed both within the cytoplasm and on the surface of thyroid cells, and that C-mediated cytotoxicity by anti-TPO autoantibodies can also be observed with untrypsinized human thyroid cells in culture *(30)*. Moreover, the presence of anti-TPO autoantibody bound

in vivo to the apical microvillar surface of human thyroid follicular cells has also been reported *(30)*. Electrondense deposits indicative of antigen–antibody complexes have been observed along the follicular basement membrane in thyroid specimens from patients with HT or GD *(78)*. It is therefore conceivable that *in situ* deposition of immune complexes might be responsible for thyroid damage through a type III immunopathologic reaction, and the importance of this mechanism in the production of thyroid lesions has been clearly established in experimental murine thyroiditis *(59)*. Circulating soluble immune complexes, including Tg and anti-Tg antibody, have been detected by several techniques *(25,79)*; in contrast, attempts to identify the thyroid microsomal antigen in serum immune complexes provided negative results *(75)*. The pathogenetic role of circulating immune complexes in AITD is still unclear. It has been postulated that, together with autoantibodies, immune complexes containing specific thyroid autoantigens may arm killer (K) cells mediating antibody-dependent cellular cytotoxicity (ADCC) (*see* "ADCC" following).

Functional Relevance of Anti-TPO Antibodies

TPO is an essential enzyme in the generation of thyroid hormone (TH), since it catalyzes the oxidation of iodide and the coupling of iodotyrosil residues of Tg. A potential interference of anti-TPO autoantibodies with TPO enzymatic activity, leading to impairement of thyroid function independently of C-dependent cytotoxicity, has therefore been envisaged. In vitro, a variable effect has been seen, ranging from no inhibition to complete inhibition *(75)* by antibodies of TPO enzymatic activity, but this effect is probably limited in vivo by the inability of antibodies to penetrate the thyroid and reach TPO at the surfaces of follicular cells facing the colloid space *(30)*.

Antibodies to the TSH Receptor

Hyperthyroidism of GD is caused by autoantibodies to the TSH receptor (TRAbs) than can stimulate thyroid activity. Moreover, autoantibodies that inhibit some biologic effects of TSH on thyroid cells have been described in sera of patients with idiopathic myxedema, which may be important in the pathogenesis of thyroid atrophy and hypothyroidism. As discussed earlier, the different biologic activities of TRAbs are probably due to different epitopes recognized in the extracellular portion of the TSH receptor.

Thyroid-Stimulating and Growth-Promoting Autoantibodies. Thyroid-stimulating antibodies (TSAbs) were historically detected by low-affinity transport system (LATS) bioassay, but a consistent number of GD patients were negative by this test even after concentration of the IgG fraction of the sera. Subsequently, a wide number of techniques based on the stimulation of adenylate cyclase in human thyroid tissue have been developed through the use of membranes, tissue slices, and monolayer primary cultures *(75)*. Differentiated rat thyroid cells in continuous culture (FRTL-5 cells) *(75)* and, more recently, Chinese hamster ovary (CHO) cells stabily transfected with human TSH receptor *(80,81)* have also been employed for sensitive assay of TSAbs. By these methods, up to 80–100% of patients with active untreated GD have positive TSAb activity. This finding strongly supports the role of this antibody in the development of Graves' hyperthyroidism, in keeping with the notion that transient neonatal hyperthyroidism is produced by transplacental transfer of maternal TSAb *(82)*. Although no clear relationship between TSAb levels and the severity of hyperthyroidism has been found in several studies *(75)*, the persistence of high circulating titers of TSAb at the end of a period of therapy with antithyroid drugs is generally considered as indicative of imminent relapse into thyrotoxicosis *(75,81)*. GD is characterized by hyperthyroidism and goiter caused by thyroid hyperfunction and hyperplasia, respectively.

The pathogenetic relevance of TSAbs in the development of thyrotoxicosis is well established. The presence of serum antibodies that stimulate thyroid growth but not thyroid function has been described in euthyroid and hyperthyroid goitrous patients (thyroid growth-stimulating antibodies [TGAbs]) *(83,84)*. Antibodies that can stimulate thyroid growth and which are indistinguishible from TSAbs are frequently found in GD patients, in agreement with the notion that the growth of human thyroid cells is a cyclic adenosine monophosphate (cAMP)-dependent phenomenon *(75,85)*. The actual existence in euthyroid goiter of TGAb distinct from TSAb has been questioned, mainly on the basis of technical drawbacks of the methods (mostly cytochemical assays) employed *(85)* and because of the difficulty in reproducing the relevant data in other laboratories *(86)*. Two recent studies comparing different methods failed to confirm the presence of TGAb independent of TSAb in patients with sporadic or endemic goiter *(87,88)*.

In addition to their thyroid-stimulating activity, TRAbs are also detected through their ability to inhibit the binding of radioiodinated TSH (TSH-binding-inhibiting antibodies, TBIAbs) to particulate or solubilized receptors *(89)*. It has been known for a long time that a minority of IgGs (mostly from patients with autoimmune hypothyroidism) show TBIAb activity but are devoid of TSAb activity. This observation led to the subsequent identification of TRAbs that could block in vitro the biologic activity of TSH (TSHBAbs).

Nature and Biological Activity of TSH-Blocking Antibodies. Most cases of spontaneous hypothyroidism in adults are due to a chronic autoimmune thyroiditis characterized by variable degrees of infiltration of the thyroid by lymphocytes, progressive destruction of follicular epithelium, and fibrous replacement of this latter tissue. This autoimmmune process may result either in thyroid enlargement (goitrous HT) or atrophy (atrophic thyroiditis or idiopathic myxedema), but eventually the thyroid gland becomes insufficient to maintain normal serum concentrations of T_4 and T_3, despite maximal drive by increased circulating TSH. The inability of the thyroid to respond with a compensatory production of hormone and tissue growth to the increased TSH may be due to the presence of TSHBAbs. These antibodies may be detected through their inhibition of the biologic effects (generally cAMP production) induced by TSH in thyroid membranes, thyroid slices, or cultured cells (including CHO cells transfected with human recombinant TSH receptor) *(75,90)*. With these techniques, TSHBAbs have been detected in the sera of most patients with idiopathic myxedema, and, to a lesser degree, those with goitrous hypothyroid and even euthyroid HT *(90,91)*. TSHBAbs have been also detected in GD sera, generally mixed with TSAbs. In this case, biphasic patterns of hyper- and hypothyroidism may be observed in the affected patients, depending on the prevailing type of serum TRAb *(92)*.

Transplacental transfer of TSHBAb is also responsible for transient congenital hypothyroidism *(75,93)*. Most of these cases are represented by newborns of mothers who have hypothyroid HT, or, more rarely, other autoimmune thyroid disorders, with high serum TSHBAb activity. This syndrome is the counterpart of transient thyrotoxicosis caused by transplacental transfer of TSAb, and a complete recovery of thyroid function within the first months of life is generally observed. Complex neonatal thyroid dysfunctions caused by transplacental transfer of maternal IgGs containing mixtures of TSHBAb and TSAb have been also described *(94)*. Transplacental transfer of TSHBAb (mainly detected by cytochemical assays) has also been suggested to have a pathogenic role in permanent sporadic *(95)* and endemic *(96)* congenital hypothyroidism. As with TGAb, these data were not reproduced in other laboratories using different techniques *(75)*, and a recent study of a rather large number of patients with endemic cretinisms provided negative results for TSHBAb *(97)*.

Cellular Mechanisms

Several cellular mechanisms, such as ADCC, NK activity, and antigen-specific T-cell cytotoxicity, may be involved in the pathogenesis of thyroid damage in AITD. Furthermore, there is growing evidence that cytokines produced in the course of the autoimmune reaction may exert cytotoxic or other functional activities on thyroid cells. Additionally, apoptosis of either thyroid cells or infiltrating lymphocytes could play an important role in the destructive phenomena of AITD.

ADCC

Several types of circulating mononuclear cells (MNCs) with cytolytic potential, such as K cells, monocytes, and polymorphonuclear leukocytes, may bind and lyse target cells coated with IgG antibodies. This is the phenomenon called ADCC *(1)*. The potential relevance of ADCC as the effector mechanism of thyroid damage was first suggested by the ability of normal peripheral MNCs to lyse Tg-coated erythrocytes or ^{51}Cr-labeled human thyroid cells preincubated with sera from HT patients *(98,99)*. Anti-Tg and Anti-TPO antibodies are involved in this in vitro phenomenon. The existence of a different thyroid-cell-surface autoantigen, distinct from Tg and TPO and responsible for ADCC, has been suggested *(100)*, but this observation has not been confirmed *(101)*.

ADCC may also be mediated by NK cells (*see* next subheading).

NK Cells

NK cells are lymphocytes present in normal individuals, and which, although not previously exposed to relevant antigens, are nevertheless able to kill in vitro a variety of transformed, virus-infected, or embryonic cells in the absence of antibody *(1)*. NK cells express CD16 and CD56 antigens and are activated by IL-2, IFN-α and -β, and particularly by IL-12. These cells have two main mechanisms of target-cell killing. The first is ADCC, the second mechanism makes use of cell–cell contact in which special lectin-like NK-receptors, known as NKP-P1 receptors, recognize glycosylated surface molecules on target cells. Once activated, the NK cells lyse the target cells via the release of perforin and lymphotoxin.

With few exceptions, the prevalence of circulating NK cells (as assessed by phenotypic markers) has been typically found in AITD *(75)*. A high proportion of intrathyroidal lymphocytes from AITD infiltrates display NK activity *(102,103)*. When studied at the clonal level, most NK$^+$ lymphocytes express the CD8$^+$ "cytotoxic–suppressor" cluster determinant. CD4$^+$ T-cell clones with NK activity are found in HT but not in GD: this findings could be related to the greater destructive phenomena characteristic of HT *(104)*.

T-Cell Mediated Cytotoxicity

Cytotoxic cells recognize autoantigens directly on target cells when these antigens are present in the context of MHC class I molecules, and kill target cells through the use of perforin and other cytotoxic molecules. The importance of specifically sensitized cytotoxic T cells as direct mediators of thyroid-destructive processes is clearly established only in animal models of experimental and spontaneous autoimmune thyroiditis *(75,105)*. The unequivocal demonstration of this mechanism in humans is very difficult, since the assessment of T-cell-mediated thyroid cytotoxicity strictly requires autologous target cells. As noted earlier, T lymphocytes from AITD patients specifically proliferate in response to thyroid autoantigens, but the relationship of this proliferation to the cytotoxic activity has not been generally assessed. One CD8$^+$ cell clone exerting specific cytotoxicity toward autologous thyrocytes has been derived from AITD infiltrate *(106)*. Using the same approach, CD8$^+$ T-cell lines and clones cytotoxic for autologous thyroid cells can be derived from glands

of mice with EAT *(107)*. Taken together, these findings support the concept that a direct involvement of autosensitized T lymphocytes may be an important mechanism in thyroid-autoimmune lesions. The relative prevalence of thyroid-specific cytotoxic T cells within the thyroid infiltrate is still unknown, but indirect evidence suggests that they could be only a minority (<10%) of the total lymphoid cells *(104)*. It is conceivable that thyroid-specific lymphocytes, including cells mediating direct cytolytic activity, represent the first infiltrating cells in the initial course of the AITD. Subsequently, nonspecific T lymphocytes with high cytolytic potential could be enrolled and activated to amplify and maintain the effector mechanisms leading to thyroid destruction.

Mechanisms Mediated by Cytokines

As discussed earlier in greater detail, cytokines are polypeptides produced by different immune cells in response to antigenic stimulation, and exert pleiothropic, redundant, synergic, and sometimes antagonist action. Cytokine production plays a major role in thyroid autoimmunity, in view of the ability of these molecules either to amplify the immune response by inducing a cytokine cascade through the actions of different immune cells and to exert direct functional effects on thyroid epithelial cells (TECs) *(75)*. Once activated by autoantigen recognition, thyroid-specific T lymphocytes produce IL-2, with the induction of T-cell proliferation and differentiation, IFN-γ production, and activation of NK cells. Later, activated lymphocytes produce other cytokines with further amplification and perpetuation of the immune response (cytokine cascade). Several studies aimed at characterizing the phenotype and function of thyroid-infiltrating T cells have provided evidence for $CD8^+$ cytolytic T cells and $CD4^+$ T lymphocytes displaying a pattern of cytokine production (IFN-γ, TNF-α, IL-2) typical of TH1 cells. This pattern is observed in all AITD, but the TH1 profile and the cytolytic activity is more evident in infiltrates from living HT patients than from those with GD *(108,109)*. Besides their effects on immune cells, locally produced cytokines could exert other important actions in AITD. First, some of them (IFN-γ and TNF-α) can stimulate HLA class II expression on TECs. Although there is overwhelming evidence that this abnormal expression is a secondary phenomenon that is not relevant in the initial triggering of thyroid autoimmunity, the abnormal class II expression may play a crucial role in the amplification and progression of AITD. This concept will be discussed later in more detail. The second important mechanism exerted by cytokines is that some of them, especially those produced by infiltrating macrophages, such as TNF, are probably directly cytotoxic to thyroid cells and/or are able to interfere with function of TECs *(75,110,111)*. This concept derives from a large number of in vitro experiments done on thyroid cell cultures exposed to different cytokines. Extensive reviews of this topic have been published, and to which the reader is referred for details *(111–113)*. The relevance of cytokines in the pathogenesis of Graves' ophthalmopathy is discussed later in this chapter.

Apoptosis

Apoptosis or "programmed cell death" is a process distinct from necrosis, which represents the regulated activation of a preexisting cell-death program encoded by the genome *(114)*. Apoptosis is induced either by the loss of trophic molecules or by proapoptotic signals derived from ligand binding to a membrane receptor. The apoptotic machinery of the cells includes specific receptors (called "death receptors"), adaptor molecules, the caspase cascade, the mitochondria, and the Bcl-2 antiapoptotic proteins *(115)*. The death receptors belong to the TNF-receptor gene superfamily and contain a homologous cytoplasmatic sequence of 80 amino acids (death domain) bridging the death receptors and activating

(through death-effector domains) caspase 8. Caspase 8 in turn sequentially activates other caspases, a group of cysteine proteases cleaving proteins at specific peptide sequences containing an aspartate residue *(116)*. This cascade of caspases ultimately activates enzymes that progressively digest the cell and its genetic material, producing the classical morphologic changes of apoptosis (chromatin condensation, cytoplasmic shrinkage, and plasma-membrane blebbing) *(117)*. In apoptosis there is also a specific endonuclease cleavage of DNA that, unlike the random cleavage of necrosis, occurs only once for every turn of the DNA around the chromatin, leading to the typical "ladder effect," with DNA fragment multiples of 180 bases *(117)*. The best characterized death receptor is represented by CD95 or Fas, which is activated by the CD95 ligand (CD95L) or the Fas ligand (FasL), but other molecules, such as TNF-related apoptosis-inducing ligand (TRAIL) and its specific receptor (death receptors 4 and 5) *(118)*, are also important.

In the past decade it has become apparent that the thyroid-autoimmune destructive mechanisms described above induce the death of TECs mainly through the induction of apoptosis *(119–121)*. Normal thyroid glands show a low level (<1%) of apoptosis, which probably represents the expression of slow basal thyroid-cell turnover. Immunohistochemical examination of HT glands shows a high percentage (up to 20–30%) of apoptotic TECs, especially at the periphery of infiltrated areas *(119,121)*. The extent of apoptosis is variable, possibly in relation to different phases of the disease and/or to the extent of the lymphocytic infiltration *(119,121)*. The process is mainly restricted to TECs, with intrathyroidal lymphocytes tending to remain intact *(119)*. A lower level of apoptosis, intermediate between that seen in HT and normal glands, is observed in GD *(119)*. TSH-receptor stimulation has been found to inhibit Fas expression *(122)*; thus, the apoptotic pathway could be involved mainly in HT rather than in GD, where TSAb would act like TSH to diminish Fas expression. On the other hand, other investigators have found no effect of TSH on Fas expression by TECs in culture *(123)*.

While the involvement of apoptosis in the autoimmune death of TECs can be considered to have been conclusively demonstrated *(119–121)*, the precise mechanism(s) used by TECs to signal apoptosis remain a matter of controversy *(124)*. Apoptosis induced by Fas/FasL is the most widely studied apoptotic system in the thyroid gland. In spite of several discrepancies, it is currently accepted that Fas is constitutively expressed at low levels in normal TECs, and that its expression is markedly increased in HT glands, possibly as a consequence of proinflammatory cytokines (IL-1β, TNF-α, and IFN-γ) released from the infiltrating lymphocytes. The expression of Fas on TECs is expected to facilitate apoptosis after interaction with FasL expressed on activated cytotoxic T lymphocytes and NK cells *(125)* (Fig. 5). Figure 5 also depicts an alternative hypothesis suggested after the report by Giordano et al. *(126)* of constitutive FasL expression on TECs obtained from autoimmune (HT) and nonautoimmune thyroid glands. According to this view, when the expression of Fas is stimulated by the action of IL-1β or other proinflammatory cytokines on TECs, thyroid-cell death could be induced by neighboring TECs expressing FasL through suicidal or fratricidal pathways. This hypothesis has been criticized due to the difficulty in confirming FasL expression by normal or even autoimmune TECs *(120)*. These discrepancies are probably due to technical problems related to the specificity of the antibodies employed to detect FasL and, to a lesser degree, to other factors, including culture conditions and differences in the clinical phases of the AITD-affected thyroid glands *(120)*. At present, the weight of evidence suggests that FasL is expressed on TECs under some pathologic conditions (including multinodular goiter and thyroid carcinoma) without a clear correlation

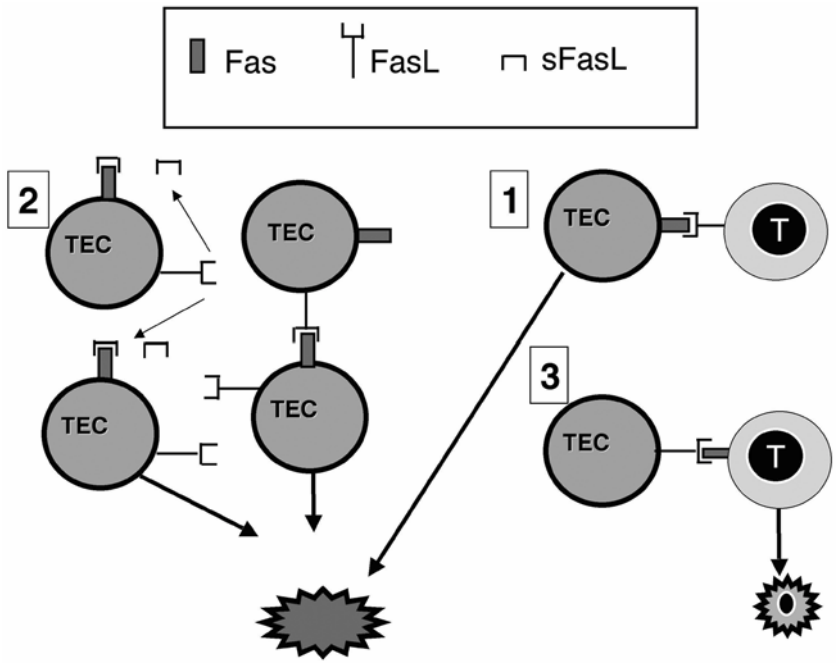

Fig. 5. Potential involvement of apoptotic pathways in thyroid autoimmunity. (1) The binding of activated T lymphocytes expressing Fas-L to Fas-expressing TECs induces TEC apoptosis. (2) Simultaneous expression of Fas and Fas-L on TECs induces "fratricidal" apoptotic death by TEC–TEC interaction; the cleavage of soluble Fas-L from the TEC plasma membrane may induce fratricidal or suicidal apoptosis by paracrine or autocrine mechanisms, respectively. (3) Expression of Fas-L on TECs could also lead to apoptotic death of autoreactive activated T cells expressing Fas.

with the pathogenesis of the disease *(120,121)*. Moreover, recent data appear to argue against the hypothesis of Fas-mediated thyroid suicide/fratricide *(120)*. Transgenic mice with TECs overexpressing FasL do not display increased apoptosis in their thyroid glands, and develop a milder form of EAT *(127)*. Moreover, the antithyroid drug methimazole (which is believed to have thyroid-specific immune-suppressor activity; *see* "The Thyroid–Immune System Relationship" later) induces the expression of FasL on TECs *(128)*. Taken together, these data support a different hypothesis for the relevance of the Fas/FasL system in thyroid autoimmunity *(129)*. According to this view, the expression of FasL on TECs, in combination with hyperexpressed MHC class II molecules, could allow the elimination by apoptosis of Fas-expressing, thyroid-specific T lymphocytes.

Thyroid cells are also able to produce a soluble form of Fas (sFas), resulting from alternatively spliced Fas mRNA lacking exons 3, 6, and 7 *(121)*. sFas is supposed to inhibit Fas-FasL interaction and protect target cells from apoptosis *(121)*. Increased serum sFas (possibly deriving from the thyroid gland) has been found in patients with active hyperthyroid GD *(130)*, suggesting a protective effect of TECs and, possibly also of B cells producing TSAb, against apoptosis *(121)*, in keeping with the hyperplastic–hyperfunctioning nature of GD. A soluble form of FasL (sFasL) also derives from cleavage of FasL at the cell membrane by a metalloproteinase *(121)*. sFasL is believed to induce apoptosis of Fas-expressing target cells, and could therefore be involved in autocrine and paracrine thyroid suicidal or fratricidal apoptotic pathways (Fig. 5).

Although very important, the Fas/FasL system alone may not be sufficient to induce thyroid apoptosis. Recent data also support a role in apoptosis for the TRAIL-mediated pathway *(120)*. Both death receptors 4 and 5 are present on TECs, and TRAIL is expressed after exposure to proinflammatory cytokines *(120)*. Thus, models similar to those envisaged for Fas/FasL can be conceived for the involvement of TRAIL in destructive HT *(120)*. The specific pattern of cytokine secretion may be an important factor in the switch toward either thyroid destruction or the killing of intrathyroidal lymphocytes. As far as the TRAIL system is concerned, it would appear that the presence of IFN-γ favors protection of TECs and immune-cell apoptosis, whereas IL-1β and TNF-α provide the best conditions for the apoptotic death of TECs *(120)*.

Apoptosis may be increased in affected thyroid glands affected by autoimmune disease only not as the result of enhanced proapoptotic signals, but also by a presence of reduced antiapoptotic molecules. A decrease in the antiapoptotic molecules Bcl-2 and Bcl-X_L has been reported in GD glands and in TECs from HT patients *(119)*. This decrease is probably a consequence of the action of proinflammatory cytokines *(119)*.

In conclusion, it would appear that the increased thyroid-apoptotic activity found in AITD (and particularly in destructive forms of HT) is the consequence of multiple mechanisms involving a destabilization of the fine balance between proapoptotic and antiapoptotic molecules, rather than the result of activation of a single specific death-receptor ligand.

ETIOPATHOGENESIS OF AITD

Factors Potentially Involved in Triggering AITD

Theoretical possibilities that would account for the development of an organ-specific or antigen-specific autoimmune disease would include the following:

1. An antigenic stimulus (however initiated, including molecular mimicry) that precipitates and/or sustains the disorder. This could result from increased circulating concentration of antigen resulting from cell destruction or viral infection, and leading to a high circulating antigen concentration that overwhelms low-dose antigen tolerance. Partial degradation or denaturation of antigen by viral attack or other means, as well as cross-reaction with bacterial or viral epitopes, could be also involved.
2. The induction of thyroid autoimmunity by such antigenic stimuli not in all cases, but only in persons with an underlying immune abnormality. This immune abnormality may have an important genetic background, owing to the inheritance of specific HLA, TCR, or other genes encoding proteins involved in the processing and/or in presentation of the antigen.
3. Somatic mutations of TCR genes and Ig genes that could lead to clones of autoreactive lymphocytes. This possibility is highly improbable, however, since somatic mutations of TCR genes occur very rarely, and monoclonal or oligoclonal activation is not present in autoimmune diseases.
4. Inherent defects in immunoregulation of T and B cells, resulting either from hereditary or environmental factors.
5. Self-reactive T and B cells resulting from failure of clonal deletion and from abnormal maturation of the immune system, leading to the persistence of autoreactive fetal lymphocytes.
6. Abnormal expression of MHC class II molecules on TECs, which has been proposed as the primary event necessary for thyroid autoimmunity, although this is presently believed to be mainly secondary to activation of the cytokine.
7. Finally, environmental factors such as iodine and hormones (stress hormones, steroids) may alter immunoregulation and contribute to AITD development.

Abnormal Antigenic Stimulus

Thyroid damage could lead to the release of sequestered antigens and induce an immune response. Tg, once considered a secluded autoantigen, is actually a normal component of plasma. Less is known about the actual plasma concentration of TPO and TSH receptor. However, as discussed below, viral subacute thyroiditis is clearly associated with massive thyroid damage and antigen release, but is not followed by the development of progressive thyroid autoimmunity. External and internal irradiation are associated with GD through a brief increase in thyroid-autoantibody production, but whether and to what extent this phenomenon is caused by autoantigen release or effects of radiation on thyroid-infiltrating lymphocytes remains unclear.

In humans, no significant inherited or acquired intrinsic abnormality of thyroid autoantigens has so far been identified *(131)*, with the exception of minor allelic differences in TSH receptor, which are generally unrelated to the development of AITD *(132)*. However, a genetic abnormality of thyroid cells has been found to be crucial for the development of AITD in the obese-strain (OS) chicken model of spontaneous hereditary AITD *(133)*. This abnormality is manifested by thyroid autonomy, increased thyroid uptake of iodide, and increased thyrocyte expression of HLA-DR in response to IFN-γ. By contrast with this model, no such abnormalities can be demonstrated in humans with AITD. Both in patients in remission after antithyroid drug treatment for GD, and in AITD xenografts in nude mice, the thyroid cells can be shown to be functioning normally, under normal physiological control, and with normal suppressibility by exogenous TH *(26,134)*. Moreover, neither thyroid cells in tissue culture derived from human AITD *(135)* nor nude mouse xenografts in vivo, respond in an excessive manner to IFN-γ either alone or in combination with TNF-α, in terms of HLA-DR expression *(136)*. One can only conclude that the human AITD thyroid cells are intrinsically normal, with no evidence of a genetic thyrocyte abnormality.

Even in the absence of intrinsic antigenic abnormalities, autoimmunity could result from a physiologic response of a normal immune system to autoantigen released in an inflammatory response to virus infection or to another antigen expressed in the target tissue *(137)*. Furthermore, a viral or microbial antigen with structural homology to an autoantigen (molecular mimicry) might initiate production of cross-reacting autoantibodies and a subsequent immune response directed against autologous cell structures *(138)*. Although several potential ways whereby infections might induce AITD have been envisaged *(138)*, their actual relevance in human disease is very questionable, as discussed below.

Viral Infections and AITD

The most obvious example of what appears (almost certainly) to be a viral infection of the thyroid gland is subacute De Quervain's thyroiditis. The clinical and laboratory features of this entity have been thoroughly described elsewhere *(26)*. There are many descriptions of the immunologic and serologic phenomena that appear during the course of this illness, only to disappear gradually as the inflammatory lesions subside; these include the appearance of thyroid autoantibodies (including TRAbs) and thyrocyte HLA-DR expression in at least a minority of patients *(139)*. Yet AITD only rarely follows. Thus, two points should be made from these observations. First, that lymphocytes in normal persons have the genes and capacity to produce thyroid autoantibodies, including TRAbs in response to the increased release of thyroid antigen from inflamed and damaged thyroid cells. Second, that this excess antigen release into the immune system will not itself lead to AITD.

Bottazzo et al. *(140)* first reported "aberrant" expression of HLA class II antigen (HLADR) on the cell surfaces of AITD thyrocytes, which they subsequently proposed *(141)* as resulting

from viruses that stimulated intrathyroidal T lymphocytes to produce IFN-γ, which would in turn induce thyrocytes to express HLA-DR, thus enabling them to directly present antigen, hence inducing AITD. Indeed, transfection of thyroid cells in culture by viral vectors has been reported to induce weak HLA-DR expression by thyrocytes, although not at the magnitude observed in AITD *(142)*. Moreover, Ciampolillo et al. *(143)* have demonstrated the presence of HIV gag sequences by Southern blot analysis in the thyroid glands of GD patients but not in normal glands or thyroid neoplasms. Wick et al. *(144)* have reported that antibodies against the gag 2 protein of the human foamy virus binds to GD thyroid tissue, as well as to retrobulbar fibroblasts from patients with Graves' ophthalmopathy. However, there is much evidence against the theory that viruses induce AITD. First, BB rats delivered by a cesarean-section and raised in a germ-free (gnotobiotic) environment still develop insulin-dependent diabetes (and AITD) despite being free of viruses *(145)*. Second, the presence of HIV gag sequences in the thyroid glands of GD patients could not be confirmed by two other groups *(146,147)*. Third, three other centers could not demonstrate the presence of foamy virus (human spumaretrovirus [HSRV]) sequences in GD thyroid tissues or peripheral blood leukocytes in frequencies greater than in normal control subjects *(148–150)*. Moreover, if HLA-DR expression by AITD thyrocytes was a result of virus infection of thyroid cells, it would not be expected that thyrocyte HLA-DR would quickly disappear from those cells in tissue culture *(135)* or after xenografting of AITD tissue into nude mice *(134)*, as indeed it does.

Bacterial Infections and AITD

As mentioned earlier, several authors have proposed that microbial infections, including those of bacterial origin, may play a role in precipitating AITD *(151)*. Although several organisms have been suggested, the greatest focus has been on *Yersinia enterocolitica (151)*. Indeed, evidence of cross-reactivity between antigens from these microorganisms and thyroid-cell-membrane antigens (including TSH receptor) has been reported *(152)*, although other groups have recently failed to confirm this *(153,154)*. If there is cross-reactivity between bacterial and thyroid antigens (molecular mimicry), this would not necessary imply that such cross-reactivity plays any role in the induction of AITD; in fact, the evidence suggests otherwise. For example, in a study of patients with active *Y. enterocolitica* infections (some with thyroid stimulating properties) were frequently found, yet there were no examples of thyroid dysfunction *(154)*. Thus, unusual or excessive antigen presentation, even if the antigen has homology with human thyroid antigens, does not appear sufficient to induce AITD. The situation seems analogous to that of subacute thyroiditis, in which there is a transient, appropriate response (of a normal immune system) to the liberation of antigen, but AITD does not follow *(139)*. Thus, once again, AITD depends on an abnormal immune system.

Genetic Predisposition to AITD

Familial clustering and twin studies strongly suggest a genetic predisposition to AITD. Evidence for a family history of thyroid disease has been found in more than 40% of patients with GD *(155)*, and a high prevalence of antithyroid antibodies (45–55%) *(156)* and overt thyroiditis (20–35%) *(157,158)* has been documented for a long time. Concordance rates for GD in identical twins have been reported to be as high as 30–40%, as compared with 3–9% in dizygotic twins *(159)*. The rather low concordance rates for monozygotic twins imply that in addition to the genetic background, environmental factors play an etiologic role in AITD. As subsequently discussed in more detail, the genetic predisposition to AITD derives from

Table 6
Associations Between HLA and Autoimmune Endocrinopathies and Other Related Autoimmune Disorders[a]

		Frequency, %		
Condition	*HLA*	*Patients*	*Controls*	*Relative risk*
Idiopathic Addison's disease	D/DR3	60	26.3	6.3
Graves' disease	D/DR3	56	26.3	3.7
Insulin-dependent diabetes mellitus	D/DR3	56	28.2	3.3
	D/DR4	75	32.2	6.04
	D/DR2	10	30.5	0.2
Myasthenia gravis	D/DR3	50	28.2	2.5
Sjögren's syndrome	D/DR3	78	26.3	5.7
Atrophic thyroiditis	DR3	64	23.8	5.7
Goitrous thyroiditis	DR5	53	26.3	3.1
Pernicious anemia	Dw5	25	5.8	5.4
Subacute thyroiditis	Bw35	70	14.6	13.7

[a]The relative risk indicates how many times more frequently the disease develops in individuals carrying the HLA antigen in question than in individuals lacking the antigen. Adapted from ref. *161*.

several susceptibility loci, which is typical of complex polygenic disorders. Two different strategies have been applied to determine the identity of these loci: one is represented by population-based case–control studies, and the other by linkage studies.

Population-based studies assess the association of a marker allele with a disease by comparing the frequency of the allele in a population affected by that particular disease with the frequency found in a disease-free population. Initial studies, done largely with serologic methods, identified the HLA class II antigen DR3 on chromosome 6p as significantly associated with GD *(160)* and with the atrophic form of HT. The association of AITD with the HLA class I B8 antigen reported in early investigations was subsequently shown to be the consequence of strong linkage disequilibrium between B8 and DR3. Other class II antigens (DR4 and DR5) have been found in association with the goitrous form of HT. As summarized in Table 6, these associations have also been found in several other organ-specific autoimmune diseases *(161)*. Further insights into the relationship between HLA class II polymorphism and AITD susceptibility come from more recent studies reporting full molecular characterization of HLA antigens *(162,163)*, summarized in Table 7. However, the existence of a significant association between other class II antigens (DP and DQ) and AITD independently of DR3, continues to be debated because of the strong linkage disequilibrium between these molecules. Taken together, the data emerging from HLA associations in AITD are complex, and the contribution of HLAs to the overall genetic predisposition is low, because the relative risk (RR) conferred by any particular HLA allele is generally from 2 to 4, which is largely below the RR of 20 calculated from family studies *(162,163)*. Thus, genes outside the HLA system should contribute to the predisposition to these disorders. Consequently, other genes have been investigated in association studies, including these encoding the immunoglobulin heavy chain, the T-cell receptor, the TSH receptor, and the IL-1 receptor antagonist, but the results have been inconclusive. More consistent results derive from recent studies of the immunoregolatory gene encoding the cytotoxic T-lymphocyte antigen 4 (CTLA-4), located on chromosome 2q33. This protein is expressed on the surface of activated T cell and competes with CD28 for the costimulatory

Table 7
HLA Class II Alleles Identified by Sequence-Specific Oligonucleotide Probes Significantly Related to AITD in Association Studies

Allele	*Relative risk*
DQB1*0201	3.3
DQA1*0501	2.5–3.8
DRB1*0304	2.7
DQB1*0301/4	1.9

Data from refs. *235–237*.

receptors B7-1 and B7-2. Binding of B7 to CTLA-4 delivers negative signals to the T cell and reduces T-cell expansion, cytokine production, and immune responses. The CTLA-4 gene was first investigated as a candidate gene for GD *(164)*, and the significant association found was confirmed by subsequent case-control studies in patients with GD *(165,166)* and HT *(166)*. Unfortunately, as for HLAs, low RRs have been found for the CTLA-4 gene, suggesting that it has only a minor independent role in AITD.

A different approach for association studies is represented by linkage studies in families. Linkage is defined as the tendency of genes to be inherited together as a consequence of their physical proximity on a single chromosome. If a chromosomal marker is inherited together with a disease (cosegregation), and the cosegregation is statistically significant, the marker and the disease are said to be linked. This method, successfully used in studies of type 1 diabetes mellitus and multiple sclerosis, requires the identification of potential candidate predisposition genes. For this reason, linkage studies in AITD have initially focused on the HLA complex. In one large family with AITD, no evidence of linkage to HLAs was found *(167)*, and similar findings were subsequently obtained *(168)*. To date, only one study *(169)* has shown a positive linkage of HLAs with HT, in a subset of patients with type 1 diabetes mellitus. Because type 1 diabetes mellitus is linked to HLAs, it is doubtful whether these data apply to the larger population of HT families without diabetes.

Some authors have suggested that classical linkage analysis of MHC-HLA genes has failed to replicate case–control data because its ability to detect genes of modest effect is limited. To overcome this problem, the use of a different methodologic approach, called linkage disequilibrium analysis, has been proposed in families with AITD. This method evaluates the transmission of alleles from heterozygous parents to one or more offspring. We would expect all alleles to have a 50% chance of being transmitted to offspring. However, if one of the alleles is associated with a disease, its expected transmission to diseased offspring would be greater of 50%. Using this method in GD patients in the United Kingdom, Heward and coworkers *(170)* found a significant increase in the frequency of HLA-DRB1*0304, -DQB1*02, -DQB1*0304, -DQA1*0501, and the haplotype -DRB1*0304-DQB1*02-DQA1*0501 in offspring with GD than in unaffected controls. Although the relevance of the HLA complex in family studies needs further evaluation, these antigens cannot in any case represent major independent susceptibility genes for AITD. Similarly to the low RRs found in association studies, family linkage analysis has been extended to several candidate genes outside the HLA complex (the genes for the thyrotropin receptor, thyroglobulin, thyroperoxidase, immunoglobulin heavy chain, T-cell receptor, IL-1 receptor antagonist gene, different insulin-dependent diabetes mellitus [IDDM]-related genes), but this has provided unsatisfactory results *(163,171)*.

Linkage analysis has also been then applied to whole-genome screening without any previous knowledge of candidate genes, thanks to the advances made by the human genome project, and this approach may allow mapping of new genes. A recent series of studies *(172–176)* used the whole-genome-screening approach to dissect the genetic susceptibility in families with AITD. Six loci were identified that showed evidence of linkage with AITD: the AITD-1 gene, located on chromosome 6p, was linked with both GD and HT; the GD-1 gene, located on chromosome 14q31 *(172,174)*, the GD-2 gene, located on chromosome 20q11.2 *(174)*, and the GD-3 gene, located on chromosome Xq21.33-22 *(175)*, were linked with GD alone; the HT-1 gene, located on chromosome 13q32, was linked with HT; and the HT-2 gene, located on chromosome 12q22, was linked with HT only in European and not in North American families.

In summary, despite strong epidemiologic evidence for a genetic component in the etiology of AITD, few hereditary risk factors have been consistently identified, although this includes some new candidate genes. Unfortunately, despite all of the susceptibility genes so far identified, none entirely accounts for the large hereditary effect of AITD. Thus, more genetic determinants must be hidden in the folds of the human genome, and will most likely be detected in the near future.

Abnormal Immunoregulation in AITD

Role of T-Lymphocytes

The central role of T lymphocytes in AITD was discussed earlier. It has been suggested that only one, or at most a few, T-cell clones may be involved at the beginning of AITD, as shown by the restricted usage of TCR V genes by autoreactive T lymphocytes. In this regard, Davies et al. *(177)* have reported limited variability of TCR Vα and Vβ, genes in intrathyroidal T cells from hyperthyroid GD patients, and similar but less marked results for TCR Vα genes in autoimmune thyroiditis. This finding might be considered surprising, given both the proclivity of an autoimmune lesion to recruit an expanded population of nonspecific T-lymphocyte clones into the affected tissue over time and the difficulty in attempting to study AITD within a few days after its onset *(178)*. In this context, McIntosh et al. *(179)* have failed to confirm the findings of Davies et al.

Studies of the numbers and function of T cells in the peripheral blood in AITD have been divided into those relating to CD4 (helper/inducer) and to CD8 (suppressor/cytotoxic) cells, since their functions and responses are quite different, and often contrary *(180)*. Although the phenotype of suppressor T-cells may vary under different circumstances, CD8 cells can be clearly shown to contain suppressor activity in humans *(181)*. Moreover, $CD8^+$ $CD1lb^+$ cells have suppressor activity but no cytotoxic activity *(182)*, and may therefore be characterized as suppressor (regulatory) T lymphocytes. Fortunately, the findings in studies of suppressor function, and of the activation of suppressor cells in vitro, parallel those for $CD8^+$ cells, and thus $CD8^+$ cells may be used in experiments as "suppressor" T-cells under appropriate circumstances *(183)*. Studies of suppressor T cells have been of two quite different types: those of generalized (nonspecific) suppressor T-cells, and those of antigen-specific suppressor T-cell function *(26)*.

Although clonal deletion of autoreactive T lymphocytes in the thymus plays an important role in the development of tolerance to self-antigens, many autoreactive T lymphocytes reach the periphery, where they remain unresponsive to self-antigens *(184)*. Both "anergy" and active suppression have been postulated to explain this finding. Anergic T lymphocytes can certainly be identified in the periphery, but this cannot account for the observation that

adoptive transfer of T lymphocytes from mice tolerant to a given antigen reduces the immune response to that same antigen in syngeneic recipients *(185)*. The best explanation for this observation is that there exist T lymphocytes that can suppress immune responses.

It has therefore been suggested that AITD could derive from a reduced number/activity of T-suppressor cells *(186,187)*. Several reports have described a reduction in the numbers, activation, and function of nonspecific suppressor T lymphocytes during the hyperthyroid phase of GD, with these deficits tending to normalize as thyroid function improves *(188)*. Reduced nonspecific T-suppressor-cell number/function is also observed in many other autoimmune diseases *(189,190)*, suggesting that AITD may share a generalized nonspecific immunoregulatory defect with other autoimmune diseases. On the other hand, with some exceptions, T-cell findings in euthyroid autoimmune thyroiditis are usually normal *(188)*. Furthermore, a reduction in nonspecific T-suppressor cells has also been found in toxic nodular goiter (a nonimmune form of hyperthyroidism), and an inverse correlation has been observed between the numbers of nonspecific T-suppressor-cell numbers and serum thyroid hormone concentrations *(191)*. Thus, it has been suggested that the reduction in nonspecific T-suppressor lymphocytes is not a primary or specific event, but is a phenomenon secondary to the hyperthyroidism itself *(192)*; however, by its additive effect, it may act to perpetuate and amplify the disease.

In addition to findings of non-specific suppressor T-lymphocyte abnormalities, several studies support the presence of an organ-specific defect in suppressor T-lymphocyte function in AITD, unrelated to thyroid function *(186)*. These studies are generally based on functional in vitro assays showing reduced thyroid-antigen-specific activation of $CD8^+$ T-cell subsets from peripheral mononuclear cells of AITD patients. This phenomenon is seen both in HT and GD, when Tg and TPO are used as antigens *(186)*, and has been reported to be specific for GD when TSH receptor is used as antigen *(193)*. Antigen-specific T-suppressor defects have also been reported in some animal models of AITD (*see* paragraph 2.6). These results have been interpreted as an evidence that a thyroid-specific T-suppressor defect is the key immunoregulatory abnormality responsible for the loss of tolerance in AITD *(186)*. It should be noted, however, that the pathophysiologic relevance of some of the methods used to detect antigen-specific T-suppressor cells is still debated, owing to the difficulty in reproducing results and that the nature of antigen-specific suppressor activity is controversial *(194,195)*. It has recently been suggested that a likely explanation for many suppressor phenomena is the reciprocal inhibition of TH1 and TH2 cells by the specific cytokines produced by each: this mechanism has been actually shown to be responsible for exacerbating or inhibiting autoimmune disease in animal models *(195)*.

Besides stemming from abnormal immunoregulatory T-lymphocytes, the disorder of immunoregulation responsible for the development of AITD could be the consequence of both abnormal autoantigen processing and presentation by professional APCs and/or by thyroid cells themselves, and of the action of various cytokines and other locally produced immunoregulatory molecules.

Role of APC, Cytokines, and Other Immunoregulatory Molecules

It is apparent that the immunologic disturbance is based on the presentation of antigen by APCs (e.g., monocytes, macrophages, dendritic cells, and possibly, thyrocytes [*see* following in this subheading]) and the corresponding responses of T and B lymphocytes. These cellular interactions depend on the TCR, MHC molecules, and a variety of costimulatory molecules that facilitate the immune response. In addition, the production of various cytokines by

elements of the immune system in a coordinated fashion, and at close range to the target cell, is an essential ingredient in completing the pathophysiologic picture.

Autoimmunity could be triggered by inherited or acquired abnormalities in the function of professional APCs. In keeping with this concept, it would appear that autoimmune responses may be favored by the particular differentiation pathway of dendritic cells, which can be initiated by certain locally produced cytokines or by intrinsic defects in genes controlling dendritic-cell function *(196)*. In animal models of autoimmune thyroiditis, dendritic cells and some subclasses of macrophages appear to have an important role in Tg presentation during the initial phases of thyroiditis *(197)*. The precise role of dendritic cells and other APCs in human AITD remains to be defined.

As discussed earlier in this chapter, the analysis of TECs and thyroid infiltrates of AITD glands shows many abnormalities that may be important in the process of antigen presentation. Probably the best known and most debated such abnormality is the abnormal expression of MHC class II antigens (HLA-DR) on the surface of thyroid cells. This observation led originally to the hypothesis that HLA-DR expression could result in an ability of TECs to function as APCs, and that this could be the primary abnormality in thyroid autoimmunity *(140,198)*. This hypothesis was soon abandoned as evidence accumulated that expression of HLA-DR (as well as DP and DQ antigens) was a secondary phenomenon resulting from local cytokine (particularly IFN-γ and TNF-α) production, augmented by TSH stimulation *(199)*. This concept stems from the transient nature of HLA-DR expression on TEC. These molecules in fact disappear from thyroid cells when the immune environment is removed, either in tissue culture or in nude mice, and can be restored by the addition of appropriate cytokines *(136,200)*. Furthermore, Iwatani et al. *(201)* have shown that the expression of HLA-DR antigen on thyroid cells in culture depends on the presence not only of helper T cells, but also of monocytes, implying that a full induction of the cytokine cascade is needed *(201)*. Moreover, there is nothing unique about the response of the thyroid cells in AITD to IFN-γ or other cytokines (e.g., TNF-α), since normal thyroid tissue responds at least as well as or better than AITD thyroid tissue when treated with these agents, both in terms of HLA-DR expression and ICAM-I expression *(200)*. In experimental animal models, thyroid gland first displays thyroid lymphocytic infiltration, and only subsequently do TECs appear to express class II molecules *(202)*. Therefore, it would appear that the expression of these molecules on the TECs depends entirely on the local cytokine environment, in turn dependent on the local immunocyte infiltration *(25)*. On the other hand, class II expression on TECs is involved in further steps in the autoimmune process, since HLA-DR-positive thyroid cells are able to behave as APCs for T-lymphocytes, provided that they have been prestimulated by the appropriate costimulatory signal (B7 molecules) *(203)*. Interestingly, in the absence of B7, DR-positive TECs do not stimulate but instead induce anergy in potentially autoreactive but still naive T cells, which are dependent on these costimulatory molecules *(202)*.

Although the primary event triggering thyroid autoimmunity remains elusive, several cytokines and other locally produced immunoregulatory substances could be involved in the autoimmune process from the initial presentation of autoantigen to the full activation of the autoimmune response. The local production, in the initial phase of thyroid autoimmunity, of particular chemokines that can selectively attract TH1 cells could be responsible for the peculiar TH1-like pattern of the thyroid infiltrate in AITD discussed earlier in this chapter. Chemokines are chemotactic cytokines that control the attraction of leukocytes to tissue, upregulating the inflammatory response *(1)*. The relative position of a Cys tandem

defines chemokines as having any one of four structural motifs (CXC, CC, C, and CX3C). Chemokine receptors are heptahelical receptors coupled to G proteins. Human TH1 and TH2 cells express chemokine receptors differentially, and accordingly migrate differentially in response to different chemokines. Chemokines such as IFN-inducible protein (Ip-10) and monokine induced by IFN-γ (Mig) are CXC chemokines that are inducible by IFN-γ and which display potent lymphocyte chemotactic activity. The highly organized infiltrate present in thyroid glands with AITD suggests that several chemoattractant proteins are probably involved in a multistep inflammatory model. Recent studies also suggest the direct involvement of thyroid follicular cells in the production of Ip-10 and Mig, which would then mediate the local accumulation of T cells in AITD *(204,205)*; furthermore, increased concentrations of Ip-10 have been found in the serum of patients with active GD *(205)*. Overexpression of Ip-10 and Mig by TECs is the consequence of high levels of proinflammatory cytokines, mainly IFN-γ.

Various other molecules are probably important also for the development of AITD. These include lymphocyte function-associated antigens (LFA-1, LFA-2, LFA-3), which act as accessory molecules that increase the binding of T cells to APCs *(1)*. In contrast to normal TECs, thyrocytes of autoimmune thyroid glands express ICAM-1 which, interacting with LFA-1, enhances lymphocyte activation *(206–208)*. ICAM-1 is expressed more consistently and at higher levels on TECs from HT than from GD glands *(207,209)*, possibly as a consequence of the greater cytokine production by the HT infiltrate. Furthermore, TECs from HT glands also express LFA-1, leading to potential bidirectional interactions between TECs and infiltrating mononuclear cells *(209)*. Similarly to HLA-DR, the appearance of ICAM-1 on TECs in AITD is believed to be mainly a secondary event *(210)*. B7-1 antigen (which could provide an important costimulatory signal) has been found on TECs from HT glands *(211)*, while neither B7.1 nor B7.2 molecules are found in TECs from GD glands *(212)*. The mechanism responsible for this different expression is unknown, but it could again be the consequence of the different cytokine milieu found in the two diseases. Thyroid cells can produce some other immunologically active molecules as a result of cytokine stimulation or complement attack; these include prostaglandin-E_2 (PGE_2), IL-6, and IL-8, thus further increasing thyrocyte–immunocyte signaling *(213)*.

Antiidiotype Antibodies

Antiidiotype antibodies are believed to play a role in the physiologic regulation of Ig production. Although anti-Tg antibodies can be generated in animal models by experimental manipulation of the idiotype–antiidiotype network *(214)*, there is scant evidence that a disturbance of the anti–idiotype network is actually involved in human AITD. Igs from some GD patients are able to bind TSH *(215)*, a finding that has been interpreted as suggestive of the presence of antiidiotype antibodies to TRAbs, or, conversely, that antiidiotype antibodies to antibodies directed to TSH may function as TRAbs, however, either possibility remains to be proven. Antiidiotype antibodies to anti-Tg and anti-TPO antibodies have been very difficult to demonstrate in sera of AITD patients or normal subjects *(216,217)*, suggesting that antithyroid-idiotype antibodies are rarely produced at a detectable level.

Environmental Factors and Thyroid Autoimmunity

Exogenous administration of large amounts of IL-2 *(218)*, IFN-α, or other cytokines *(219–222)* for the treatment of cancer or viral hepatitis may lead to the appearance of thyroid autoantibodies and thyroid dysfunction (mainly hypothyroidism). A similar effect has been reported with highly active antiretroviral therapy for acquired immune deficiency syndrome

(AIDS) *(223)*. The relevance of these observations to the spontaneous development of AITD is unclear, given the high pharmacologic doses of drugs employed. However, GD is associated with increased serum IgE concentrations *(224)*, and relapse of hyperthyroidism has been described after attacks of allergic rhinitis *(225)*. These observations suggest that a preferential activation of TH2 cells may sometimes lead to stimulation of TSAb production, causing hyperthyroid GD. The role of dietary iodine in stimulating thyroid autoimmunity in genetically predisposed individuals is well documented in animal models *(226,227)*. In these models, iodine appears to exert its effects through interference with almost all stages of the immune process. Iodine-induced alterations in thyrocyte metabolism and thyroid-cell necrosis are probably involved in the process of antigen presentation and in the effector autoimmune mechanisms leading to thyroid-cell damage. Moreover, iodine has direct effects on the antigenicity of Tg *(226)*, on thymus development *(228)*, and in the development and function of various immune cells, including T and B lymphocytes, macrophages, and dendritic cells *(227)*. Iodine exerts a similar effect by iodine in human AITD, although in this the evidence is only circumstantial *(75,229)*. Lithium has been suspected of inducing thyroid autoimmunity, but the data could be confounded by the increased frequency of positive thyroid antibodies in patients with major depression, who are often treated with this drug *(75,230)*. Cigarette smoking is associated with an increased risk of GD and Graves' ophthalmopathy: the mechanisms involved in this phenomenon are unknown, but may include abnormal immunologic stimulation *(231)*. A large number of anecdotal reports have associated the onset of GD with physically or psychologically stressful events, although this association has been difficult to prove *(232)*; however, stress can probably trigger autoimmunity via perturbations of the neuroendocrine network, including alterations in glucocorticoid secretion *(233)*. Several environmental toxins and polluants (polybrominated biphenyls, phenols, thiocyanate, hydroxypyridines, substituted dihydroxybenzenes, and resorcinol derivatives) have been proposed as potential inducers of AITD *(229)*, but the available data are scanty and nonconclusive.

Endogenous hormones, such as corticosteroids, estrogens, androgens, prolactin, and many others, are able to modulate the immune reaction and could therefore play a role in triggering/exacerbating thyroid autoimmunity *(234,235)*. A condition known to strongly interfere with the expression of AITD, mainly through changes in endogenous hormone concentrations, is pregnancy. This important topic will be discussed in more detail next.

Pregancy and Thyroid Autoimmunity

Pregnancy is characterized by a clinical improvement in both GD and HT, associated with a decrease in both the prevalence and titer of circulating antithyroid autoantibodies *(236)*. In particular, remission of hyperthyroidism with pregnancy is observed in about 50% of GD patients; in patients with goitrous HT and hypothyroidism, partial recovery of thyroid function and reduction of the goiter size is often observed during the third trimester of pregnancy. The reverse is observed in the immediate period after birth, which is associated with marked exacerbation of thyroid-autoimmune reactions. In this period, up to 70% of GD patients show a relapse of hyperthyroidism, whereas hypothyroidism recurs in those with HT who have had a partial recovery of thyroid function during pregnancy. However, the most frequent and characteristic postpartum thyroid dysfunction is represented by postpartum thyroiditis (PPT). PPT is characterized by a course of self-limited destructive thyrotoxicosis followed by recovery of thyroid function, and is typically observed in women genetically predisposed to thyroid autoimmunity, often with detectable serum antithyroid antibodies but normal thyroid function before and during pregnancy *(237)*. This latter expression after

pregnancy of an asymptomatic autoimmune thyroiditis is rather frequent among women of reproductive age, with a prevalence of antithyroid antibodies in euthyroid, unselected pregnant women of 6%–19.6% *(238,239)*. Higher prevalence of antithyroid antibodies (up to 25–30%) are found in particular subgroups, such as members of families with an increased prevalence of AITD and/or other endocrine-autoimmune disorders, particularly type I diabetes mellitus *(240)*. PTT is also more often observed in women with a previous history of HT or GD, but the greatest risk factor is a previous episode of PPT. Up to 30–70% of women with history of PPT will develop a new episode of PPT after future pregnancies *(241)*. The strict link with AITD, as well as several clinical and immunopathologic data, strongly support the concept that PPT has an autoimmune pathogenesis and may be considered a variant of HT *(241)*. Thus, the most probable cause of PPT is represented by postpartum exacerbation of humoral and cellular thyroid autoimmunity.

When all of its possible etiologies are taken together, the occurrence of transient or persistent autoimmune thyroid dysfunction is a frequent event in the first postpartum year, being observed in 5–10% of the general population *(237)*. The clinical presentation of postpartum thyroid dysfunction is complex and may fall into the five categories of *(237)*: (1) transient destructive thyrotoxicosis with low radioiodine (RAI) uptake followed by transient hyperthyroidism; (2) transient hyperthyroidism with high, normal, or low RAI uptake; (3) persistent hyperthyroidism with high RAI uptake (true GD); (4) transient or persistent hypothyroidism without preceding transient thyrotoxicosis; and (5) preceding destructive thyrotoxicosis followed by stimulatory (GD) hyperthyroidism. Most cases of destructive-induced thyrotoxicosis develop from 1–3 mo postpartum, while persistent stimulatory hyperthyroidism usually develops later (3–4 mo).

The mechanism(s) responsible for the changes in expression of thyroid autoimmunity during pregnancy and in the postpartum period are only incompletely known, and may include several of the general immunosuppressive mechanisms described above. Changes in NK-cell number/activity and in the proportions of different T-lymphocyte subsets have been particularly suggested, but the relationship of these changes to the clinical course of AITD is unclear. For example, women with PPT display normal peripheral NK-cell activity *(242)*. Interestingly, circulating concentrations of α_2-pregnancy-associated glycoprotein (PAG) are significantly higher in pregnant patients with GD and HT undergoing remission than in those with active disease *(243)*. Independently the underlying mechanism(s), the decrease in the circulating antithyroid antibody titer in the second part of gestation, and the postpartum increase is a well-documented phenomenon *(237)*. This behavior has been observed for anti-Tg, anti-TPO (formerly antimicrosomal), and anti-TRH-R autoantibodies. A decrease in TSAbs is the most probable cause of the frequent remission of hyperthyroidism observed in pregnant women with GD, while the increase in TSAb titers explains the relapse of Graves' hyperthyroidism often observed immediately postpartum *(244)*.

THE PATHOGENESIS OF GRAVES' OPHTHALMOPATHY AND DERMOPATHY

The ophthalmopathy of GD (GO) is still of unknown etiology *(245,246)*. It may precede, accompany, or follow hyperthyroidism, and most patients (but not all) with GO manifest hyperthyroidism at some time. However, about 5% of patients with definite "euthyroid Graves' ophthalmopathy" have no past or present history of hyperthyroidism. GO is occasionally associated with HT and primary myxedema *(26)*. Although the majority of

patients with euthyroid Graves' ophthalmopathy, who manifest no clinical evidence of thyroid dysfunction, do exhibit subclinical abnormalities in thyroid function or positive antithyroid antibody titers, there remains a small group who have no detectable laboratory evidence of thyroid abnormality whatsoever.

Conversely, even minimal ocular disease, as evidenced by computerized axial tomography, ultrasonography, or increased intraocular pressure on upward gaze, can be demonstrated in many GD patients with no obvious clinical eye signs *(247)*. Nevertheless, there remains a group of GD patients who show no ocular abnormalities even with such careful testing *(26,245)*.

It is widely accepted that GO is an autoimmune disorder *(246,248,249)*. This concept is mainly supported by the associated histopathologic changes, consisting of an increased volume of the extraocular muscles, orbital connective and adipose tissues, edema of the extraocular muscles from increased production of the hydrophilic glycosaminoglycans (GAGs) present in orbital tissue, and marked infiltration of immunocompetent cells (T lymphocytes, macrophages and, to a lesser extent, B lymphocytes) *(250)*.

According to the best-supported pathogenetic hypothesis *(246)*, the orbital infiltrate is initiated by autoreactive $CD4^+$ T lymphocytes recognizing an antigen shared by the thyroid and the orbital tissues (fibrobasts, fat cells, and the perimysium of extraocular muscle). This process may be facilitated either by circulating or by locally produced adhesion molecules, the expression of which may be induced by cytokines *(251)* and which may be related to the activity of the disease *(252)*. In keeping with this scenario is the finding of a biased usage of the TCR Vβ gene *(253)*, which supports the concept of an antigen-specific immune reaction *(250)*. As described for the thyroid infiltrate in GD, $CD4^+$ T lymphocytes could, after antigen recognition, secrete cytokines that amplify the immune response either by activating other T-cell subsets or activating autoantibody-producing B cells *(113,249)*. Phenotypic and functional analysis of T-cell clones from the orbital tissue of GO patients has revealed a predominance of T cells with a TH1 profile *(254,255)*, yet a TH2 profile of cytokine production has also been reported *(256,257)*. These differences might be related to different stages of disease development or disease activity levels *(258)*, and/or to different methodologic approaches.

Cytokines (mainly IFN-γ) induce the expression on cultured retroocular fibroblasts of both MHC class II molecules and heat-shock protein-72 (HSP-72), which may favor antigen recognition, and of ICAM-1, which may stimulate T-cell recruitment *(113,249)*. In addition, cytokines stimulate GAG synthesis and secretion from fibroblasts *(259)*, with consequent fluid attraction into the retroorbital space and development of periorbital swelling, proptosis, and extraocular muscle swelling *(246)*. The observed expansion of the orbital tissue volume content could also be the consequence of cytokine-induced proliferation of fibroblasts *(113,249)*. Orbital fibroblasts may contribute to perpetuating the ongoing immune reaction in the orbit by inhibiting apoptosis of infiltrating T cells *(260)*.

The unresolved questions in the foregoing pathogenetic hypothesis are: (1) the nature of the antigen shared by the thyroid and the orbit, and (2), the type of orbital cell carrying the autoantigen recognized by T cells.

All thyroid autoantigens (Tg, TPO and TSH-receptor) have been considered as potential candidates for the shared antigen involved in GO. The presence of Tg in orbital tissues has been postulated for a long time, on the basis of lymphatic connections of the orbit with the thyroid gland *(261)*, but the evidence supporting this hypothesis has been conflicting *(246,248)*. Quite recently, however, Tg has been clearly detected in orbital tissues only from

patients with GO *(262)*, but the evidence supporting a pathogenic role for anti-Tg autoimmunity in GO is still lacking. It has been hypothesized that anti-Tg autoimmunity could be involved in the pathogenesis of GO through molecular mimicry of acetylcholinesterase (ACHE) for a Tg epitope *(29)*. The rationale for this suggestion is represented by the high number of endplate muscle fibers rich in AChe that are present in extrinsic ocular muscles *(29)*. This attractive hypothesis, however, could not be confirmed by subsequent studies.

The most probable candidate as a shared antigen causing GO is the TSH receptor, the autoantigen involved in development of the TRAbs responsible for Graves' hyperthyroidism *(263)*. TRAb transcripts have been demonstrated by RT–PCR in the orbital tissues of patients with Grave's opthalmopathy *(264,265)*, but the extreme sensitivity of this technique may allow the amplification of virtually any gene owing to illegitimate transcription *(266)*. Intact and variant mRNA transcripts for TSH receptor have been demonstrated in orbital tissues (fibroblasts or adipose cells, mainly from GD patients) by other techniques including Northern blot analysis *(267)*, *in situ* hybridization with antisense oligonucletide probe for the extracellular domain of the TSH receptor *(268)*, and liquid hybridization analysis *(269)*. The presence of TSH-receptor-like immunoreactivity has been shown in orbital and pretibial fibroblasts with antibodies directed at the extracellular domain of the TSH receptor *(270)*. More specifically, Ludgate and coworkers *(271)*, using monoclonal antibodies to the TSH receptors produced by genetic immunization *(271)*, detected immunostaining in fibroblast-like elongated cells and in adjacent clusters of adipocytes in orbital bioptic samples of GO patients, but not in orbital-tissue specimens from pseudotumor or in extraocular muscle samples. The presence of circulating IgG and IgA antibodies able to react with the extracellular domain of the TSH receptor expressed as a fusion protein in bacteria has been reported in some GO patients who had negative conventional tests for circulating autoantibodies to TSH receptor *(271)*.

Further support for involvement of the TSH receptor in the pathogenesis of GD stems from experimental animal models. Direct evidence for the production of TRAb by the autoimmune GO infiltrates is provided by the detection of TRAbs in the sera of nude mice with orbital-tissue xenografts from GD patients *(272)*. An experimental model of GO was recently developed by the genetic immunization of mice with recombinant human DNA *(271)*. In this study, transfer of syngeneic, TSH-receptor-primed splenocytes to BALB/c or NOD mice led to destructive thyroiditis with a TH1 cytokine profile in autoimmune-prone NOD mice, while BALB/c mice developed a TH2 response together with the appearance of TSAbs *(273)*. Ocular changes similar to those found in GO were observed in the majority of BALB/c mice, but not in NOD mice *(271)*. Finally, as discussed in "Animal Models of Thyroid Immunity" earlier, the same investigators succeeded in obtaining some outbred NMRI mice immunized with cDNA for the TSH receptor, both hyperthyroidism and ocular changes suggestive of GO, providing the first complete model of experimental GD *(76)*. These results, while strongly supporting the concept of TSH receptor as the shared thyroid–orbital antigen in GO, also suggest the existence of a specific genetic predisposition toward orbital autoimmunity in mice. A genetic predisposition in humans distinct from that of GD has been suggested, but not definitively proven. A genomic point mutation in codon 52 of the extracellular domain of the TSH receptor, leading to a proline-for-threonine substitution, was found in 2 of 22 GO patients and in no normal subjects *(274)*. The role of this polymorphism in the pathogenesis of GO is still unclear.

Another possible explanation is that the thyroid-orbital cross reacting antigen might be located on eye-muscle cells. A 64-kDa antigen shared by the thyroid and the orbit was

reported by Salvi et al. *(275)*, but the specificity of this antigen is uncertain, since it has also been found in other tissues *(276,277)*. Serum antibodies present in about two thirds of GO patients have been found to react under nondenaturing conditions with a 64-kDa protein expressed in eye muscle cells but not in skeletal muscle *(278)*. These antibodies were not found in sera of patients with HT or in euthyroid controls *(278)*. This 64-kDa protein has been partly sequenced and identified as the flavoprotein subunit of mitochondrial succinate dehydrogenase, with a corrected molecular mass of 67 kDa *(279)*. Serum antibodies to purified succinate dehydrogenase appear to have clinical relevance, since they were detectable in 67% of patients with active GO, 30% of patients with stable eye disease, 30% of Graves' disease patients without clinically apparent ophthalmopathy, and only in 7% of normal subjects *(279)*. Furthermore, the appearance of these antibodies has been reported as predictive of subsequent development of GO *(280)*.

Other eye-muscle autoantigens possibly involved in GO include the calcium-binding proteins calsequestrin (63 kDa) and sarcalumenin (53 kD), expressed in extraocular muscle and skeletal muscle, but not in the thyroid *(281,282)*; a different 63–64-kDa protein (D1 protein), cloned from a thyroid cDNA expression library *(276)*, which is expressed in extraocular muscles, skeletal muscles, thyroid, testis, and other tissues *(283)*; and a novel eye-muscle protein called G2s (220 kDa), expressed in extraocular muscles, skeletal muscles, and thyroid *(282)*. Patients with GO also have a higher prevalence of circulating antibodies directed against two porcine eye-muscle antigens (64 kDa and 95 kDa) than do patients without eye involvement or normal controls *(284)*. On the other hand, serum antibodies reacting with several extraocular-muscle antigens may be detected in patients with nonspecific orbital inflammation *(285)*. An antigenic role for eye-muscle cells in GO is also suggested by the coexpression of HLA-DR and HSP-70 in eye muscle cells from GO patients *(286)*. Taken together, the foregoing data support the concept that, with the possible exception of G2s membrane protein, most of the eye-muscle antigens named above are intracellular, ubiquitous, and probably devoid of the disease specificity expected in an organ-specific autoimmune disorder *(263)*. Antibodies to these antigens might therefore represent a secondary phenomenon resulting from eye muscle damage and subsequent antigen exposure.

Tallstedt et al. *(287)* have shown evidence that GO is more often aggravated by ^{131}I therapy for GD than it is by other modalities of treatment, and Bartalena et al. *(288)* have shown that such worsening after ^{131}I therapy can be prevented with corticosteroid therapy. These observations are in accord with the notion discussed above that thyroid–orbital antigenic cross-reactivity is responsible for GO. However, another possibility is that GO might represent instead a genetically related, closely linked, overlapping autoimmune disease, but separate from Graves' hyperthyroidism *(26,245)*.

The nature of the Graves' dermopathy is even more elusive than that of GO. The term "pretibial myxoedema," although describing the common site of the clinically apparent lesions, is actually a misnomer, since the pathologic changes are diffuse throughout the subcutaneous tissues *(26)*. Gravity and vascular arrangements dictate where the lesions appear most obviously. Since the fundamental abnormality in Graves' dermopathy relates to the laying down of glycosamino glycans (as in GO), the main cellular focus of the disease appears to be the fibroblast *(26,289)*. GD patients with pretibial myxedema show the highest levels of serum TSAb; this finding strongly supports a pathogenic connection with TSH receptor autoimmunity *(26,290)*, although the direct proof for this connection remains to be understood.

THE THYROID–IMMUNE SYSTEM RELATIONSHIP: *IMPLICATIONS FOR GD AND HT THERAPY*

Effect of Thyroid Function on the Course and Clinical Expression of AITD

As previously discussed in this chapter, thyroid autoimmunity may occur within the spectrum of thyroid activity ranging from hyperthyroidism (as in GD) to hypothyroidism (as in idiopathic myxedema) and through euthyroidism (as in several cases of HT). This implies the possibility of an interaction between the primary autoimmune disorder and secondary abnormalities of thyroid function.

Several studies have shown that treatment of hyperthyroid GD with antithyroid drugs (ATDs) of the thionamide group, such as methimazole (MMI), carbimazole (CMI), and propylthiouracil (PTU), is associated with a significant decrease in TRAb, anti-Tg, and anti-TPO antibody titers *(75)*. This effect is specific for thyroid antibodies, since administration of MMI does not affect serum gastric parietal-cell antibody, which is often present in patients with GD *(291)*. ATD treatment is also associated with a progressive reduction of circulating, activated (DR^+) peripheral T cells *(292)* and with a transient increase in the proportion of the circulating DR^+ suppressor/cytotoxic T-cell subset, which may be responsible for the decrease in antithyroid autoantibody titers *(292)*.

In apparent contrast with the above findings is a documented, marked decrease in serum anti-Tg and anti-TPO antibodies in patients with idiopathic myxedema or hypothyroid HT subjected to levothyroxine treatment *(293)*. Interestingly, in the latter study *(293)*, we documented no significant effect of levothyroxine in patients with euthyroid HT and normal circulating TSH concentrations before therapy.

The mechanism by which correction of hyperthyroidism and hypothyroidism is associated with a decrease in thyroid-autoimmune phenomena is still matter of controversy, but three main possibilities may be envisaged, as detailed below.

1. The observed effect could be the direct consequence of normalization of thyroid hormone levels. In hyperthyroid patients this view is mainly supported by studies providing evidence that thyrotoxicosis by itself may affect the immune system, possibly through a decrease in the number and function of generalized T-suppressor lymphocytes. The role of thyroid function itself is also suggested by the observation that TSAb titers decline in GD patients treated with $KClO_4$ *(294)*. In contrast to MMI (*see* next item), $KClO_4$ has no recognized immunosuppressive effects *(295)*.
2. ATDs could exert an inhibitory effect on the thyroid-autoimmune reaction. This hypothesis (which has been extensively reviewed *[75]*), stems from the observation that MMI at high concentrations ($\geq 10^{-5}$ *M*) inhibits pokeweed mitogen-driven anti-Tg and anti-TPO antibody synthesis by peripheral blood mononuclear cells from AITD patients without affecting total Ig secretion *(296)*. The relevance in vivo of this phenomenon is still unclear, since other authors have reported that similar MMI concentrations are also able to inhibit total Ig synthesis by cultured lymphocytes *(297)*. Furthermore, the plasma MMI concentrations currently obtained in vivo during ATD treatment are well below 10^{-5} *M*. However, since MMI is concentrated within the thyroid, it has been hypothesized that the major effect of this drug could be exerted at the level of intrathyroidal lymphocytes, which are indeed a major source of serum antithyroid antibodies *(298)*.

   A direct suppressive effect of MMI on thyroid-autoimmune reactions, independent of changes of thyroid status, has been postulated from the results obtained in HT patients treated with MMI and levothyroxine to maintain constant serum thyroid hormone concentrations

(299). The immunosuppressive activity of MMI could be the consequence of the inhibition of macrophages, resulting in defective antigen presentation *(300,301)*. The inhibition of macrophage functions is probably related to the peroxidase blocking activity of MMI, leading to reduced scavenging of oxygen free radicals *(302)*. With some exceptions *(303)*, ATDs do not directly prevent the expression of HLA-DR *(304)* or TPO *(305)* on the surface of thyroid cells. However, ATDs inhibit the production by TECs of several substances (reactive oxygen metabolites, prostaglandin E_2, and cytokines), and this phenomenon could account for the thyroid-specific (auto)immune suppression exerted by these drugs *(306)*. As mentioned, ATDs have been shown to upregulate Fas ligand expression *(128)*, a phenomenon that may attenuate the autoimmune response by inducing apoptosis of T lymphocytes expressing Fas.

3. Antithyroid drugs and/or thyroid hormone may interfere with the immune system by reducing thyroid-autoantigen availability/presentation. Administration of thyroid hormone is associated with a decrease in serum TSH, with a consequent reduction in thyroid-tissue stimulation and thyroid-autoantigen expression *(75)*. This could account for the decrease in serum antithyroid antibodies observed in hypothyroid HT patients after prolonged treatment with levothyroxine *(293)*. Both TSH and TSAb appear to enhance thyroid-cell TPO expression through the same cAMP-dependent mechanism *(307)*. Moreover, both TSH *(308,309)* and TSAb *(304)* enhance γ-IFN-dependent HLA-DR expression by thyroid cells. It could therefore be envisaged that both TSH and TSAb are very important in self perpetuation mechanisms of HT and GD, respectively.

   A potential role of MMI in modulating either the basal expression of thyroid autoantigens or the expression of such autoantigens induced by thyroid stimulators has also been proposed, but the results obtained are rather contradictory *(75)*.

Nature of the Remission of GD

More than one type of clinical remission occurs in GD *(26)*. Destruction of sufficient thyroid parenchyma with ^{131}I or thyroidectomy may prevent recurrence. Conversely, spontaneous, continuous immunologic thyroid destruction by concomitant HT may bring about euthyroidism or even hypothyroidism. Hypothyroidism may ensue from a change in the nature of a TSAb from a stimulating to a blocking antibody. In contrast, another important type of remission is one in which all immunologic stigmata of the disease disappear, including the goiter, thyroid antibodies, TSAb, and evidence of sensitization of T lymphocytes *(310)*.

It is conceivable that this last form of remission might occur only in patients with a less severe defect in immunoregulation, in whom GD was precipitated by some environmental insult adversely influencing the immune system. This abnormality could then be reversible when the environmental disturbance disappears, with the adverse effect of hyperthyroidism on the immune system being eliminated by restoration of euthyroidism through appropriate treatment (antithyroid drugs, ^{131}I, or thyroidectomy) *(26)*. Moreover, rest, the passage of time, the recovery from infection, the use of sedation, and other nonspecific measures will each serve to permit the partly disturbed immunoregulatory system to be restored to its previous functional efficiency *(26)*.

On the other hand, those persons with a presumed severe defect would not be expected to undergo immunologic remission, no matter how long their antithyroid drugs were continued (*see* next subheading). Only those remissions associated with spontaneous or iatrogenic thyroid destruction would occur in this group *(26)*.

Implications for Therapy of GD

Despite greatly improved understanding of the immune basis of GD, few substantial changes have been made in its management over the past generation. However, the selection of patients for specific therapies, and the effects of different treatments, have benefited from new knowledge.

As discussed earlier, it has been suggested that ATDs are themselves immunosuppressive, and treatment with high doses of such drugs (in association with levothyroxine to prevent hypothyroidism) has been reported to favor remission in GD *(311)*. The clinical relevance of these observations is, however, very questionable. It is actually difficult to reconcile this notion with the fact that many patients continue to display immunologic activity throughout the course of therapy, no matter what dosage of an antithyroid drug is used and no matter how well hyperthyroidism is controlled. This does not accord with an expected pharmacologic effect of these agents, in which a dose response should affect the immune system but does not. Recent studies have failed to confirm any improvement in the remission rate of GD in patients treated with high doses of ATD *(312,313)*. Moreover, no differences have been observed in a variety of immune parameters with high- versus low-dose ATD regimens, the control of hyperthyroidism being the crucial factor in all cases *(312,314,315)*. Thus, the normalization of thyroid hormone levels is attended by normalization of the suppressor/helper T-cell ratio *(292,312)*, reduced activation of $CD4^+$ helper T cells *(316)*, and the normalization of circulating soluble IL-2 receptor *(317,318)*. In any event, because of the relatively short duration of action of ATD, it is difficult to comprehend how a long-term remission would persist after cessation of therapy. The action of the drugs on thyroid cells (rather than on immune cells), normalizing thyroid function and restoring euthyroidism, probably better explains remissions. ATD effects on thyroid hormone production and other thyroid-cellular activities then reduce "thyrocyte-immunocyte signaling," restoring the previous (precarious) state of immunoregulation and tending to produce a remission *(199)*.

The *de novo* use of ^{131}I therapy for GD is also associated with immunologic perturbations, namely a transient rise in the titers of TSAbs and other thyroid autoantibodies, followed by an ultimate decline in these titers *(319)*. This is almost certainly because of the liberation of thyroid antigens from the damaged thyroid parenchyma, stimulating the already disturbed immune system *(26)*.

Additionally, subtotal thyroidectomy is often associated with a decline in TSAb activity, perhaps because most of the offending thyroid-committed lymphocytes are removed with the gland. Recurrences after surgery would have to be associated with sufficient remaining thyroid parenchyma to be able to respond to TSAb, and sufficient remaining thyroid-committed lymphocytes to mount the immune attack *(26)*.

Natural Course of Autoimmune Thyroiditis and Effect of Thyroid Hormone Therapy

The thyroid-functional state and serology in thyroiditis can vary markedly over time, even with temporary or permanent remissions *(26)*. This is particularly evident in subclinical or minimal autoimmune thyroiditis. Although a full explanation of these variations is as yet forthcoming, it seems obvious that the variations are in the immune system, secondarily affecting thyroid function. These perturbations in immunoregulation probably reflect the *milieu interieure* or immune environment (i.e., effects of the environment on a day-to-day basis). Nevertheless, there is some tendency for autoimmune thyroiditis to progress gradually to more severe destruction, consistent with the known effect of aging on the immune system *(320)*. In subclinical hypothyroidism associated with thyroid autoantibodies, about 5% of

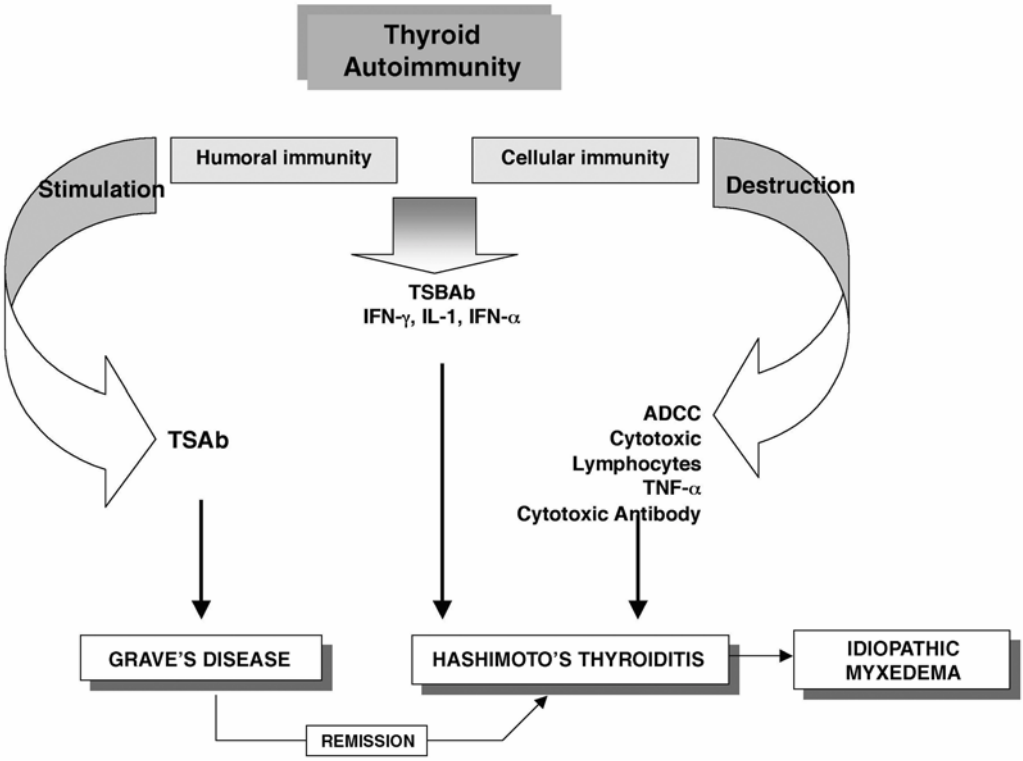

Fig. 6. Schematic representation of the sequence of events leading to the development of thyroid autoimmunity.

patients per year will go on to develop overt hypothyroidism *(26)*. About 20% of elderly women will have demonstrable autoimmune thyroiditis *(26)*. Males have a much lower incidence. The important effects exerted by pregnancy on the clinical course of asymptomatic autoimmune thyroiditis have already been discussed.

Thyroid hormone treatment is rational treatment for patients with hypothyroidism, and this has an added advantage beyond merely normalizing thyroid function in patients whose hypothyroidism is caused by autoimmune thyroiditis. In those patients with increased levels of TSH, such therapy was found to reduce thyroid autoantibody titers, almost certainly through a reduction in thyroid antigen presentation via reduced thyrocyte stimulation *(26)*. Since it is known that increased TSH stimulates increased thyrocyte HLA-DR expression and increased thyroid antigen expression, it may be inferred that a reduced level of TSH will do the reverse *(26)*. Theoretically, this effect should also reduce the autoimmune pathologic process, although this desirable effect has not actually been documented.

SUMMARY

Figures 6 and 7 summarize the chain of events leading to the development of thyroid autoimmunity and AITD. In normal subjects, autoreactive thyroid-specific lymphocytes are efficiently controlled by central tolerance in the thymus, by peripheral tolerance, and by active suppression (Fig. 6). A very low level of autoreactivity to thyroid autoantigens occurs as a physiologic process, since not all lymphocytes reacting with thyroid autoantigens are clonally deleted. The genetic background (e.g., the expression of HLA-DR3 or -DR5 antigens) may increase the number/activity of autoreactive cells able to escape immunological

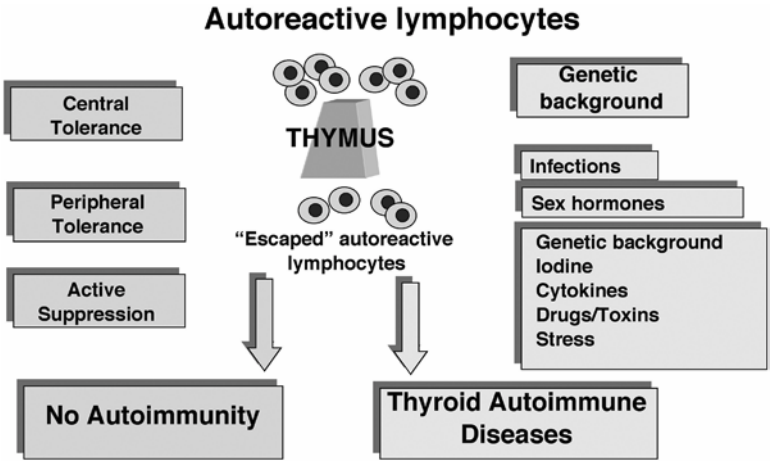

Fig. 7. Progression and clinical outcome of AITD.

tolerance. Exogenous factors, possibly represented by viral or bacterial infections (through molecular mimicry, cytokine/chemokine release, and other mechanisms); neurendocrine factors (including stress-related hormonal changes); iodine intake; drugs; and toxins may trigger a low level of thyroid-autoimmune response in genetically predisposed individuals (Fig. 6). This process may not proceed for many years, or may even regress, probably through the action of a series of active suppressor circuits including the reciprocal regulation of TH1 and TH2 subsets, and possibly through inherited defects in antigen-specific suppressor-cell function. When suppressor factors are unable to control the ongoing thyroid autoimmunity, the intensity of the immune response increases with the appearance of specific effector mechanisms that can induce thyroid hyperfunction (TSAb), thyroid hypofunction (TSHBAb and cytokines), and cell destruction (NK, K, and cytotoxic T cells; cytotoxic antibodies; and proapopototic cytokines). At this stage, AITD becomes clinically evident (Fig. 7); meanwhile, high local cytokine (mainly IFN-γ and TNF-α) concentrations lead to abnormal expression on the TEC surface of several molecules, such as MHC class II antigens, adhesion molecules, Fas, Fas-L, and others capable of augmenting and perpetuating both thyroid-specific and nonspecific immune reactions. In this phase, hyperthyroidism may further contribute to immune dysfunction by reducing the activity/number of nonspecific suppressor lymphocytes. AITD is now fully expressed, and the resulting thyroid dysfunction will depend upon the balance between stimulating and blocking/destructive mechanisms (Fig. 7). At this stage of disease the spectrum of thyroid autoreactivity may further widen, including the recognition of epitopes cross-reacting with thyroid autoantigens and nonspecific T-cell stimulation in genetically predisposed individuals. This may lead to the appearance of new autoimmune manifestations such as Graves' ophthalmopathy and pretibial myxedema.

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8 Problems in the Management of Hypothyroidism

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Contents

INTRODUCTION

Hypothyroidism is the most common endocrine deficiency state. Overt hypothyroidism affects 2% of adult women and 0.2% of adult men, and mild hypothyroidism is present in an additional 7–15% of older adults *(1)*. The most common underlying cause is autoimmune (Hashimoto's) thyroiditis, followed by previous thyroid radiation with ^{131}I or external-beam therapy. Certain medications can cause hypothyroidism, particularly when given to patients with underlying autoimmune thyroiditis, including lithium carbonate, amiodarone, other iodine-containing compounds, the cytokines interferon (IFN)-α and interleukin (IL)-2, and the antithyroid thionamide drugs. Transient hypothyroidism occurs in forms of thyroiditis caused by presumptive viral infection (subacute thyroiditis) or autoimmunity (postpartum, painless, or silent thyroiditis). Congenital hypothyroidism (CH) caused by thyroid agenesis or defective hormonogenesis affects one in 4000 newborns. Rarely, hypothyroidism results from hypothalamic or pituitary disorders causing deficiencies in thyrotropin-releasing hormone (TRH) and thyroid-stimulating hormone (TSH, thyrotropin) respectively.

Clinical manifestations of overt hypothyroidism can include fatigue, lethargy, cold-intolerance, unexplained weight gain, hoarseness, edema, constipation, skin dryness, arthralgias, myalgias, muscle cramps, headache, impaired mentation, depression, and menorrhagia. Physical findings can include changes in mental status ranging from dullness of affect to coma, bradycardia, diastolic hypertension, dry skin and hair, skin pallor, keratinic discoloration, hoarseness and dysarthria, and delayed relaxation time of the deep tendon reflexes. In neonates with CH, hypertelorism, macroglossia, and umbilical hernia are classical findings. In patients with underlying thyroiditis, there may be a firm goiter, but the thyroid can be normal in size or nonpalpable as well. Occasionally, the diagnosis of hypothyroidism is first suspected on the basis of abnormal laboratory values for routine

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hematologic or chemical parameters, including anemia, hyponatremia, hypercholesterolemia, and hyperprolactinemia.

The first problem in "managing" hypothyroidism is recognizing it, since most of its clinical features and general laboratory abnormalities are nonspecific and insensitive *(2)*. Indeed, they may be absent altogether, even in patients with overt hypothyroidism. This has led some authorities to recommend that individuals with a significant risk of hypothyroidism (e.g., older persons and postpartum women) be routinely screened for thyroid dysfunction *(3)*. Once suspected, the diagnosis can usually be readily confirmed or excluded by measurement of the serum TSH and FT_4) concentrations *(4,5)*. Measurement of TSH is the most sensitive single test since the TSH concentration is elevated in all patients with primary hypothyroidism, even if the serum FT_4 remains within the low-normal range, a condition termed "mild" or "subclinical hypothyroidism." TSH measurement alone may be misleading in the diagnosis of central (pituitary or hypothalamic) hypothyroidism and in the rare condition of inherited resistance to thyroid hormone (TH). The underlying cause of hypothyroidism is usually obvious clinically and seldom requires additional testing. However, circulating thyroid autoantibodies (e.g., antibodies to thyroid peroxidase [TPO] and thyroglobulin [Tg]) confirm the presence of autoimmune thyroiditis, and in patients with central hypothyroidism, additional testing for other elements of hypopituitarism and cranial radiologic studies are indicated.

THYROXINE THERAPY

For the vast majority of patients, treatment of hypothyroidism is straightforward. Levothyroxine sodium (levothyroxine or L-T_4) is the drug of choice *(6,7)*. Its plasma protein binding and consequent long circulating half-life permit administration of a single daily dose, which is reliably absorbed under most circumstances. Physiologic conversion of administered levothyroxine to triiodothyronine (T_3) produces a stable concentration of this latter iodothyronine, which is more biologically active, in the circulation and target tissues. Changes in the absorption and metabolism of levothyroxine can occur in a number of settings, which can be readily recognized by well-informed physicians and patients (*see* below). Patients typically begin to respond to treatment with levothyroxine in 10–14 d, and achieve biochemical and clinical equilibrium on a given dose by 4–6 wk. Treatment can be monitored with serum TSH measurement, which is particularly valuable in the assessment of patients with nonspecific symptoms. Additional serum FT_4 measurement alters management in only 4% of patients *(8)*. However, the FT_4 level can be helpful in defining the degree of overtreatment when the serum TSH level is low, and is essential in the management of patients with central hypothyroidism. Side effects of levothyroxine are related to iatrogenic thyrotoxicosis and to special settings in which the restoration of euthyroidism may cause clinical problems (*see* "Complications of Restoring Euthyroidism" following).

PROBLEMS IN THE MANAGEMENT OF HYPOTHYROIDISM

Optimizing the L-Thyroxine Dose

Despite the apparent simplicity of treating hypothyroidism with levothyroxine, many patients are suboptimally managed. In a cross-sectional study of almost 26,000 participants

[1]The abbreviations "T_4" and "T_3" are used in this chapter to designate thyroid hormones that are endogenously produced or, regardless of origin, are in the circulation and target tissues. "L-T_4" or "levothyroxine" and "L-T_3" refer to thyroid hormone preparations for pharmacological use.

in a regional health fair, 1525 were incidentally taking thyroid medications; among these individuals, 40% had an abnormal serum TSH concentration, approximately evenly divided between those with TSH values suggesting under- and overtreatment *(1)*.

In patients with primary hypothyroidism, the serum TSH concentration is widely considered the "gold standard" in defining the appropriate dose of levothyroxine *(9)*. Assessment of the clinical and biochemical responses to any new dose of levothyroxine should be done after 4–6 wk because of the relatively long, 7-d circulating half-life of thyroxine. Clinicians should also appreciate that the distribution of TSH values in a normal population does not follow a normal bell-shaped curve, but is rather skewed toward the lower end of the analytical normal range. Consequently, many experts advocate prescribing a levothyroxine dose that will suppress the serum TSH concentration to the 0.5–2.0 mIU/L range in younger, otherwise healthy patients *(10)*. The following detailed discussion addresses important considerations in estimating the correct dose of levothyroxine for a particular patient and anticipating factors that can interfere with optimal long-term thyroid hormone (TH) replacement.

Initial Treatment Dose

Since side effects of thyroxine are almost exclusively related to iatrogenic thyrotoxicosis, it is generally best to begin with a dosage of levothyroxine at the lower end of the anticipated requirement range for a given patient, and then, if needed, titrate upward to the final dose. Several clinical factors can help estimate a hypothyroid patient's ultimate levothyroxine dose requirement and the appropriate initial dosage to employ. The optimal dose of levothyroxine is related to lean body mass; except for very obese individuals, this can be estimated from total body weight. On the basis of studies employing the formulations of levothyroxine used in North America throughout the 1990s, patients with no endogenous TH production have been shown to require from 1.6–1.8 μg/kg/d *(11)*. Patient age is another important consideration, since the rate of endogenous thyroxine clearance is more rapid in children, who may require as much levothyroxine as 3.8 μg/kg/d, and is slower in the elderly, whose levothyroxine dose requirement may be 1.0 μg/kg/d or less *(12)*. Particularly because of the risk of underlying heart disease in older persons, as discussed subsequently, a lower initial dose of levothyroxine is appropriate in this population. The severity of hypothyroidism can also predict the final dose requirement for levothyroxine. Patients with mild hypothyroidism, in whom the serum TSH is increased despite a normal serum FT_4 concentration, require on average only one-half the usual dose of levothyroxine for supplemental treatment (i.e., 0.8 μg/kg/d) *(13)*. The underlying cause of hypothyroidism affects the final dose requirement; patients with postsurgical hypothyroidism typically require more levothyroxine than those with autoimmune thyroiditis, presumably because of residual endogenous TH production. Similarly, patients with radioiodine-treated Graves' disease (GD) may need a lower dosage of levothyroxine because of residual gland function driven by persisting thyroid-stimulating immunoglobulins (TSIs). Sometimes, ongoing fluctuations in the underlying TSI level can also cause a changing levothyroxine dose requirement in these patients. If the specific indication for treatment entails more than simple hypothyroidism, a higher levothyroxine dose may be justified. For example, suppression of a goiter in autoimmune thyroiditis might warrant a levothyroxine dose intended to suppress the serum TSH level to the 0.1–0.5 mIU/L range, and postoperative management of epithelial thyroid cancers often includes a dose sufficient to suppress the serum TSH to less than 0.1 or even less than 0.01 mIU/L.

Physiologic Events and Illness

Aging can reduce the levothyroxine dose requirement because of a declining rate of thyroxine clearance. This can explain the development of iatrogenic thyrotoxicosis with the

same dosage of levothyroxine used to optimally treat a patient earlier in life. Pregnancy causes an increased dose requirement for levothyroxine in approx 75% of women *(14)*. The cause of this increased dose requirement is unknown, but has been attributed to increased deiodination by placental monodeiodinase, an increased circulating level of thyroxine-binding globulin (TBG), and/or increased renal clearance of iodothyronines. It is vital to detect and respond to this change, since inadequately treated hypothyroidism during pregnancy has been associated with complications affecting the mother (e.g., preeclampsia) and fetus (e.g., abortion, stillbirth, and subtle impairment of neuropsychological development) *(15,16)*. The increased dose requirement may develop during the first, second, or third trimester of pregnancy, necessitating repeated monitoring. The magnitude of this increased levothyroxine dose requirement can range from 25% to more than 100% of the prepregnancy dose. Patients typically return to their pre-pregnancy dose requirement after delivery. There is no evidence that breastfeeding necessitates an increased dose of levothyroxine.

In patients with progressive thyroid dysfunction caused by underlying autoimmune thyroiditis, previous radioiodine (RAI) therapy, or external-beam cervical irradiation, a further slow decline in TH levels over time can lead to an increasing dose requirement for levothyroxine. Patients with small-bowel disease *(17)* or a jejunoileal bypass *(18)* may have partial impairment of levothyroxine absorption, and those with nephrosis or those undergoing dialysis may have accelerated T_4 clearance *(19)*. Patients who have serious sustained systemic disorders (e.g., myocardial infarction, sepsis, burns, or surgical illnesses) typically also have an accelerated T_4 disposal rate. If this results in an increase in their serum TSH concentration, their dose of levothyroxine should usually be increased to compensate for accelerated clearance. In patients undergoing surgery from which prompt recovery is expected, the omission of one to four days of treatment with levothyroxine is inconsequential. Longer periods of perioperative oral intake restriction justify replacement with intravenous levothyroxine in a dose that should usually be equivalent to the previous oral dosage, with accelerated thyroxine clearance in the setting of illness justifying the modestly greater bioavailability of parenteral than of oral levothyroxine.

Drug Interactions and Thyroxine Formulations

A number of drugs and dietary supplements have been shown to reduce absorption of levothyroxine when administered simultaneously with or in close proximity to it *(20)*. The mineral supplements iron *(21)* and calcium carbonate *(22)* are the supplements most commonly used by the population of patients with hypothyroidism, and are often not reported as "other medications" unless the patient is specifically asked. Simultaneous ingestion of soy products similarly can interfere with absorption of levothyroxine *(23,24)*, but evidence that high-fiber diets can do so is controversial *(25,26)*. The bile-acid sequestrants cholestyramine and colestipol *(27)*, the antacid phosphate-binder aluminum hydroxide *(28)*, and the duodenal ulcer drug sucralfate *(29)* all interfere with absorption of levothyroxine. There is incomplete information about the minimal time between dosing of levothyroxine and these other agents required to avoid interference, but 4–6 h is probably sufficient *(30)*. Just as simultaneous use of these agents with levothyroxine can lead to its inadequate absorption and insufficient circulating and tissue levels of thyroxine, so can their discontinuation cause iatrogenic thyrotoxicosis with a dose of levothyroxine previously needed for full replacement.

Several anticonvulsant medications, including phenobarbital *(31)*, phenytoin *(32)*, carbemazepine *(33)*, and valproic acid, can accelerate thyroxine metabolism by increasing hepatic cytochrome P450 enzyme activity. The antituberculous agent rifampin also increases the rate of thyroxine catabolism *(34)*. The action of these agents can take several weeks to

fully develop, and dissipates over weeks after their discontinuation. The degree to which the dose of levothyroxine must be increased after their discontinuation varies widely, but can be substantial (e.g., 100%). In general, an initial increase in the dose of levothyroxine by 25–50 μg/d is appropriate, with serum TSH reassessment in 4 wk.

Approximately one-third of postmenopausal women who have begun estrogen replacement therapy have been shown to have an increased levothyroxine dose requirement, which is typically modest and may at least in part be necessitated by the accompanying increase in circulating TBG *(35)*. Conversely, androgen therapy for breast cancer in women with previously stable levothyroxine-treated hypothyroidism led to iatrogenic thyrotoxicosis *(36)*.

A number of branded and generic formulations of levothyroxine are marketed in the United States, and for a variety of reasons, a new formulation is not infrequently substituted for another during lifelong treatment of a patient with hypothyroidism. Older reports have described a decreased levothyroxine content in some generic products *(37)*, and reformulation of one thyroxine brand in the 1980s led to an increase in the bioavailable dose of thyroxine *(11)*. Whether there are significant differences in the bioavailability of current levothyroxine preparations has been one of the most controversial issues in contemporary clinical thyroidology *(38)*. The several studies addressing this issue have yielded conflicting results, and all have had methodologic shortcomings (e.g., inclusion of subjects with endogenous thyroid function, inadequate sample size, lack of TSH assay data, and/or failure to ensure compliance). Reviewing these data in detail is beyond the scope of this chapter. However, it should be noted that the U.S. Food and Drug Administration has not determined that levothyroxine formulations are interchangeable. Because of the scientific uncertainty about this matter, many thyroid specialists recommend that patients remain on a given preparation from one prescription refill to the next. Furthermore, when substitution does occur, it is prudent to reassess the serum TSH concentration after 4–6 wk of treatment with the new formulation.

Identifying and Managing Poor Compliance

Faithful adherence to prescribed levothyroxine therapy is essential for the optimal treatment of hypothyroidism. In the early phase of treatment, memory impairment associated with TH deficiency can interfere with compliance, and ensuring faithful long-term adherence to any drug regimen is challenging. The fact that on average, only 260 d per year of levothyroxine prescriptions written are actually filled suggests the magnitude of this problem *(39)*. Inadequate compliance obviously leads to incomplete therapy for TH deficiency. Furthermore, unrecognized noncompliance when a patient's laboratory testing indicates inadequate treatment may prompt an unnecessary dose increase, which has the potential for causing iatrogenic thyrotoxicosis if compliance later improves.

A number of clues suggest noncompliance with levothyroxine therapy for hypothyroidism. First, an unexpectedly high dose requirement suggests the possibility of previous dose escalation resulting from inadequate adherence. Second, there may be a pattern of erratic and nonsensical relationships between the prescribed levothyroxine dosage and resulting thyroid function tests (e.g., a serum TSH level of 2 mIU/L with a levothyroxine dosage of 125 μg/d, and then a TSH of 7 mIU/L with a levothyroxine dosage of 150 μg/d). Including a flow sheet in hypothyroid patients' medical records, with recording of the patient's levothyroxine doses and serum TSH levels over time, can help detect such patterns. Third, patients who improve their compliance in anticipation of an upcoming appointment and laboratory testing may have an increased serum TSH with a FT_4 concentration in the mid- to upper third of the normal range, owing to the more rapid rise in circulating thyroxine than a decrease in TSH

that occurs with reinstitution of therapy. A more accurate estimate of patient adherence to therapy can often be obtained by asking, "How many doses of your thyroid medication do you miss in a typical month?"

Patients' adherence to levothyroxine therapy can be improved by suggesting that they take their medication at a regular time of the day when an activity occurs, such as toothbrushing or hair grooming. It is not essential that levothyroxine be taken in the morning if this is a hectic period. Patients should be instructed to make up missed doses, either later in the day or even on the following day. Forgetful patients can even make up an entire week's missed doses on Sunday. Some patients find a 7-d pill container useful for ensuring compliance.

Central Hypothyroidism

Because central hypothyroidism is caused by an inadequate TSH level or biologic action, its serum concentration cannot be used to ascertain the optimal levothyroxine dose in patients with hypothalamic or pituitary disorders that secondarily cause hypothyroidism. Clinicians must rely on their knowledge of the factors generally predicting the levothyroxine dose requirement (e.g., weight, age, pregnancy, and medications), the patient's clinical status, and the serum FT_4 concentration. The interpretation of symptoms and signs potentially related to hypothyroidism can be complicated in patients who have other elements of hypopituitarism (e.g., adrenal insufficiency causing fatigue and weight loss, growth-hormone deficiency causing weakness and increased body fat, and hypogonadism in males causing decreased strength and stamina). Simply increasing the dose of levothyroxine will not obviously alleviate complaints attributable to another hormonal deficit. It is particularly important to ascertain whether adrenal insufficiency is present, since, as noted subsequently, it may be exacerbated by reversal of TH deficiency alone.

To ensure that TH replacement is adequate in patients with central hypothyroidism, it is usually advisable to use a levothyroxine dose that maintains the serum FT_4 in the upper one-half of the normal range. The serum T_3 concentration and a number of target-tissue responses to TH have been proposed as additional measures of optimal levothyroxine therapy, including basal oxygen consumption and circulating proteins (e.g., serum-soluble interleukin (IL)-2 receptor, ferritin, sex-hormone-binding globulin) *(40)*. However, it has not been convincingly shown that their use produces a better outcome than does consideration of clinical findings and the FT_4 level alone.

Persistent Symptoms in Patients with Treated Hypothyroidism

The frequency with which both euthyroid and hypothyroid individuals experience the nonspecific symptoms associated with TH deficiency (e.g., fatigue, weight gain, and constipation) *(2)* makes these complaints a common event among hypothyroid patients who are receiving thyroxine-replacement therapy. Conversely, symptoms associated with iatrogenic thyrotoxicosis (e.g., insomnia, anxiety, heat intolerance, tremor, and palpitations) are also common and nonspecific. Patients can be counseled to consider four straightforward criteria in deciding when to suspect that their symptoms might be due to a suboptimal dose of levothyroxine, as follows: (1) Is the symptom a new one for the patient? (2) Has the symptom persisted for more than 2 wk? (3) Are two or more symptoms consistent with TH deficiency or excess, respectively, present? (4) Has there been a change in the patient's health or medications known to alter the levothyroxine requirement, as described previously?

For the clinician, the first step in assessing such symptoms is to recheck a serum TSH concentration. When iatrogenic thyrotoxicosis is being considered, the FT_4 level should be measured as well. If the serum TSH is elevated, clinicians should consider possible

Table 1
Checklist for Levothyroxine-Treated Hypothyroid Patients With Increased Serum Levels of TSH

Inadequate prescribed dosage
Noncompliance
Dispensing error
Improper dose
Formulation change
Drug interaction
Reduced levothyroxine absorption
Iron compounds
Calcium carbonate
Cholestyramine
Amphogel
Sucralfate
Accelerated levothyroxine clearance
Phenytoin
Carbemazepine
Phenobarbital
Valproic acid
Rifampin
Decreased residual gland function
Autoimmune thyroiditis
Postirradiation
Pregnancy
Postmenopausal estrogen therapy
Systemic illness

contributions by one or more of the factors discussed above and enumerated in Table 1. In patients with treated primary hypothyroidism and persistent symptoms of TH deficiency with a serum TSH greater than 2.5 mIU/L, a modest increase in the dose of levothyroxine (e.g., 12.5–25 μg/d, may be justified. In symptomatic patients with treated primary hypothyroidism and a serum TSH in the lower one-half of the normal range), augmenting the levothyroxine dose is unlikely to relieve the complaint *(41)* and may well cause iatrogenic thyrotoxicosis. In these patients, attention should be focused on potential nonthyroidal explanations for the complaints.

Another controversial consideration in persistently symptomatic hypothyroid patients is whether to supplement levothyroxine therapy with L-triiodothyronine (L-T_3). One prospective, placebo controlled, double-blind clinical trial showed that partial replacement of levothyroxine dose with L-T_3 was associated with improvement in certain neuropsychological and quality-of-life parameters *(42)*. This study has been criticized for several shortcomings: some study patients had residual endogenous gland function; the ratio of administered L-T_3 to levothyroxine was nonphysiologically high; some patients had a low serum TSH concentration while taking combined therapy, indicative of iatrogenic thyrotoxicosis; and there were sample size and biostatistical limitations. In general, it has been argued that combined levothyroxine plus L-T_3 therapy often leads to mild iatrogenic thyrotoxicosis (*see*

next subheading) and always to fluctuating serum T_3 concentrations owing to the shorter, 1-d circulating half-life of T_3. Combination therapy also increases the cost, need for compliance, and complexity of monitoring. Nonetheless, some clinicians believe this can be a useful maneuver in some patients. Whenever combination therapy is used, a physiologic molar ratio of levothyroxine to L-T_3 (~14 to 1) should be employed, multiple daily L-T_3 doses should be used to minimize nonphysiologic fluctuations in the endogenous T_3 level, and the TSH level should be monitored to ensure that iatrogenic thyrotoxicosis does not develop.

Side Effects of Levothyroxine

Iatrogenic Thyrotoxicosis

The common side effects of levothyroxine and L-T_3 therapy are attributable to TH excess. Symptoms and signs of overtreatment are similar to those of endogenous thyrotoxicosis, but may be harder to recognize because of their milder degree and accommodation to their longer duration. Particularly in older persons and those coincidentally taking β-adrenergic blocking agents, classical sympathomimetic symptoms may be absent. In addition to clinical thyrotoxicosis, two organ systems are particularly sensitive to even modest TH excess: the heart and the skeleton.

Even minimal TH excess has been associated with a threefold increase in the risk of atrial fibrillation, especially in older persons *(43)*; subtle changes in inotropic and lusitropic ventricular functions; and modest concentric left ventricular hypertrophy *(44–46)*. The potential clinical consequences of these effects are symptomatic palpitations, heart failure, and embolic cerebrovascular accident attributable to atrial fibrillation; and exacerbation of ventricular dysfunction when there is underlying primary cardiac disease. When treatment of hypothyroidism is the goal, careful adjustment of the dose of levothyroxine can prevent these problems. When suppression of TSH secretion is also intended, as it is in postoperative thyroid-cancer management, β-adrenergic blockade can prevent changes in ventricular function and structure *(47,48)*.

Iatrogenic thyrotoxicosis, like endogenous hyperthyroidism, has been associated with bone-mineral loss; however, the preponderance of evidence suggests that optimal levothyroxine therapy for hypothyroidism is not associated with such loss *(49,50)*. The transient increase in bone turnover observed in some studies following the initiation of levothyroxine therapy for hypothyroidism probably represents only short-term disequilibrium in bone turnover during the return to steady-state conditions. When suppression of TSH secretion is also intended, as it is in postoperative thyroid-cancer management, calcium supplementation and bisphosphonate therapy can prevent significant bone loss.

In patients with treated primary hypothyroidism, monitoring of the serum TSH level is the most sensitive approach to detect excessive TH therapy. Complications such as atrial fibrillation and osteoporosis have been shown to occur with doses of TH that suppress the serum TSH concentration to less than 0.1 mIU/L, even when the serum FT_4 remains within the normal range. FT_4 measurement can help in defining the degree of hormone excess and the extent to which the dosage of levothyroxine should be reduced. When the serum TSH is low in patients receiving supplemental L-T_3 therapy, the serum T_3 measurement is also required, and this should generally be done from 2–4 d after dosing to assess peak serum T_3 concentrations.

Mild iatrogenic thyrotoxicosis in otherwise healthy subjects can be addressed by reducing the daily dose of levothyroxine without interrupting therapy. The long half-life of T_4 will produce a smooth transition. In patients with more severe thyrotoxicosis, temporary

discontinuation of levothyroxine for several days will accelerate restoration of euthyroidism. When patients are symptomatic, β-adrenergic blocking agents may be temporarily used.

Complications of Restoring Euthyroidism

Among serious problems occurring with treatment of hypothyroidism, the most common is exacerbation of ischemic heart disease (IHD). The effects of TH on the cardiac conducting system and myocardium lead to acceleration of heart rate and augmentation of myocardial contractility, both of which increase the myocardial oxygen requirement *(51)*. For patients with limited coronary arterial reserve, this can result in onset or worsening of angina, myocardial infarction, dysrhythmias, and death *(52)*. Recognizing the potential for this complication is important because: (1) hypothyroidism and ischemic heart disease are both common in older persons; and (2) hypothyroidism itself is associated with certain risk factors for atherosclerosis (e.g., hypercholesterolemia, hypertension, hyperhomocysteinemia, and increased oxidizable low-density lipoprotein [LDL] and lipoprotein-a [Lpa]). Because the response of hypothyroid patients with heart disease is unpredictable, caution in prescribing levothyroxine therapy is advisable whenever the patient has risk factors for IHD and/or is more than 60 yr old. Levothyroxine should be initiated in a 12.5–50 μg/d dose, which can then be advanced in 12.5–25 μg increments at 4–6-wk intervals under close monitoring of clinical variables and serum TSH levels, and sometimes serial ECG monitoring. Initiation or augmentation of β-adrenergic blockade is sometimes indicated as well *(53)*. The older recommendation that a compromise in the levothyroxine therapeutic dose of levothyroxine be accepted *(54)* (i.e., allowing the TSH to remain modestly elevated) no longer seems advisable for several reasons. First, most studies have shown that LDL-cholesterol remains higher in the mildly hypothyroid state *(55)*. Second, modest TSH elevation has been associated epidemiologically with a higher prevalence of previous myocardial infarction, independent of hypercholesterolemia *(56)*. Third, one study has shown that progression of coronary disease after angioplasty occurs significantly more often when hypothyroidism is not fully treated *(57)*. Consequently, if the gradual introduction of TH therapy does exacerbate IHD, patients should be aggressively evaluated with arteriography and revascularized. Percutaneous transluminal angioplasty has not been shown to have any increased morbidity or lesser efficacy in hypothyroid patients than in general *(58)*. Cardiac surgery in hypothyroid patients has been associated with several complications, which are described below, but not a higher mortality *(59)*.

Adrenal cortical insufficiency can accompany hypothyroidism and, when unappreciated, can be exacerbated by TH therapy in the following three settings: (1) coincident autoimmune (idiopathic) adrenal failure and autoimmune (Hashimoto's) thyroiditis; (2) combined TSH and adrenocorticotrophic hormone (ACTH) deficiency in hypopituitarism; and (3) functional impairment of the hypothalamic–pituitary–adrenal axis response to stress in severe hypothyroidism *(60)*. Another potentially confusing circumstance is the patient with adrenal sufficiency in whom there is a modestly elevated serum TSH concentration that glucocorticoid replacement can restore without any TH treatment at all. Older reports of acute adrenal crisis occurring after institution of TH replacement attributed this phenomenon to accelerated clearance of residual cortisol as euthyroidism was restored. Practically speaking, coexisting adrenal insufficiency should be suspected in hypothyroid patients under the following circumstances: (1) there are clinical and laboratory findings consistent with adrenal insufficiency and not typical of hypothyroidism alone (e.g., fever, weight loss, nausea and vomiting, hypotension, hyperpigmentation, eosinophilia, hyperkalemia, or hypoglycemia); (2) other clinical findings suggest the possibility of polyendocrine

failure (e.g., hypocalcemia or known hypoparathyroidism, premature ovarian failure, pernicious anemia, vitiligo, or alopecia areata); (3) the patient has known or suspected central hypothyroidism; and (4) hypothyroidism is severe (TSH greater than 80 mIU/L) or is complicated (*see* the following subheading). Whenever adrenal insufficiency is deemed a serious consideration, it should be excluded with appropriate basal and ACTH-stimulated cortisol measurement. Patients with less convincing clues to adrenal insufficiency should, at the very least, be monitored closely during the first 4 wk of levothyroxine treatment.

A number of other side effects have been reported with treatment of hypothyroidism. Rapid reversal of hypothyroidism has been reported to complicate management of asthma *(61,62)* and urticaria, both of which can sometimes be ameliorated by hypothyroidism. Pseudotumor cerebri has been reported as a complication during the early phase of treatment of hypothyroidism in children *(63,64)*. In these settings, treatment with levothyroxine should be initiated in a cautious manner resembling the approach taken in patients with ischemic heart disease. Levothyroxine treatment of hypothyroidism can also have pharmacokinetic effects (e.g., accelerating renal digoxin clearance and augmenting the anticoagulant effect of coumarin through faster catabolism of vitamin K-dependent clotting factors). Transient hair loss is a common occurrence after the institution of thyroid medication, and the alarm it causes can undermine patients' adherence to therapy. Additionally, some patients experience a mysterious syndrome of acute sympathomimetic symptoms—typically including anxiety, insomnia, palpitations, and tremor—that has its onset within hours after the first or second dose of levothyroxine. Sometimes, these symptoms are misinterpreted as allergy to levothyroxine, which is a poorly documented phenomenon if it exists at all. The time course of these symptoms contrasts with the pharmacokinetics of levothyroxine, which predict a slow rise in target-tissue concentrations and effects. One report suggests that coexisting anemia may play a role in some patients *(65)*. The condition can usually be managed by reassurance of the patient, correction of anemia when present, and the temporary discontinuation and then resumption of levothyroxine in a very low, 25 μg/d dosage. Occasionally, β-adrenergic blockade may also be useful in easing such symptoms during the first few weeks of levothyroxine therapy.

Managing Mild and Severe Hypothyroidism

Mild (Subclinical) Hypothyroidism

Mild hypothyroidism refers to the thyroid state of individuals in whom there is an increased serum TSH concentration while the serum FT_4 level remains within the normal range. This syndrome has also been called "subclinical hypothyroidism," "prehypothyroidism," and "decreased thyroid reserve," each of which terms has shortcomings. Subclinical hypothyroidism implies that there are no clinical manifestations of this thyroid state, which several studies have disproven. Prehypothyroidism suggests that all patients with isolated serum TSH elevations will develop overt hypothyroidism, which is untrue. Decreased thyroid reserve accurately describes the gland's inability to increase TH production when it is required (e.g., during pregnancy), but does not characterize the clinical and metabolic status of affected patients. The prevalence of mild hypothyroidism has been reported to be 3–17% in various populations, with a higher prevalence and incidence in women and older persons *(66)*. Its most common causes are essentially the same as those of overt hypothyroidism: autoimmune thyroiditis, previous thyroid irradiation, and medications such as lithium carbonate and amiodarone. Other causes of isolated TSH elevation that should be distinguished from mild hypothyroidism include the transient increase in TSH during recovery from nonthyroidal illnesses *(67)*, renal failure *(68)*, adrenal cortical insufficiency

Table 2
Indications for Treatment of Mild Hypothyroidism

Prevent progression to overt hypothyroidism
Reverse associated hypercholesterolemia
Reverse somatic and neuropsychological symptoms
Optimize thyroid status of pregnant women
Improve myocardial function in patients with intrinsic heart disease
Goiter

(69), prolonged environmental cold exposure *(70)*, and central hypothyroidism, in which a minority of patients have modestly increased immunoassayable TSH.

There are several potential indications for the treatment of mild hypothyroidism, which have been discussed elsewhere *(13,71)*; (Table 2). Early institution of levothyroxine therapy in patients destined to have progression of hypothyroidism prevents its overt development. Patients at particular risk of progression have been shown to include those older than 60 yr, those with a history of previous neck surgery or irradiation, pregnant patients, patients with a TSH >10 mIU/L, and those with circulating thyroid autoantibodies indicative of underlying autoimmune thyroiditis *(72)*. In patients with mild hypothyroidism and hypercholesterolemia, the majority of clinical trials have shown a reduction in circulating total and LDL-cholesterol concentrations with TSH-normalizing levothyroxine therapy *(55)*. For patients in whom mild hypothyroidism is associated with somatic and neuropsychological symptoms, the majority of clinical trials have again shown a significant benefit of levothyroxine therapy *(73–76)*. In pregnant women, the presence of even mild hypothyroidism has been associated with subtle impairment of their children's subsequent neuropsychological development *(16)*. Reversal of the modest changes in ventricular function may be an indication for levothyroxine therapy in patients with intrinsic heart disease *(77,78)*. Lastly, mildly hypothyroid patients with goiter may respond with a decrease in gland size to TSH-suppressive therapy.

When a patient with an increased serum TSH level is detected in the course of case-finding in clinical practice or population screening, clinicians should consider the differential diagnosis and potential underlying causes, as described above; assess the patient for clinical indications to treat; and obtain follow-up laboratory testing, including a confirmatory TSH assay, TH_4 measurement to identify overt hypothyroidism, and if there is not yet another clinical indication for treatment, obtain a fasting lipid profile and screen for thyroid autoantibodies. The treatment of choice is levothyroxine, as it is for overt hypothyroidism, although the dose requirement is typically only 50% of that for patients with frank hypothyroidism. Therapeutic monitoring and potential side effects are the same as those in patients with overt hypothyroidism.

Severe and Complicated Hypothyroidism

When TH deficiency is profound and prolonged, serious and potentially life-threatening complications can ensue. Myxedema coma refers to the well-known syndrome of multisystem failure occurring in such cases *(79,80)*. However, more focused complications of hypothyroidism, predominantly affecting one or two organ systems, are even more common, including hypothermia, congestive heart failure, ventilatory failure, ileus, delirium, and dementia. Hypothyroidism with these complications is more common in the elderly and persons with underlying diseases rendering an organ system vulnerable. Complications of hypothyroidism are often precipitated by intercurrent illnesses, such as sepsis or cerebrovascular accident,

Table 3
Medical Considerations in Management of Complicated Hypothyroidism

Complications	*Pathogenesis*
Congestive heart failure	Impaired ventricular systolic and diastolic functions
Ventilatory failure	Blunted hypercapneic and hypoxic ventilatory drives
Hyponatremia	Impaired renal free water excretion; possible SIADH
Ileus	Bowel hypomotility
Medication sensitivity	Reduced clearance rate for sedative, analgesic, and anesthetic agents
Hypothermia and lack of febrile response to sepsis	Decreased calorigenesis
Delirium, dementia, seizure, stupor, and coma	Decreased actions on the central nervous system, often with metabolic encephalopathy from superimposed hyponatremia and hypercapnea
Adrenal insufficiency	Functional impairment of hypothalamic–pituitary–adrenal stress response, or associated intrinsic adrenal or pituitary disease
Coagulopathy	Uncertain

SIADH, syndrome of inappropriate secretion of antidiuretic hormone.

or by the administration of sedatives, potent analgesic drugs, or anesthetic drugs that are cleared less rapidly. The diagnosis of myxedema should be considered in all of these clinical settings. In patients with primary hypothyroidism, measurement of the serum TSH can establish the diagnosis. Central hypothyroidism is more difficult to exclude in severely ill patients, who often have thyroid-function-test abnormalities characteristic of the euthyroid sick syndrome *(81)*. In these patients, identifying other elements of hypopituitarism, as well as central nervous system imaging, may be required to include or exclude hypothyroidism.

There are two essential components of management for patients with myxedema coma and other forms of complicated hypothyroidism: TH replacement and intensive general medical care. Controversies about the optimal approach to TH therapy, such as whether to give levothyroxine, L-T_3, or a combination of the two, and whether a loading dose should be given, have not been resolved by randomized clinical trials *(82,83)*. Given the rarity of complicated hypothyroidism, it is unlikely that they ever will be. Trials have compared early target-tissue responses to intravenous levothyroxine and L-T_3 therapies in patients with moderate, uncomplicated hypothyroidism. During the first week of therapy, myocardial, ventilatory, renal-water-clearance, and metabolic-rate responses were all more rapid and complete with L-T_3 at 50 μg/d than with levothyroxine at 100 μg/d *(84,85)*. Three general types of TH regimens have been advocated: (1) an intravenous levothyroxine loading dose of 300–500 μg, followed by a weight-appropriate daily maintenance dose of levothyroxine; (2) intravenous L-T_3 at 50–75 μg/d; or (3) a combination of intravenous levothyroxine and oral or intravenous L-T_3.

Sustained vigilance for and meticulous care of the individual complications of hypothyroidism are probably more important than which thyroid hormone regimen is chosen. The major medical issues to be addressed in affected patients are enumerated in Table 3. In addition, an underlying precipitant should be aggressively sought and treated. Once the acute phase of illness has passed, it is important to remember patients' persistent sensitivity to sedative medications, which can sometimes precipitate late ventilatory failure.

A related problem is management of the preoperative patient found to have hypothyroidism. Several studies have shown that these patients in fact tolerate surgery relatively well *(86–89)*. Nonetheless, it is almost always prudent to postpone elective procedures until TH replacement can be initiated for 4–8 wk. For patients requiring emergency surgical procedures, the hypothyroid state can lead to certain perioperative complications, including higher incidences of intraoperative hypotension, perioperative heart failure, and postoperative gastrointestinal and neuropsychiatric complications, and a lower prevalence of fever with postoperative infection. If anticipated, these complications can be recognized and managed.

Transient Hypothyroidism and Discontinuation of Levothyroxine Therapy

Most patients with hypothyroidism will require lifelong levothyroxine therapy. There are, however, certain conditions in which TH deficiency is temporary. A small minority of patients with autoimmune thyroiditis may recover thyroid-gland function, at least in the short run. Subacute thyroiditis and lymphocytic thyroiditis typically cause hypothyroidism that resolves after 2–8 wk. Medication-induced hypothyroidism is usually reversible when the offending agent is discontinued *(90)*. The children of women with autoimmune thyroiditis may have transient hypothyroidism in the neonatal period from transplacental transfer of thyroid autoantibodies *(91)*. RAI therapy causes reversible rather than permanent hypothyroidism in a minority of treated hyperthyroid patients *(92)*. There are other patients for whom continued TH treatment is no longer appropriate (e.g., those no longer requiring TSH-suppressive therapy for goiter or benign nodules, and those for whom TH had been prescribed inappropriately for weight loss or fertility). If these patients have no intrinsic thyroid disease, gland recovery can be anticipated even after decades of inappropriate therapy. The relatively long 7-d half-life of thyroxine makes it reasonable to discontinue levothyroxine medication completely rather than taper the dose. Intrinsic thyroid function can be reassessed after 4–6 wk. Serum TSH is the most reliable marker of recovery, except in patients with recently treated thyrotoxicosis and those with central hypothyroidism.

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9 Management of the Various Causes of Thyrotoxicosis

Douglas S. Ross, MD

Contents

INTRODUCTION

Disorders That Cause Thyrotoxicosis

A number of different clinical entities can cause thyrotoxicosis. It is essential that the correct etiology be identified, since appropriate therapy depends on the mechanism of the thyrotoxicosis. Table 1 lists the various causes of thyrotoxicosis.

Thyrotoxicosis commonly results from *de novo* synthesis of thyroid hormone (TH) within the thyroid gland. Patients with the disorders that cause this will have an increased 24-h radioiodine (RAI) uptake and can be managed with therapy directed against the thyroid gland, such as thionamides, RAI, or surgery. In contrast, thyrotoxicosis with a low 24-h RAI uptake indicates either inflammation and destruction of thyroid tissue, with release of preformed hormone into the circulation, or an extrathyroidal source of TH. Thyrotoxicosis resulting from thyroid inflammation is not associated with new hormone synthesis, and thionamide therapy is not appropriate in this situation.

Beta-Blockers in the Treatment of Thyrotoxicosis

β-Adrenergic blocking agents are useful for ameliorating the symptoms of thyrotoxicosis regardless of the etiology. In many tissues, thyrotoxicosis increases the number of β-adrenergic receptors *(1)*. The resulting increase in β-adrenergic activity is responsible for many of the symptoms of thyrotoxicosis, and it explains the ability of β-blockers to ease

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Table 1
Disorders that Cause Thyrotoxicosis

Thyrotoxicosis with a high radioiodine uptake
Due to thyrotropin receptor antibody
Graves' disease
Hashitoxicosis
Due to thyroid autonomy
Toxic adenoma
Toxic multinodular goiter
Due to HCG
Hydatiform mole
Choriocarcinoma
Due to TSH
TSH-producing pituitary adenoma
Resistence to thyroid hormone
Thyrotoxicosis with a low radioiodine uptake
Subacute thyroiditis
Subacute granulomatous thyroiditis
Subacute lymphocytic thyroiditis
Postpartum thyroiditis
Amiodarone
Radiation thyroiditis
Palpation thyroiditis
Ectopic thyrotoxicosis
Factitious
Struma ovarii
Functional metastatic follicular thyroid cancer

HCG, human chorionic gonadotropin; TSH, thyroid-stimulating hormone.

palpitations, tachycardia, tremulousness, anxiety, and heat intolerance *(2)*. Propranolol in high doses (>160 mg/d) can also decrease the plasma triiodothyronine (T_3) concentration by as much as 30% *(3)*, via inhibition of the 5′-monodeiodinase that converts thyroxine (T_4) to T_3. Propranolol is highly lipid soluble, allowing it to become sufficiently concentrated in tissues to inhibit monodeiodinase activity. In comparison, atenolol, alprenolol, and metoprolol cause minimal reductions in plasma T_3 levels, and sotalol and nadolol cause no reduction *(4)*. Despite this theoretical advantage of propranolol, its short half-life as compared to that of atenolol or nadolol limits its clinical utility. Furthermore, if the inhibition of 5′-monodeiodinase is felt to be clinically important for a patient with severe thyrotoxicosis, it is better achieved by the addition of an iodinated radiocontrast agent to the medical regimen (*see* "Iodinated Radiocontrast Agents . . ." following).

Beta-blockers should be given to most thyrotoxic patients who do not have a contraindication to their use. Patients with relative contraindications to β-adrenergic blockade may better tolerate β_1-selective agents, such as atenolol or metoprolol. The author prefers to use atenolol in doses of 25–50 mg once daily for most patients, and higher doses given twice daily for patients with more severe disease. Because of its ability to inhibit the conversion of T_4 to T_3, propranolol is preferred for a patient who is symptomatic, allergic to thionamides, and unable to use iodinated radiocontrast agents until after RAI therapy.

Beta-blockers are essential for the preoperative treatment of Graves' disease (GD) in patients who are allergic to thionamides. The longer-acting agents, such as atenolol, provide

for more constant intraoperative and postoperative control, and minimize the need for intravenous beta-blockers during the period in which the patient is unable to take oral medications *(5)*. Because there may be an increased spontaneous abortion risk in pregnant thyrotoxic patients treated with propranolol and a thionamide as opposed to a thionamide alone *(6)*, the use of beta-blockers to treat thyrotoxicosis during pregnancy should be limited to more symptomatic patients.

TREATMENT OF HYPERTHYROIDISM WITH A HIGH RADIOIODINE UPTAKE

Autoimmune thyroid disease (AITD) and autonomous thyroid tissue are the major causes of excess new hormone synthesis by the thyroid. Trophoblastic disease and thyroid-stimulating-hormone (TSH)-mediated hyperthyroidism are rare causes of thyrotoxicosis and are discussed separately below. GD, the most common form of hyperthyroidism, is an autoimmune disorder resulting from anti TSH-receptor antibodies (TRAbs) (also called thyroid-stimulating immunoglobulins [TSIs]), which stimulate thyroid-gland growth, TH synthesis, and TH release *(7)*. "Hashitoxicosis" is a term used to describe AITD that initially presents, as does GD, with hyperthyroidism and a high RAI uptake caused by TRAbs *(8)*. This is followed by the development of hypothyroidism caused by infiltration of the gland with lymphocytes and a resultant autoimmune-mediated destruction of thyroid tissue resembling that seen in chronic lymphocytic thyroiditis (Hashimoto's thyroiditis [HT]). Treatment of GD and Hashitoxicosis is similar, in Hashitoxicosis hypothyroidism may intervene, making further therapy unnecessary.

Toxic adenoma and toxic multinodular goiter are the result of focal and/or diffuse hyperplasia of thyroid follicular cells, whose functional capacity is independent of regulation by TSH. Treatment of hyperthyroidism resulting from thyroid autonomy is similar to that of hyperthyroidism from TRAbs, except that in toxic adenoma or toxic nodular goiter, thionamides are useful for acute control of the hyperthyroidism, with RAI or surgery usually required for definitive therapy, whereas patients with GD or Hashitoxicosis may develop remission during thionamide therapy.

Iodine-induced hyperthyroidism develops after a patient receives an iodine load from intravenous contrast medium, topical iodine-containing antiseptics (e.g., povidone–iodine [Betadine]), or iodine-rich drugs such as amiodarone *(9)*. Affected patients most often have underlying thyroid autonomy, leading to *de novo* synthesis of excess hormone. The RAI uptake may be misleadingly low owing to dilution of the RAI tracer used to measure the uptake. Because iodine-induced hyperthyroidism may be self-limiting if the source of iodine is discontinued, definitive treatment may not be necessary. RAI ablation of the autonomous tissue may not be possible for several weeks after iodine exposure, since the exogenous iodine will limit entry of RAI into the thyroid gland.

Treatment of Graves' Hyperthyroidism and Toxic Adenoma or Toxic Multinodular Goiter

Two approaches can be taken to the treatment of thyrotoxicosis caused by GD: (1) control of the hyperthyroidism with thionamides (antithyroid drugs) over a prolonged period, with the hope of achieving a remission; or (2) definitive therapy by ablation of thyroid tissue with RAI or by surgical removal of the gland. Patients with toxic adenoma or toxic nodular goiter may

be treated with thionamides to achieve a euthyroid state before definitive treatment with RAI or surgery, but are not expected to achieve a remission.

Thionamides

The thionamides were found in 1943 to inhibit TH synthesis. They are actively transported into the thyroid gland, where they inhibit both the organification of iodine to tyrosine residues on thyroglobulin and the coupling of iodotyrosines *(10)*. Propylthiouracil (PTU) and methimazole (MMI) (Tapazole) are the two thionamide drugs available in the United States, and carbimazole is widely used in Europe.

Pharmacology. The dose regimens for MMI and carbimazole are interchangeable, since carbimazole is completely metabolized to MMI. Both drugs are given orally, are rapidly absorbed, and reach peak plasma concentrations in 1–2 h. Both drugs can be prepared for rectal administration *(11,12)* and PTU has been prepared for intravenous use *(13)*. PTU, but not MMI, inhibits the 5′-monodeiodinase that converts T_4 to T_3. However, MMI has a number of important pharmacokinetic advantages over PTU. The half-life of MMI is 4–6 h, as opposed to only 75 min for PTU. The intrathyroidal MMI concentration, which can reach a thyroid-to-plasma ratio of 100:1, remains at therapeutic levels for up to 20 h, which is considerably longer than does PTU. This is demonstrated by perchlorate discharge testing, which assesses inhibition of iodine organification. Twenty-four hours after a single 15-mg dose of MMI, perchlorate was found to discharge 37% of the RAI tracer; in contrast, perchlorate discharged only 8.6% of the RAI at 24 h after a 300-mg dose of PTU *(14)*. The pharmacokinetic advantages of MMI predict the outcome of clinical trials demonstrating that it is effective in a single daily dose *(15,16)*, whereas PTU is more effective in divided doses *(17)*. Additionally, despite a short-term advantage of PTU in blocking the conversion of T_4 to T_3, there is clear evidence of the superiority of MMI in the long-term treatment of hyperthyroidism. In one study, for example, MMI (10 mg three times per day) normalized the plasma T_3 concentration at an average of 5.8 wk after the beginning of therapy, as compared to 16.8 wk for PTU (100 mg three times per day) *(14)*. MMI is also less likely than PTU to be associated with failure of RAI ablation when antithyroid drugs are administered before RAI therapy *(18)*. Patients receiving thionamides require several weeks to achieve euthyroidism, because thionamides block only *de novo* hormone synthesis. Thus, already-formed TH stored within the colloid space must be utilized before clinical improvement is seen.

PTU is less soluble than MMI, and is bound to plasma proteins. It crosses the placenta one-fourth as well as MMI and is concentrated in breast milk one-tenth as well *(19)*. PTU may be preferred during pregnancy and nursing, although both thionamides have been used during nursing. Mothers who took up to 20 mg of MMI daily had nursing infants with normal thyroid function and normal growth and development *(20)*.

Dosing Regimen. Traditionally, initial therapy has been with relatively high doses of thionamides: 30–40 mg of MMI in single or divided doses or 300 mg of PTU in divided doses. The dose is tapered to maintenance levels, usually 5–15 mg/d of methimazole or 50–100 mg twice daily of PTU, as thyroid function test results begin to become normal. The higher initial doses are probably unnecessary in most patients. In one trial, for example, MMI in doses of 15 mg/d, 30 mg/d, or 10 mg three times a day produced a euthyroid state in the same average time *(15,16)*. Because some patients with severe hyperthyroidism or very large glands may require maintenance doses in excess of those noted above, such patients should still be started on the higher doses of thionamides to assure an appropriate response to therapy. However, because the side effects of the thionamides are dose-dependent, patients with small glands and mild hyperthyroidism can be started on 10–20 mg of MMI daily.

The dose should be increased if the hyperthyroidism is not ameliorated within 6 wk. When high doses of MMI are used, they should be divided and given at intervals to minimize gastrointestinal side effects, and then combined into a single daily dose if tolerated. It is unusual for any patient to require more than 40 mg/d of MMI; such patients are frequently noncompliant.

Toxicity. Both PTU and MMI cause rash, urticaria, arthralgias, arthritis, fever, nausea, or vomiting in approx 13% of patients *(21)*. If one drug is not tolerated, the other can be substituted for it, but up to 50% of patients experience cross-sensitivity *(22)*.

Agranulocytosis is a rare but serious complication of thionamide therapy, with a prevalence of 0.2–0.5%. In one study, agranulocytosis was more frequent in elderly patients taking MMI in doses >40 mg/d; the prevalence of agranulocytosis with PTU was dose-independent *(23)*. Most cases of agranulocytosis occur within 3 mo of starting treatment. However, a recent Japanese study found that the development of agranulocytosis was independent of dose, age, duration of treatment, or second exposure to a thionamide *(24)*. Controversy exists about the utility of monitoring the white-blood-cell (WBC) count during thionamide treatment. Most clinicians in the United States do not recommend periodic monitoring. However, a recent study in Japan was able to identify 78% of cases of thionamide-induced agranulocytosis before the onset of symptoms by checking blood counts every 2 wk for the first 2 mo of therapy *(25)*. A WBC count should be obtained before treatment, since leukopenia without agranulocytosis may be seen in GD as a result of antineutrophilic antibodies *(26)*. Patients who are taking a thionamides and who develop a fever or sore throat should have an immediate WBC count with a differential count, and should discontinue the medication until the results of these tests are available. Recovery from agranulocytosis usually takes a few days; granulocyte colony-stimulating factor may be useful adjunctive therapy in severe cases *(27)*, although in a nonrandomized trial the time to recovery was unaffected by its use *(28)*. Morbidity and death from serious infections can occur with thionamide-induced agranulocytosis, especially if the agranulocytosis is prolonged. MMI has also been associated with aplastic anemia *(29)*.

Hepatic toxicity is a rare complication of thionamide therapy. PTU can cause fulminant hepatic necrosis; death may occur in up to 20% of patients and appears unrelated to dose or duration of therapy *(30)*. Transient elevations in transaminases may occur in up to one-third of patients taking PTU; this abnormality may be associated with focal areas of hepatic necrosis on liver biopsy *(31)*. MMI, in comparison, is occasionally associated with cholestatic jaundice. Patients taking MMI who develop pruritis should have their liver function tested.

Previously vasculitis was thought to be a rare complication of thionamide therapy *(32)*. Recent reports, however, have described a 38–67% incidence of antineutrophil cytoplasmic antibodies (ANCAs) in patients taking PTU *(33,34)*; ANCAs were not found in any patients taking MMI in these studies (although there are rare case reports of ANCA-associated vasculitis in patients taking MMI). Most patients with ANCAs were asymptomatic, many had arthralgias or myalgias, and none had renal disease. The long-term consequences of PTU-related, ANCA-associated vasculitis are unknown.

Use of MMI, but not of PTU, during pregnancy has rarely been associated with a scalp defect, aplasia cutis, in the neonate. PTU, which also has the advantage of not crossing the placenta as well as MMI, may therefore be preferred in hyperthyroidism complicating pregnancy. Both thionamides can cause fetal goiter and hypothyroidism.

Monitoring Therapy. Patients taking thionamides require careful monitoring to prevent hypothyroidism. It is also important to periodically monitor both the plasma T_3 and T_4

concentrations, since T_3 levels may be elevated when T_4 levels have returned to normal. The plasma T_3-to-T_4 ratio is particularly high in Graves' hyperthyroidism, and patients with exaggerated "T_3-predominant" GD may respond less well to therapy and are less likely to attain prolonged remission *(35)*. Some cases have been reported in which patients taking thionamides, especially PTU, have elevated T_3 levels in association with subnormal serum T_4 levels *(36)*.

TSH levels may be misleading during the treatment of hyperthyroidism *(37)*. Pituitary TSH production is suppressed by the hyperthyroid state, an effect that can persist for weeks to several months after TH levels have become normal. As a result, a subnormal plasma TSH concentration may be seen despite normal or even low plasma TH levels in patients beginning thionamide therapy. TSH measurements are useful only after steady-state conditions have been achieved. Sufficient thionamide should be given to maintain a normal serum TSH during long-term therapy, since subnormal serum TSH levels despite normal T_4 and T_3 concentrations (subclinical hyperthyroidism) during thionamide therapy are associated with increased bone turnover *(38)*.

Remission in GD. There is controversy about whether clinical remission of hyperthyroidism in GD in patients treated with thionamides derives from direct immunomodulatory effects on the underlying autoimmune process *(39)* or from effects caused by diminished TH production. According to the latter hypothesis, the major action of the thionamides is to reduce TH levels, which secondarily modulate thyrocyte activity, thereby reducing thyroidal antigen presentation to intrathyroidal T lymphocytes and diminishing anti-TSH-receptor-antibody production *(40)*. In one study, perchlorate, a drug unrelated to the thionamides that competes with iodine for thyroidal uptake, also reduced anti-TSH-receptor antibody titers as the hyperthyroidism resolved, supporting the latter hypothesis *(41)*.

Thionamide treatment is generally begun in patients with Graves' hyperthyroidism, toxic adenoma, or toxic nodular goiter, and who have moderate to severe symptoms, to rapidly attain a euthyroid state in preparation for definitive therapy with RAI or surgery. However, patients with GD who want to avoid or defer definitive therapy can continue taking thionamides for prolonged periods with the hope of attaining a permanent remission. It is best to agree on a tentative duration (e.g., 1–2 yr) of thionamide therapy at its beginning, and then to reexamine treatment options and goals at the end of this period. However, the thionamide can be stopped at any time, to allow patients to proceed with RAI or surgery. On the other hand, occasional patients will continue taking thionamides for years or decades.

The rate of persistent remission of GD following cessation of thionamide therapy varies from 13–80%, but is usually 20–30% in the United States after 1–2 yr of therapy. The remission rate may be higher for women than for men: the respective rates in an English study were 40% vs 20% *(42)*. Remissions were also more likely in patients over 40 yr old. It has been proposed that the level of iodine consumption can influence the remission rate, but this remains controversial *(43)*. It is also uncertain whether remissions associated with thionamide therapy are due to the drug or to the natural history of the disease. The spontaneous remission rate in a group of hyperthyroid patients treated with propranolol alone was 31%, which was similar to that seen after thionamides *(44)*. Although these patients probably had, on average, mild hyperthyroidism (which has been associated with higher rates of remission), this observation supports the hypothesis that the thionamides primarily buy time for a spontaneous remission to occur. Remission is more likely in patients with low or undetectable titers of anti-TSH-receptor antibodies at the onset of GD *(45)*. It is also more common in females, patients with mild hyperthyroidism, patients with small glands or with glands that shrink during the course of thionamide therapy, and those with high titers of

Table 2
Findings Associated with Relapse
During Thionamide Therapy of Graves' Disease

Subnormal serum TSH levels
Flat response of serum TSH to TRH administration
Elevated thyrotropin receptor antibody levels
High thyrogolobulin levels during concurrent L-thyroxine therapy
High radioiodine uptake during concurrent L-thyroxine therapy
High ratio of serum T_3 to T_4
HLA-D3 and high thyrotropin receptor antibody levels
HLA-D3 allele DQA2U
High serum IgE concentrations
Hypoechogenicity on thyroid ultrasound

TSH, thyroid-stimulating hormone; TRH, thyrotropin-releasing hormone; HLA, human leukocyte antigen.

thyroid autoantibodies *(46,47)*. Remission rates also increase with the duration of therapy, rising in one study from 25% after 2 yr to 75% after 11 yr *(48)*.

Patients with GD can achieve a spontaneous remission through any of three mechanisms. (1) A decrease in the titer of anti-TSH-receptor antibodies, or TSIs, may correlate with remission. (2) Autoimmune-mediated destruction of functioning thyroid tissue, from extensive lymphocytic infiltration, similar to that seen in chronic lymphocytic thyroiditis (Hashimoto's thyroiditis), may prevent the gland from responding to anti-TSH-receptor antibodies, and can result in euthyroidism or ultimately spontaneous hypothyroidism (also called "burnt out" GD). Circulating titers of anti-thyroid peroxidase (anti-TPO) antibodies (also referred to as antimicrosomal antibodies) correlate with the degree of lymphocytic infiltration, and hyperthyroid patients with higher titers are found to have higher rates of spontaneous "remission" *(3,46)*. Remission can also occur because of the appearance of TSH receptor-blocking antibodies (also called thyroid-stimulating-blocking immunoglobulins), which occupy the TSH receptor and block the stimulatory action of TSH or of stimulating immunoglobulins *(49)*. These patients can develop hypothyroidism spontaneously, and occasionally fluctuate between hyperthyroid and hypothyroid states, depending on the relative titers of stimulating and blocking immunoglobulins.

There is extensive literature regarding various tests that can be done during treatment with thionamides to predict whether an individual patient has achieved permanent remission (Table 2). None of the tests listed is sufficiently accurate to be widely useful *(50–56)*. In the United States, most clinicians taper or discontinue thionamides and then follow the patient carefully for evidence of recurrent hyperthyroidism. Patients with subnormal plasma TSH concentrations after several months of thionamide therapy are not in remission, and the thionamide should not be discontinued unless a decision has been made to proceed with definitive therapy. Recurrent hyperthyroidism is initially manifested by an increased plasma T_3 concentration or a suppressed plasma TSH concentration. It can occur as early as the first 10 d after discontinuing thionamides, or as late as several months. Prolonged remission is likely if the patient remains euthyroid for 6 mo after stopping thionamides. Late relapse occurs in only 8–10% of these patients.

A study in Brazil concluded that higher doses of thionamides resulted in higher remission rates in GD *(57)*. The patients receiving high-dose therapy required cotreatment with TH

to prevent hypothyroid symptoms. The remission rate was 75% in the group receiving high-dose thionamides and TH, versus 42% in the group receiving low doses of thionamides alone. However, four studies have failed to find a benefit of higher doses of thionamides *(58–61)*. A European multicenter trial found identical rates of remission in patients treated with either 10 mg or 40 mg of MMI, with both groups cotreated with levothyroxine *(58)*. A Swedish study found identical remission rates in patients treated with 60 mg of MMI and levothyroxine, versus those begun on 30 mg of MMI that was then tapered to lower maintenace doses *(59)*. A Japanese study found no difference in remission rates between patients treated with 30 mg or 15 mg of MMI; subsequent rates of relapse were also identical *(60)*. However, another Japanese study found higher remission rates in GD when thionamide therapy was combined with levothyroxine *(62)*. The relapse rate was less than 2% in patients cotreated with levothyroxine, versus 35% in those given placebo. Nevertheless, seven ensuing published studies have failed to confirm these findings *(63)*.

RAI

RAI is ultimately the most commonly recommended treatment for hyperthyroidism in the United States. Sixty-nine percent of North American thyroid specialists chose RAI for early therapy in a hypothetical patient with Graves' hyperthyroidism. RAI is less popular elsewhere, being chosen as first-line therapy by only 22% and 11% of European and Japanese thyroid specialists, respectively *(64)*. RAI is administered as sodium ^{131}I in an oral solution or capsule. The RAI is rapidly incorporated into thyroid tissues, and β-particle emissions result in extensive local tissue damage. The net effect is elimination of thyroid function over a period of 6–18 wk or longer.

GD. The primary goal of RAI therapy in GD is to cure the hyperthyroidism. It is controversial whether RAI should be given in a dose sufficient to induce hypothyroidism or in a lower dose in an attempt to achieve a euthyroid state *(50)*. Complete ablation of the thyroid gland cures the hyperthyroidism in 90% of patients after one dose. It also causes permanent hypothyroidism in 70% of patients within 1 yr *(65)*. Thus, hypothyroidism is the expected outcome of RAI therapy, and thyroid function can be accurately regulated by titrating the levothyroxine replacement dose. Although maintenance of endogenous thyroid function may appear desirable, there are a number of disadvantages associated with the low-dose RAI regimens used for this. Less than one-third of patients so treated remain euthyroid at 10 yr after therapy *(66)*. Additionally, low-dose therapy is more likely to result in treatment failure, which may require several subsequent courses of RAI over a 6–24-mo span *(67,68)*. During this period of poorly controlled hyperthyroidism, there may be a permanent reduction in bone density *(69)*. Many patients who were formerly thought to have been euthyroid have, when assessed with third-generation TSH assays, in actuality been found to have subclinical hyperthyroidism with its associated risks of atrial fibrillation and reduced bone density *(70)*. The development of hypothyroidism after low-dose RAI therapy occurs at a rate of 2–3%/yr, and may be accompanied by an insidious onset of symptoms. Additionally, thyroid remnants in patients who achieve euthyroidism may regrow under continued stimulation by TSH receptor-stimulating antibodies, resulting in recurrent hyperthyroidism.

There is potential concern about the effect of RAI on the ophthalmopathy associated with GD. A randomized trial found an increased risk of new or worsening ophthalmopathy in patients treated with RAI rather than with surgery or thionamides *(71)*. This complication occurred primarily in patients who required more than one RAI treatment, and in most patients resulted in an extra 1–2 mm of proptosis. Earlier studies did not demonstrate a relationship between the choice of therapy for hyperthyroidism and progression of

Graves' ophthalmopathy *(72,73)*. However, several investigators report acute worsening of ophthalmopathy following the administration of RAI, which can be ameliorated by the prophylactic administration of corticosteroids *(74)*. It may be prudent to defer RAI treatment in patients with moderate to severe Graves' ophthalmopathy until their orbits have been stable for a year.

Toxic Adenoma and Toxic Multinodular Goiter. The dose of RAI is less controversial for patients with a toxic adenoma or toxic nodular goiter. Areas of focal autonomy take up RAI well, while uptake is limited in adjacent and contralateral thyroid tissue because of suppression of TSH by the hyperthyroid state. As a result, RAI tends to destroy only the autonomous areas, and most patients remain euthyroid following RAI administration *(75)*. Patients who do develop hypothyroidism usually do so because RAI uptake was not suppressed in the contralateral lobe *(76)* or because of coexistent CLT *(77)*. Rarely, patients with toxic nodular goiter who receive RAI therapy subsequently develop hyperthyroidism associated with anti-TSH receptor antibodies *(78)*.

Dosing Regimens. The optimal dosing regimen of RAI for ablating the thyroid gland is uncertain. Fixed doses of 5, 10, or 15 mCi are common in GD, and a recent analysis suggested 10 mCi as the optimal dose *(79)*. In one study 67% of patients were cured of their hyperthyroidism after 5 mCi, and 41% became hypothyroid; after 10 mCi, 85% were cured and 61% became hypothyroid *(80)*. Another approach, which the author prefers, is to individualize the dose of RAI based on gland size and the 24-h RAI uptake *(81)*. One prospective, randomized trial compared a fixed dose with a calculated dose and recommended individual dose calculation because of the dependence of outcome on gland size *(82)*. However, another study found that a semiquantitative fixed-dose regimen of 5 mCi for small glands, 10 mCi for medium glands, and 15 mCi for large glands was as effective as calculating the individual dose *(83)*. A common calculation is to deliver 80–160 μCi/g tissue by adjusting the dose for differences in the measured 24-h RAI uptake (for example, for a 45-g gland with a 72% uptake: 45 g × 150 μCi/g/0.72 = 10 mCi). This approach may require one extra trip to the hospital, but by revealing the RAI uptake just prior to treatment, it assures the appropriateness of therapy, as well as the etiology of the hyperthyroidism in newly diagnosed patients. It also avoids undertreatment that occurs when a fixed dose is used in a patient with a large gland and an uptake that is not high. The doses of RAI used to treat toxic adenoma or nodular goiter are as much as twofold greater than in GD (up to 200 μCi/g), since the RAI is primarily taken up only in the autonomous tissue *(81)*.

Pretreatment with Thionamides. RAI can be administered as initial therapy for hyperthyroidism in young patients and those with mild hyperthyroidism, especially if they are adequately treated with beta-blockers. However, many patients are first pretreated with a thionamide to attain euthyroidism. There are several advantages to pretreatment. RAI takes approx 12–18 wk to induce a euthyroid state in most patients. Thionamides will correct the hyperthyroid state after 4–8 wk. Thus, patients who are poorly tolerating hyperthyroidism should first receive thionamides. Also, RAI may induce a transient exacerbation of hyperthyroidism by causing inflammation and release of TH into the circulation (radiation thyroiditis) *(84)*. It is therefore prudent to deplete TH stores with a thionamide in elderly patients or those with known cardiac disease. The thionamide must, however, be discontinued 3 d before RAI administration (to prevent impairment of RAI uptake by the thyroid), and then restarted 3 d later *(50,85)*. Exacerbation of hyperthyroidism following the inititation of RAI therapy may occur in part because of the necessary temporary cessation of thionamide therapy *(86)*. Two studies suggest that thionamide therapy after RAI administration is associated with a lower rate of late hypothyroidism *(87,88)*. However, pretreatment with

PTU, but not MMI, increases the rate of treatment failure by more than threefold *(89,90)*; patients treated with PTU prior to thyroid ablation should be given higher doses of RAI.

Toxicity. RAI appears to be quite safe, and its only long-term side effect is the induction of hypothyroidism *(91)*. One percent of patients treated with RAI do get radiation thyroiditis, which can cause relatively severe thyroid pain and last as long as 2–3 wk, and which may be associated with exacerbation of hyperthyroidism. Nonsteroidal antiinflammatory agents are usually sufficient analgesics in such cases, but corticosteroids may be required. Many large studies, including a prospective study of 35,593 patients with 21 yr of follow-up, have failed to show an increased overall risk of cancer or leukemia after RAI thereapy *(92)*. However, there was an increased risk of thyroid cancer in patients receiving RAI for toxic nodular goiter: the standardized cancer mortality ratio was 2.77, representing 19 excess deaths out of 2950 cancer deaths *(92)*. It is possible that some of these cancers reflect the known increased rate of thyroid cancer among patients with nodular goiter. A smaller study, of 7417 patients, also found no overall increased risk of cancer with RAI therapy, but there was a slight excess of both thyroid and small-bowel cancers *(93)*. One study noted an increased incidence of benign adenomas, but not malignancies, in adolescents given RAI *(94)*, leading to the recommendation that it be used as second-line therapy in children. Pregnancy is a contraindication to RAI therapy. Fetal thyroid tissue is present by 10–12 wk and would be destroyed by the RAI dose used to treat hyperthyroidism, resulting in cretinism. Birth defects are not more common after RAI *(95)*. The gonadal dose is about 3 rad, a range similar to that for a hysterosalpingogram or a barium enema *(96)*. The estimated risk of genetic damage is 0.005%, which is lower than the spontaneous risk of genetic abnormality of 0.8% *(91)*. Pregnancy should be delayed 4–6 mo after RAI administration *(50)*; however, unintended pregnancies during this interval can be allowed to proceed to term.

Surgery

GD. With the recognized safety and increasing acceptance of RAI, surgery has become unpopular as definitive therapy for GD and is recommended by only 1% of American thyroidologists *(64)*. Fear of potential (and unsubstantiated) carcinogenic or teratogenic effects of RAI is the most common reason why patients choose surgery. Surgery may be recommended for patients with very large glands who might require multiple doses of RAI over a period of months to years, patients with goiters causing upper-airway obstruction or severe dysphagia, and patients with a coexisting nonfunctional ("cold") nodule. In this last case, RAI may be preferred even if a fine-needle aspirate demonstrates that the nodule is benign. Surgery might also be preferred over RAI in patients with significant ophthalmopathy. Other indications for surgery include moderately severe hyperthyroidism complicating pregnancy in women allergic to thionamides, and patients in whom rapid resolution of hyperthyroidism and/or treatment is necessary.

The extent of surgery in GD is an area of controversy that parallels arguments for low- versus higher-dose RAI therapy. Thyroid remnants smaller than 4 g are associated with postoperative hypothyroidism in 27–99% of cases and which may be subclinical *(97)*; these patients require permanent levothyroxine replacement therapy. Larger thyroid remnants, of 7–8 g, appear to prevent hypothyroidism. However, many of these patients have subclinical hyperthyroidism with the attendant risk of reduced bone density and atrial fibrillation. In addition, 9–12% of these patients develop recurrent overt hyperthyroidism *(98)*. RAI is the treatment of choice for surgical failures, since repeat surgery is associated with an unacceptable risk of complications. This presents a significant problem for those patients who initially chose surgery because of concerns about RAI exposure. In such patients, hypothyroidism should be the goal of initial surgery.

Toxic Adenoma and Toxic Multinodular Goiter. Surgery is more popular than RAI therapy for patients with toxic multinodular goiter, especially in patients with very large glands or glands with both autonomous (hot) and nonfunctioning areas, since normalization of serum TSH levels following RAI may result in renewed goitrogenesis. Large areas of cystic degeneration or substernal extension of goitrous tissue are other possible indications for surgery *(99)*.

Complications. Complications of thyroid surgery include transient and permanent hypoparathyroidism and recurrent laryngeal nerve palsy. Permanent problems should occur in less than l% of patients. Surgeons with specific expertise in thyroid disease should be chosen to avoid these complications.

Preoperative Preparation for Surgery. Preparation of hyperthyroid patients for thyroid surgery ideally begins with thionamide therapy to achieve euthyroidism. Patients with GD are also given 10 d of iodine (e.g., saturated solution of potassium iodide [SSKI] 10 drops daily) before surgery to reduce gland vascularity *(100)*. Several alternatives are available for patients who are allergic to thionamides. Unless contraindicated, a beta-blocker should be given in sufficient dosage to maintain a pulse rate below 80 beats/min with exercise (*see* "Beta-Blockers in the Treatment of Thyrotoxicosis," earlier) *(101)*. Iodine, in addition to reducing gland vascularity, will reduce TH levels over a period of 10 d (*see* next subheading). Alternatively, ipodate or iopanoic acid can rapidly reduce TH levels both by diminishing T_4-to-T_3 conversion and by supplying a source of iodine (*see* next subheading) *(102)*. If thionamides are not coadministered, iodides and iodinated radiocontrast agents should be used only for patients with GD, since iodine provides substrate for new hormone synthesis and may worsen hyperthyroidism in toxic nodular goiter.

Iodinated Radiocontrast Agents, Iodine, and Other Therapies

Ipodate and iopanoic acid (two iodine-containing drugs marketed as oral cholecystographic agents) have found increasing use in the treatment of hyperthyroidism, although at present only iopanoic acid is available in the United States *(103)*. These agents are the most potent blockers of 5′-monodeiodinase, thus impairing the conversion of T_4 to the more potent T_3. Ipodate is more potent than PTU or potassium iodide in blocking the conversion of T_4 to T_3, and results in a rapid reduction in plasma T_3 levels *(104,105)*. Doses in most studies have ranged from 500–1000 mg given once daily. Although ipodate and iopanoic acid have been used as monotherapy for the treatment of GD *(106)*, they are not as effective as thionamides, and recent studies suggest a higher relapse rate when therapy is discontinued than that seen with thionamides *(107)*. Ipodate and iopanoic acid may also induce hyperthyroidism that is resistant to conventional doses of thionamides *(108)*. Whereas the iodine released from these agents potentially provides the added advantage of blocking TH release, iodine-containing drugs should not be used as monotherapy in patients with toxic adenoma or toxic multinodular goiter, since the iodine may provide substrate for *de novo* hormone synthesis by the autonomous thyroid tissue, leading to more severe hyperthyroidism. These drugs can be used in this setting only if TH synthesis is first blocked by the administration of a thionamide.

The major uses of the iodinated radiocontrast agents have been in the treatment of severe hyperthyroidism or "thyroid storm," and in the preoperative preparation of patients who are allergic to thionamides. For example, in severe hyperthyroidism the combination of MMI and ipodate is more effective than MMI alone or MMI plus potassium iodide, since it may normalize the plasma T_3 concentration within 5 d *(109)*. Similarly, the triad of a radiocontrast agent, beta-blocker, and corticosteroid *(102)* can render a preoperative patient euthyroid within 5 d. These agents may also be effective during the hyperthyroid

phase of subacute thyroiditis (*see* below) *(110)*, or in patients with an acute levothyroxine overdose *(111)*.

Iodine elixirs, such as SSKI or Lugol's solution, replaced burnt-sponge extract in the 19th century as treatment for exophthalmic goiter. Iodine continues to have a minor role in the treatment of hyperthyroidism. Pharmacologic amounts of iodine improve the hyperthyroid state by blocking TH release. There is also abnormal autoregulation of iodine economy. In normal subjects, the administration of pharmacologic amounts of iodine leads to temporary inhibition of further iodine organification in the thyroid gland, a phenomenon called the Wolff–Chiakoff effect *(112)*; escape eventually occurs, allowing TH synthesis to proceed. In autoimmune thyroid disease, however, iodine-induced blockade of iodine organification persists and can result in hypothyroidism. This effect is even more pronounced after RAI treatment of Graves' hyperthyroidism *(113)*. The overall inhibitory effect of iodine on plasma TH levels is maximal after about 10–14 d of treatment. This is often followed by a return to higher levels, although the benefit may be more prolonged, especially in patients who have received RAI *(114)*. Iodine is at present primarily used in the preoperative preparation for thyroidectomy in GD (*see* above) *(100)* in patients with severe hyperthyroidism or thyroid storm, and as adjunctive therapy following the administration of RAI in GD (especially in patients who wish to avoid taking or who are allergic to thionamides). SSKI, begun 1 wk after RAI administration and given daily, normalizes thyroid function several weeks earlier than in patients treated with RAI alone *(65)*. Iodine can also be used months after RAI administration to ameliorate mild persistent hyperthyroidism and to delay or prevent the need for a second dose of RAI.

Corticosteroids have traditionally been used in the treatment of severe hyperthyroidism. Their major effect is to inhibit T_4-to-T_3 conversion *(115)*. They may also have a direct effect on the thyroid or on the underlying automimune process, since they reduce the 24-h RAI uptake *(115)*. Steroids may shorten and ameliorate the hyperthyroid phase of subacute thyroiditis (*see* "Subacute Thyroiditis" following), and have been used with thionamides in hyperthyroidism occurring during amiodarone therapy *(116)*.

Perchlorate is a competitive inhibitor of iodine uptake, and has been used with thionamides to ease amiodarone-related hyperthyroidism *(117)*. Lithium blocks TH release and synthesis, and has been used with thionamides *(118)*, but its use is limited owing to its multiple toxicities. Plasmapheresis can reduce serum T_4 concentrations by 40% in patients with severe hyperthyroidism *(119)*. Ethanol injection into toxic nodules under ultrasonic guidance has recently been proposed as a nonsurgical method for destroying functioning adenomas *(120)*.

TREATMENT OF SEVERE HYPERTHYROIDISM AND THYROID STORM

Precise criteria for the diagnosis of thyroid storm have been suggested by Burch and Wartofsky *(121)*, and may include hyperpyrexia to 104–106°F; tachycardia with rates that can exceed 140 beats/min; congestive heart failure; agitation, delirium, psychosis, stupor, or coma; severe nausea, vomiting, and diarrhea; and hepatic failure with jaundice. Although thyroid storm can develop in patients with longstanding untreated hyperthyroidism, it is more often induced by an acute precipitant such as thyroid or nonthyroidal surgery, trauma, infection, or an acute iodine load. The therapeutic options for thyroid storm are essentially the same as those for uncomplicated hyperthyroidism, except that the drugs for treating it are used in higher doses and given more frequently. In addition, full support of the patient in an intensive-care-unit setting is essential, since thyroid storm is associated with a significant mortality rate. More commonly, patients with severe hyperthyroidism who are not in thyroid

storm are managed with more intensive therapy. Fever should be treated with acetaminophen rather than aspirin, since the latter can increase the free TH concentration by displacing hormones from binding proteins.

Beta-blockers should be used with caution in patients with congestive heart failure. Propranolol is often selected for initial therapy because it can be given intravenously at 1 mg/min until several milligrams have been administered or adequate β-receptor blockade has been achieved *(122)*. At the same time, oral (or nasogastric-tube) administration of propranalol is started at a dose of 60–80 mg every 4 h. An alternative regimen is to utilize the short-acting β-antagonist esmolol. A loading dose of 250–500 μg/kg is given, followed by an infusion at 50–100 μg/kg/min, allowing rapid titration of the drug while minimizing adverse reactions *(123)*.

It has been suggested that PTU is the thionamide of choice in thyroid storm because it blocks T_4-to-T_3 conversion in the periphery. However, the author prefers MMI as long as other drugs (such as iopanoic acid) are coadministered to block T_4-to-T_3 conversion. This is because MMI has a more favorable pharmacokinetic profile than PTU (*see* "Thionamides" preceding), and the iodinated radiocontrast agents are more effective than PTU in inhibiting 5′-monodeiodinase *(109)*. Larger doses of thionomides are used in thyroid storm because of the possibility of poor absorption owing to concurrent gastrointestinal dysfunction (e.g., 30 mg of MMI every 6 h, or 200 mg of PTU every 4 h, orally, via nasogastric tube, or rectally) *(11,12)*. PTU can be prepared for intravenous administration by dissolving the tablets in isotonic saline made alkaline (pH 9.25) with sodium hydroxide *(13)*.

Iopanoic acid is extremely useful in treating severe hyperthyroidism at a dose of 0.5–1 g given once daily. Because iopanoic acid contains iodine, it is essential that its use be delayed for at least 1 h after thionamide administration, to prevent the iodine from being used as substrate for new hormone synthesis. It is likely that there is sufficient iodine released from the iodinated radiocontrast agents to achieve the inhibitory effect of iodine on TH release; however, no data about this are currently available. It is therefore prudent to treat patients in thyroid storm with iodide elixirs (e.g., Lugol's solution [10 drops three times daily] or SSKI [five drops every 6 h]). Intravenous sodium iodide can be used at 0.5–1.0 g intravenously every 12 h, but is no longer widely available. Lugol's solution (10 drops) can be directly added to intravenous fluids since it is sterile *(124)*.

The use of steroids in thyroid storm improved the outcome in at least one series *(125)*. The author does not routinely use pharmacologic doses of glucocorticoids in patients with severe but not life-threatening hyperthyroidism.

HYPERTHYROIDISM COMPLICATING PREGNANCY AND TREATMENT OF HCG-MEDIATED HYPERTHYROIDISM

Pregnancy complicated by hyperthyroidism is associated with an increased rate of spontaneous abortion, premature labor, and low birth weight; it can lead to maternal toxemia or congestive heart failure *(126)*. GD is the most common cause of hyperthyroidism occurring in 0.2% of pregnancies *(127)*. Human chorionic gonadotropin (HCG) is a weak thyroid stimulator. The high concentration of HCG present during early pregnancy can, in a minority of women, lead to subclinical hyperthyroidism with normal (or slightly elevated) FT_4 levels and subnormal TSH concentrations *(128)*. This change is transient and does not require treatment. An exaggeration of this phenomenon may be seen with hypermesis gravidarum, in which severe vomiting may be associated with hyperthyroidism during early pregnancy *(129)*. Although unproven, it has been postulated that the high HCG level in such cases is

responsible for both the hyperemesis (an estrogen effect) and the hyperthyroidism. Molar pregnancy or choriocarcinoma can be associated with more severe hyperthyroidism *(130)*, and partly sialylated HCG extracted from hydatidiform moles has greater thyrotropic activity than that of HCG from a normal pregnancy *(131)*. Treatment involves evacuation of the hydatiform mole or therapy directed against the choriocarcinoma. When HCG-mediated hyperthyroidism is severe, both PTU and β-adrenergic-blocking agents may be used to ameliorate the thyrotoxicosis and associated symptoms.

Women with GD complicating pregnancy are usually treated with PTU, since MMI has been associated with a rare fetal scalp defect, aplasia cutis *(132)*, and with choanal atresia *(133)*, and because the shorter half-life of PTU may result in fewer fetal complications from thionamide therapy. Fetal blood levels of TSH are elevated in 21% of PTU-treated mothers and 14% of MMI-treated mothers *(134)*. To minimize the risk of fetal hypothyroidism, one should use the smallest dose of thionamide necessary to control hyperthyroid symptoms. Mild persistent hyperthyroidism and subnormal TSH concentrations may be desirable to prevent fetal hypothyroidism *(134)*, but maternal hyperthyroidism in the third trimester has been associated with low birth weight *(135)*. The thionamide dose therefore should be titrated monthly, aiming for T_4 levels that are consistent with the thyroxin-binding-globulin (TBG) excess seen in pregnancy. Ideally, the dose of PTU can be reduced to 50 mg twice daily or less *(136)*, or PTU can be discontinued during the third trimester when the hyperthyroidism may resolve. Doses in excess of 200 mg/d, although potentially necessary to control maternal hyperthyroidism, will frequently result in fetal goiter and hypothyroidism. Fetal thyroid status and fetal goiter are assessed by monitoring fetal heart rate and by periodic ultrasonography, which can also detect delayed fetal growth. Reassuringly, studies of the intelligence quotient of children exposed to thionamides *in utero* have failed to document a deficiency *(137)*. Maternal GD may be expected to flare up postpartum *(138)*.

RAI is absolutely contraindicated during pregnancy, since it would destroy fetal thyroid tissue after 10–12 wk of gestation *(139)*. The use of beta-blockers should be restricted to significantly symptomatic patients, since these drugs have been associated with retardation of intrauterine growth *(140)* and with an increased risk of spontaneous abortion *(6)*. Surgery during pregnancy may be necessary in women who cannot tolerate thionamides because of allergy or agranulocytosis.

Fetal Hyperthyroidism

Approximately 1% of neonates born to women with GD will be hyperthyroid because of transplacental transfer of TRAbs *(141)*. Because hyperthyroid mothers are likely to be taking PTU, this occurs more commonly in mothers whose thyroid gland has been surgically removed or ablated, but who have persistent, high titers of TRAb *(142)*. High fetal heart rate, fetal goiter, advanced bone age, and craniosynostosis are potential manifestations of fetal hyperthyroidism. Thionamides can be given to the mother to treat fetal hyperthyroidism *in utero (143)*, even when the mother is hypothyroid and receiving levothyroxine because of prior thyroid ablation.

TREATMENT OF THYROID-STIMULATING-HORMONE-MEDIATED HYPERTHYROIDISM

TSH-mediated hyperthyroidism is rare, and is caused either by pituitary resistance to TH (PRTH) or by a TSH-producing pituitary adenoma. Resistance usually results from a mutation in the TH receptor (TR) gene β-1, which inhibits the normal TRβ-1-receptor in a dominant negative manner *(144)*. Approximately 10% of patients do not have TR mutations;

mutations of genes encoding cofactors that interact with TR may be responsible for PRTH in such cases *(145)*. When the pituitary is more resistant than peripheral tissues, the result is impaired inhibitory feedback on TSH production and hyperthyroidism. Treatment of PRTH is difficult and frequently unsatisfactory. In one family, the defect may have been at the level of the 5′-monodeiodinase, since the hyperthyroidism was corrected by the administration of T_3 *(146)*. Recent studies suggest that 3,5,3-L-triiodothyracetic acid (TRIAC) has a higher affinity than T_3 for some mutant receptors in PRTH *(147)*, and several reports suggest its usefulness in some but not all patients with PRTH *(148,149)*. Octreotide *(150)* and bromocriptine *(149)* may reduce TSH production, but their effects are weak and transient. Corticosteroids do reduce TSH *(151)*, but the long-term side effects are unacceptable.

Rare activating mutations in the TSH receptor result in hyperthyroidism; in such cases serum TSH levels are suppressed *(152)*.

TSH-producing pituitary adenomas are best treated with trans-phenoidal surgery with or without postoperative radiation therapy *(153)*. Cure rates have ranged from 30–38% without radiation and 41–46% with radiation. Prior to surgery, thionamides can be given to control hyperthyroid symptoms. If transsphenoidal surgery is unsuccessful, conventional treatment with thionamides, RAI, or thyroid surgery has the theoretical disadvantage, through reduction of TH levels, of stimulating growth of the pituitary neoplasm. Octreotide is currently the most promising phamacologic agent directed against pituitary adenoma; tumor size decreased by 52% and serum T_4 and T_3 concentrations became normal in 95% of patients after 1 yr *(153,154)*. Bromocryptine has been less successful at controlling TSH secretion *(153)*.

TREATMENT OF THYROTOXICOSIS WITH A LOW RAI UPTAKE

Subacute Thyroiditis

The term "subacute thyroiditis" has been applied to a group of heterogeneous disorders that result in inflammation of thyroid tissue with transient thyrotoxicosis caused by the release of preformed hormone from the colloid space. This initial presentation is followed by a hypothyroid phase and then by recovery of thyroid function. Subacute granulomatous thyroiditis (de Quervain's thyroiditis) is a viral or postviral syndrome characterized by fever, malaise, and an exquisitely tender gland *(155)*. In comparison, subacute lymphocytic thyroiditis (painless or silent thyroiditis) is part of the spectrum of AITD *(156)* and has a particular proclivity for the postpartum period (postpartum thyroiditis) *(157)*. Other causes of subacute thyroiditis include direct chemical toxicity from amiodarone (one of several mechanisms whereby amiodarone may cause thyrotoxicosis) *(158)*, radiation thyroiditis from external radiation or RAI, and palpation thyroiditis (occurring, for example, during parathyroid surgery).

Thionamides have no role in the treatment of these disorders, since new hormone is not being synthesized. RAI is similarly inappropriate, and in fact impossible to use, since the RAI uptake is usually under 1%. For the majority of patients, control of symptoms with β-adrenergic-blocking agents is sufficient. For the small minority of patients who cannot tolerate the thyrotoxic symptoms of subacute thyroiditis, the addition of an iodinated radiocontrast agent to block T_4-to-T_3 conversion may rapidly ameliorate symptoms *(110)*. Corticosteroids have also been used to shorten the course and severity of both granulomatous and lymphocytic thyroiditis *(156,157)*. Analgesia may be the principal concern in subacute granulomatous thyroiditis *(159)* and radiation thyroiditis. The author prefers to use nonsteroidal antiinflammatory agents for analgesia in subacute thyroiditis, reserving the use of corticosteroids for patients with refractory pain. Most patients become transiently hypothyroid when the thyrotoxicosis resolves, and are treated with levothyroxine for 3–6 mo.

Ectopic Thyrotoxicosis

Ectopic thyrotoxicosis results from the factitious ingestion of TH, from struma ovarii, or from large metastatic deposits of functioning differentiated thyroid cancer. Thyrotoxicosis from an acute levothyroxine overdose can be treated with charcoal-containing gastric lavage and bile-acid sequestrants (such as cholestyramine), which interfere with the absorption of TH *(160)*. Symptoms can be ameliorated with β-adrenergic-blocking agents, iodinated radiocontrast agents that will block the conversion of T_4 to T_3 and, in severe cases, plasmapheresis or dialysis *(119,161)*.

Struma ovarii is the presence of hyperfunctioning thyroid tissue in an ovarian neoplasm. Treatment consists of ovarian surgery. Patients with symptomatic hyperthyroidism can be prepared for surgery through one of the several approaches noted previously for surgical correction of thyroid overactivity.

Large, bony metastases from follicular thyroid cancer rarely cause symptomatic hyperthyroidism. Treatment may require a variety of approaches, including thionamides, RAI, surgery, external radiotherapy, or chemotherapy.

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10 Resistance to Thyroid Hormone

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Contents

HISTORY

Fuller Albright introduced the concept of hormone resistance in the 1940s *(1,2)*. In the ensuing half century, multiple hormone-resistance syndromes were described, including resistance to thyroid hormone (RTH) by Refetoff, DeWind, and DeGroot in 1967 *(3)*. Their patient was a 6-yr-old girl evaluated after a car accident and found to have signs of hypothyroidism (stippled epiphyses, deaf-mutism, delayed bone age, and goiter) in the presence of an increased protein bound iodine (PBI, then used as a surrogate for total T_4) concentration. Subsequent investigation found that the patient had normal thyroid hormones (TH) with a normal hormone secretion rate, tissue distribution, fractional turnover, and transport proteins.

Advances in clinical chemistry allowed the syndrome of RTH to be suspected with the presence of increased peripheral TH levels (triodothyronine [T_3] and/or thyroxine [T_4]) in individuals with inappropriately normal or increased serum thyroid-stimulating hormone (thyrotropin; TSH) levels. Early reports described patients who were clinically either euthyroid or hypothyroid *(4–8)*.

In 1975, Gershengorn and Weintraub *(9)* described a girl with laboratory tests for RTH who was clinically thyrotoxic (increased ^{131}I uptake, increased basal metabolic rate, and shortened pulse-wave arrival time). They classified her condition as "selective pituitary" resistance to thyroid hormone (PRTH). Later studies demonstrated a prominent hypothalamic role in the thyrotoxic syndrome *(10)*, and the more general name of central resistance to thyroid hormone (CRTH) was proposed. In addition, Kaplan, Swartz, and Larsen described a patient with peripheral resistance to TH despite normal central sensitivity *(11)*.

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Early investigations demonstrated that patients with RTH had stereochemically normal circulating T_4 and T_3 *(4,12,13)* that underwent normal metabolism *(14,15)*, and that entered peripheral tissues normally *(12,16)*. Thus, in 1972, Refetoff et al. postulated that the defect responsible for RTH must be at the cellular level, at the site of the then-unidentified thyroid hormone receptor (TR) *(12)*.

In 1986, both Sap et al. *(17)* and Weinberger et al. *(18)* reported T_3 binding by c-*erb*A, the cellular homolog of the viral oncogene v-*erb*A. Thus, TR was identified and classified as a member of the nuclear receptor superfamily *(19)*.

EPIDEMIOLOGY

RTH is a rare disorder; fewer than 300 affected families have been reported to date *(20)*. Over the past 15 yr the number of reported cases has increased dramatically as the result of both increased awareness of the disorder and use of more sensitive TSH assays. The prevalence of RTH is unknown; evaluation of serum samples from neonates screened for congenital hypothyroidism failed to clarify this issue *(21)*.

The primary phenotype of RTH has been generalized resistance to thyroid hormone (GRTH), with few patients evaluated in detail for peripheral sensitivity *(22)*. Many patients classified as having PRTH (CRTH) in previous reviews were so assigned on the basis of subjective symptoms and/or resting heart rate. Cardiac sensitivity to TH appears to be predominantly mediated by the TRα isoform *(23–27)*, in which no RTH mutations have been identified. Thus, cardiac sensitivity to TH may not be suitable for distinguishing RTH phenotypes from one another, in that it may be preserved in both forms of RTH. The number of patients with CRTH therefore remains unknown.

Most RTH is familial. Sporadic cases account for 16% of all cases *(20)*. The disease is congenital and the inheritance is autosomal dominant, with the exception of the first reported family, in which transmission was recessive *(28)*. RTH is found with equal frequency in both sexes and is not known to cluster in particular ethnic groups.

CLINICAL MANIFESTATIONS

No pathognomonic signs or symptoms mark RTH. Individuals with the disorder require greater TH (T_3) concentrations to achieve T_3-dependent effects. Clinically, RTH can be divided into the two entities of GRTH and CRTH *(9,10,22,28–32)*. In both syndromes there is TH resistance at the level of the pituitary and hypothalamus, causing inappropriate TSH secretion and, in turn, increased TH levels *(28)*. In GRTH there is also peripheral resistance, often resulting in some evidence of tissue hypothyroidism in patients. In CRTH, however, peripheral sensitivity to TH is preserved and the individual suffers thyrotoxic symptomatology from the increased levels of circulating T_3 *(22,32)*.

Historically, the presenting sign of RTH has been goiter. Thyroid-gland enlargement is presumed to result from TSH stimulation. Because the majority of RTH patients have normal TSH levels, the goiter may be explained by biologically overactive TSH produced under the influence of excess thyrotropin-releasing hormone (TRH) released from the TH-resistant hypothalamus *(10,33)*. Goiter size in paternally inherited RTH is larger than that in maternally inherited RTH *(29)*. It has been suggested that maternal hyperthyroxinemia may protect the fetus with RTH through greater transplacental passage of TH during embryogenesis and fetal development *(34)*.

Palpitations and tachycardia are widely reported in RTH *(28)*. In early reports, it was believed that cardiac signs might flag the tissue sensitivity of isolated CRTH *(35,36)*. Current

evidence suggests that TH action on the myocardium is predominantly mediated by the TRα isoform, which is not mutated in RTH *(23–27)*. Thus, retained cardiac sensitivity to TH can be characteristic of both phenotypes of RTH.

Previously, RTH patients with cardiac hyperactivity and high serum TH levels were misdiagnosed as having Graves' disease (GD) *(37–41)*. Current TSH assays should obviate confusion because the biochemical hallmark of RTH is inappropriately nonsuppressed TSH in the presence of increased peripheral TH levels.

Approximately half of patients diagnosed with RTH are also diagnosed with attention-deficit–hyperactivity disorder (ADHD; *42*). ADHD and RTH are not genetically linked *(43–45)*, and screening ADHD patients for RTH has not proved productive. Rather, the ADHD of RTH seems related to relative brain hypothyroidism. Thus, RTH patients are observed to have a high incidence of learning disability *(28)* and speech impediment. Exogenous TH does not treat ADHD in general, but alleviates similar symptoms in patients with RTH *(42)*.

The only reported case of RTH-associated deaf-mutism was linked with a homozygous deletion of the TRβ gene *(3,46)*. Functional cochlear dysfunction is reported in mice bred without the TRβ gene *(47)*. TRα- and TRβ-2-knockout mice have normal hearing *(47)*.

Growth retardation and delayed bone age are common in RTH patients, being reported in 19–29% *(29)*. Recent TR-gene-knockout studies confirm a role for the TR gene in skeletal growth *(48–50)*.

There are reports that RTH patients suffer more frequent respiratory infections than their unaffected relatives *(29)*, and that subjects with RTH seem to have reduced immunoglobulin concentrations *(29)*. TR has been demonstrated in lymphocytes *(51)*, and TRα-knockout mice exhibit defective lymphocyte growth *(52)*.

LABORATORY MANIFESTATIONS

RTH manifests biochemically with a nonsuppressed serum level of TSH despite increased peripheral TH levels (T_4 and/or T_3). Eighty-five percent of RTH patients never treated with thyroidectomy or radioiodine (RAI) have normal serum TSH levels *(28,29)*. In addition to having increased peripheral TH levels, RTH patients have elevated levels of thyroglobulin and reverse T_3. There is no difference in TH chemistry between patients with GRTH and those with CRTH.

TSH from RTH patients is reported to have enhanced biologic activity *(33)*, which is presumed to result from TRH-mediated modifications in the oligosaccharide structure of TSH *(33,53,54)*. Almost all patients with RTH have normal levels of circulating common α-glycoprotein subunit and normal α-subunit to TSH molar ratios *(55)*.

Several stimulatory and inhibitory tests have been used to evaluate TSH secretory dynamics in RTH patients. Refetoff, et al. developed a T_3-suppression protocol to separate GRTH individuals from normals *(28,56)*. A modification of that protocol has been used to identify CRTH patients *(22)*. The T_3-suppression protocol involves administration of escalating daily doses of T_3 over 3 or 4 d and evaluation of parameters of TH action (Table 1). The administered T_3 doses are 25 μg twice daily, 50 μg twice daily, and 100 μg twice daily.

RTH patients have been noted to have either normal or hypothyroid TSH responses to TRH stimulation *(56)*. Normal individuals respond to low-dose T_3 (50 μg/d) with suppression of TRH-stimulated TSH to a level more than 75% below the baseline TRH-stimulated TSH value *(56)*. Individuals with RTH fail to suppress stimulated TSH to the same degree with the same dose. When necessary, RTH patients can be separated from hypothyroid

Table 1
Clinical Indices of Increased Exposure to Peripheral TH Action

Indices that increase	*Indices that fall*
Serum ferritin	Serum cholesterol
Serum SHBG	Serum creatine kinase
Serum transaminases	Weight
Basal metabolic rate	Deep-tendon-reflex relaxation phase

SHBG, sex-hormone-binding globulin.

patients by their prolactin responses to TRH stimulation. TRH-stimulated prolactin levels are significantly greater in hypothyroid patients than in RTH patients.

The measurement of several parameters of peripheral TH action has been proposed to quantify the degree of peripheral RTH *(57–60)*. In isolation, these parameters have not proved useful in discriminating relative tissue sensitivity to TH. However, good discrimination among normal patients, GRTH patients, and CRTH patients has been achieved with evaluation of the parameters following administration of T_3, as noted above (*see* Table 1; *22,28,61*). The response of the various indices to T_3 administration is significantly attenuated in GRTH patients.

DIFFERENTIAL DIAGNOSIS

The differential diagnosis of inappropriate TSH secretion includes TSH-secreting adenoma and RTH. Although the incidence of pituitary hyperplasia and/or adenoma formation in individuals with RTH is unknown, pituitary hyperplasia in response to longstanding hypothyroidism is well documented *(62,63)*. Whether the thyrotroph in RTH is capable of hyperplasia analogous to that seen in the corticotroph following adrenalectomy remains unclear. Gurnell et al. *(64)* reported an RTH patient with pituitary hyperplasia, which resolved with TH treatment.

Because the diagnosis of TSH-secreting tumors is often delayed, curative surgical resection remains under 50%. Early diagnosis is therefore paramount; however, the morbidity of pituitary surgery mitigates against its unnecessary use in patients with pituitary "incidentalomas." Thus, a TSH-secreting tumor must be confidently separated from RTH-associated adenoma or pituitary "incidentaloma."

It is clearly important to determine TSH, T_4, and T_3 values at steady state; measurements made in patients after recent dose changes or in cases of medication noncompliance may result in spurious values. Antibodies to TSH and to TH may produce artificial hormone concentration values and must be excluded. The evaluation then includes three standard steps (Fig. 1): (1) serum TSH α-glycoprotein-subunit measurement; (2) TRH-stimulated TSH measurement; and (3) a magnetic resonance imaging (MRI) scan of the pituitary. No one test is diagnostic of either RTH or adenoma, and all tests are therefore usually performed.

It has been observed that the concentration of α-glycoprotein subunit is often increased with a TSH-secreting adenoma owing to cosecretion of free α-subunit *(65)*. If the concentration of α-glycoprotein subunit is not elevated in absolute terms, it is at least so in molar ratio relative to the TSH level *(65)*. In RTH, by contrast, both TSH-subunit genes exhibit similar defects in regulation, and the α-glycoprotein subunit level is not reported to be increased.

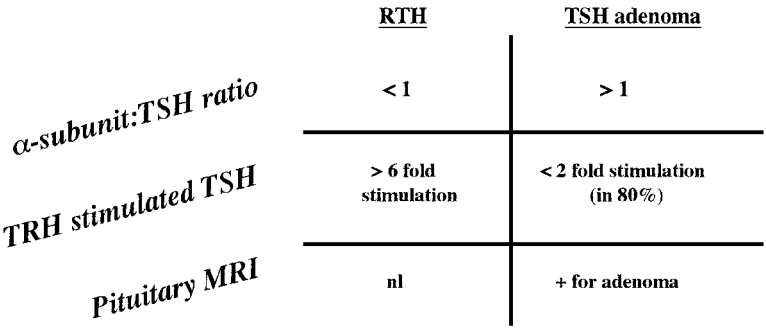

Fig. 1. Classic testing to distinguish RTH from TSH-secreting adenoma. nl, normal.

Because a TSH-secreting adenoma functions autonomously, stimulation by TRH would not be expected to alter the rate of TSH secretion. In fact, stimulation by TRH fails to cause more than a twofold rise in TSH secretion in 80% of TSH-secreting tumors *(66)*. However, 20% of patients with TSH-secreting tumors have normal TSH secretion in response to TRH stimulation. In RTH, TRH-stimulated TSH testing is often normal *(56)*; and in some RTH cases, the diminished ability of circulating TH to provide negative feedback to the pituitary may result in the exuberant TSH response seen in hypothyroidism.

MRI of the pituitary would seem an attractive test for separating the two entities of inappropriate TSH secretion. Most TSH-secreting adenomas are reported to be large and easily detected by MRI, while an RTH patient should have a normal pituitary MRI. Unfortunately, imaging is not always definitive *(67)*. At the minimum, the RTH associated incidence of nonfunctioning pituitary adenomas should equal that of the normal population. However, if RTH is associated with thyrotroph hyperplasia and this predisposed to adenoma formation, the incidence of RTH-associated pituitary tumors may prove to be even greater.

Other maneuvers used in distinguishing the two entities of inappropriate TSH secretion include evaluation of first-degree relatives of patients (TSH-secreting adenomas are not familial), attempted identification of a TRβ-gene mutation (not commercially available), and measurement of serum markers of peripheral TH action (in response to a T_3-suppression trial as outlined earlier under "Laboratory Manifestations").

MOLECULAR ASPECTS

Introduction

RTH results from mutations in the carboxyl terminus of the TH β-receptor (TRβ) *(28,68–70)*. RTH is a dominant disorder (except in one patient), in which affected individuals are heterozygous for the mutant allele. In a phenomenon called "dominant negative activity," the mutant allele interferes with the activity of the normal allele *(71–74)*. The many known RTH mutations cluster in three "hot spots" in the TRβ gene locus *(35,75–77)*.

The identification of the TR by Sap et al. and Weinberger et al. in 1986 *(17,18)* proved pivotal for studying the molecular mechanisms of RTH. Since then, research into various RTH mutations has provided an understanding of the important functional domains of the TR.

Humans have two known TR genes, TRα and TRβ, which are located on chromosomes 17 and 3, respectively (*see* Chapter 1 for more detailed discussion). These two genes have substantial structural similarities, and both generate multiple isoforms of TR by alternative splicing. These isoforms are TRα-1 and TRα-2, encoded by the TRα gene, TRβ-1 and TRβ-2 encoded by the TRβ gene. TRα-2 does not bind TH and is often called c-erbAα-2. The role

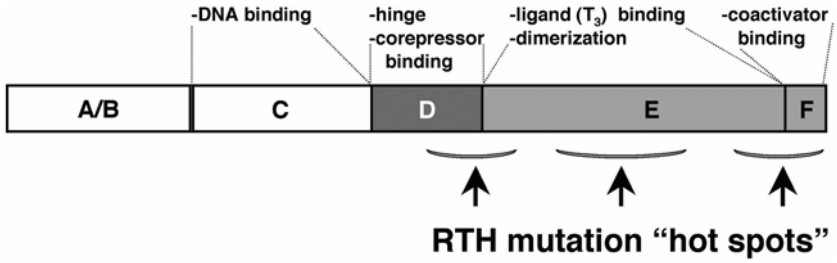

Fig. 2. Locations of RTH-mutation hot spots on a linear map of the TR.

of this protein is not clear, but it has been suggested that c-erbAα-2 may act as a negative modulator by inhibiting the action of both the TRα-1 and TRβ-1 isoforms *(78)*.

The relative expression of the two TR genes, and the distribution of their products, vary among tissues and during development. In addition, T_3 differentially regulates the expression of the two TR genes and their isoforms. TRα is widely expressed in early development, with predominant distribution in brain, cardiac muscle, skeletal muscle, kidney, liver, intestine, and brown fat. The direction of TRα expression in response to TH has been debated *(79–84)*; at present there is no consensus. The TRβ isoforms are induced later in embryogenesis. TRβ-1 is also expressed in the tissues named above. Investigators report either increased TRβ-1 expression or no change in expression with exposure to T_3 *(79,80,82–85)*. TRβ-2 is expressed primarily in the pituitary and hypothalamus, and the mRNA encoding this isoformof the receptor is downregulated by T_3 *(79)*.

In 1988, Usala et al. used restriction-fragment-length polymorphism (RFLP) analysis to demonstrate tight linkage between RTH and the TRβ locus on chromosome 3 *(68)*. Subsequently, point mutations in the TRβ gene were identified in two unrelated families *(69,70)*. Approximately 109 different mutations in the TRβ gene have since been identified in RTH patients belonging to more than 250 families *(20)*. To date, no mutations have been detected in the TRα gene. In one of the largest collections of RTH patients analyzed, 18 members of 97 families had no TRβ mutation *(20)*. Mutations in the genes for known TR cofactors have not been identified.

The TRβ gene mutations associated with RTH lie within the domain that encodes ligand-binding of the receptor region. The mutations cluster in three hot-spot regions (Fig. 2), with boundaries extending from codons 234–282, 309–383, and 429–460. Most RTH-associated mutations involve a single nucleotide substitution and result in missense mutations. In several families with RTH, nucleotide deletions or insertions result in loss of entire codons or a shift in the reading frame for the receptor *(35,75,86,87)*.

Approximately 43% of reported families with identified TRβ mutations have CpG mutations *(20)*. CpG dinucleotides are sites of point mutation resulting from the deamination of 5′-methylcytosine to thymine, which converts CG sequences to TG or CA sequences *(88)*.

Patients with RTH are usually heterozygous for mutations in the TRβ gene, in accord with the dominant pattern of inheritance of the disease. A single individual with both TRβ alleles mutated exhibited the most severe case of RTH so far reported *(86,89)*. In one kindred, RTH resulted from complete deletion of the TRβ gene *(3,46)*. The identified individual was homozygous for the complete deletion of both TRβ alleles, whereas heterozygous family members were normal.

Although there are reports of phenotypic heterogeneity for RTH among single kindreds, few families with putative CRTH have been exhaustively evaluated. Isolated central resistance

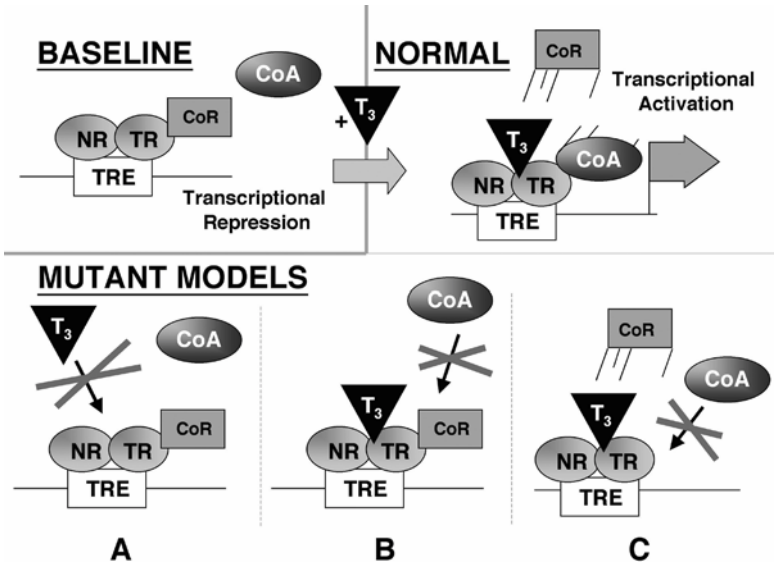

Fig. 3. Models of RTH for genes positively regulated by thyroid hormone. **(A)** Decreased T_3 binding. **(B)** Increased corepressor avidity and/or decreased corepressor dissociation. **(C)** Defective coactivator recruitment. TR, thyroid hormone receptor; NR, nuclear receptor that dimerizes with TR (usually RXR or TR); T_3, triiodothyronine; CoA, coactivator(s); CoR, corepressor(s).

to TH with preserved peripheral TH sensitivity has been documented in association with the mutations R338L, R338W, R429Q, and R383H *(22,35,36,90–92)*.

*Functional Studies of Mutant TR*β

As predicted by the localization of RTH-associated mutations in the ligand-binding domain of the TRβ gene, most mutant TRs have impaired T_3 binding (Fig. 3) *(28,35, 36,76,89,90,93–103)*. However, several RTH-associated mutations are associated with normal or near normal ligand binding, including A234T, R243Q, R243W, T277A, R338L, R338W, R429Q, R383H, and L454V *(91,104–109)*.

Using in vitro systems, TH-mediated gene transcription is impaired in the presence of mutant TRs at low levels of T_3 *(100,110)*. For most TR mutations, however, higher T_3 concentrations result in return to function at the wild-type level.

The R338L, R338W, and R429Q mutants block TH action on the centrally expressed TRβ-2 isoform, but fail to block TH action on the ubiquitously expressed TRβ-1 isoform *(91,105)*. The R383H mutation fails to block normal TH activity on stimulatory TH-response elements (TREs) found in peripheral tissues, but blocks TH action on inhibitory TREs encoded in TRH- and TSH-subunit genes *(107)*. Thus, some RTH mutations selectively interfere with the action of TH on central tissues.

A number of RTH-associated mutants are observed to bind corepressor proteins more avidly than does the wild-type TR gene (Fig. 3, *107,109,111–114*). Independent of measured T_3 binding, the A234T, R243Q, R243W, V264D, R383H, R429Q, delta 430, delta 432, and L454S mutations require greater T_3 concentrations to dissociate corepressor proteins before coactivator recruitment and gene transcription. Other RTH-associated mutants are observed to bind T_3 and corepressor normally, but recruit coactivators defectively *(104,115)*.

*The Dominant-Negative Effects of Mutant TR*β

Most patients with RTH are heterozygous for TRβ, with the mutant receptor apparently interfering with the normal receptor, a phenomenon referred to as dominant negative activity, as noted earlier. Subjects with one inactivated TRβ gene and one wild-type gene are phenotypically normal. Transfection studies designed to reconstitute the situation with normal and mutant receptors present in the same cell *(110,116)* indicate that the function of a normal receptor is markedly inhibited by the mutant receptor. In order to exert their dominant negative effects, mutant receptors must retain normal dimerization and DNA-binding properties *(28,100,110,116,117)*, although at least one exception to this has been reported *(118)*. Mutations lacking dominant negative activity may elude detection because they are clinically and biochemically silent *(119–121)*.

The ability to exert dominant negative effects within the hypothalamic–pituitary–thyroid axis is an important property of mutant TRβ proteins, and generates the characteristic biochemical and clinical features that result in detection of RTH *(100,120)*. Against this background, the variable clinical phenotype in RTH may be due to variable degrees of peripheral resistance in different patients, as well as variable resistance in different tissues within a single individual.

Differing tissue distributions of receptor isoforms may play a role in the variable phenotype of patients with RTH. The liver and pituitary predominantly express TRβ receptors *(78)*. TRα is the major isoform detected in myocardium *(122)*. Mutations in the TRβ gene are likely to be associated with hepatic resistance to TH *(123)* and nonsuppressed TSH levels, whereas the tachycardia seen in RTH may represent retention of cardiac sensitivity to TH action mediated by a normal α receptor.

Relative expression of mutant versus wild-type receptors may play a role in the degree of tissue resistance. Although one study suggested that both alleles of TRβ are equally expressed *(124)*, another study showed marked differences in the relative levels of wild-type and mutant receptor mRNA in skin fibroblasts from two RTH cases *(125)*. In one individual, temporal variation in expression of the mutant allele correlated with the degree of TH resistance in bone.

Factors unrelated to TRβ mutation may also affect the clinical and biochemical phenotype of RTH. For example, an arginine-to-histidine mutation at codon 316 (R316H) was associated with normal thyroid function in one kindred *(99)*, but in an unrelated family was associated with abnormal thyroid function *(35)*.

Putative Roles of TR Isoforms from TR Knockout Mouse Studies

The differential expression of TR genes suggests that they mediate distinct functions. The extent to which the individual genes provide unique or overlapping functions has been a matter of debate. To analyze the in vivo function of the specific TR isoforms, several groups have generated TR knockout mice. Data indicate roles for the TR isoforms that they often, but not always, fulfill for each other (Table 2).

Centrally, TH action is mediated by both TRα and TRβ, although TRβ seems to play a larger role. TRβ knockout mice have increased concentrations of TSH, delayed TSH responses to TH suppression, and increased peripheral levels of TH *(49,126,127)*, while TRα-knockout mice have increased TSH responses to TRH stimulation and increased T_4 levels *(27)*. TRα/TRβ double-knockout mice have significantly greater TSH levels than do isolated TRβ knockout mice *(49)*.

Table 2
Putative Functionally Predominant TR Isoforms in Specific Tissues[a]

Predominantly TRα	*Predominantly TRβ*	*Substantial TRα and TRβ*	*Predominantly TRβ2*
Heart	Liver	Skeletal muscle	Hypothalamus
Bone growth	Cochlea	Pituitary	Retina
Intestine			
Body-temperature regulation			

[a]Data from TR-knockout-mouse studies.

In the hypothalamus, the role of TRβ in mediating the action of TH predominantly involves the TRβ-2 isoform. Isolated TRβ-2-knockout mice have virtually the same central phenotype as complete TRβ-null mice *(127,128)*.

The action of TH on the heart is mediated primarily through TRα. Thus, TRα-knockout mice are observed to have decreased myocardial activity, including decreased heart rates, decreased mRNA expression for certain myocardial-enzyme genes, delayed myocardial contraction times, and delayed myocardial relaxation times *(23,25–27)*. Although heart rates are reported to rise in TRβ-knockout mice *(24)*, other parameters do not change *(25,27)*. TRα/TRβ double-knockout animals have the TRα-knockout phenotype *(24)*.

The action of TH on intestinal development, bone growth, and body temperature are also predominantly TRα mediated. TRα-knockout mice have decreased body temperature, decreased linear bone growth, decreased intestinal epithelial growth, decreased intestinal mucosal thickness, decreased expression of certain digestive-enzyme genes, and decreased intestinal response to T_3 administration *(23,48–50,129–131)*. In contrast, TRβ-knockout mice suffer none of the above defects. TRβ can partly substitute for TRα for some of the foregoing functions, as is demonstrated in TRα/TRβ double-knockout mice, which have more severe phenotypes of RTH than do isolated TRα-knockout animals with regard to linear growth and intestinal development *(24,49,50,129)*.

Skeletal-muscle response to TH seems to be mediated similarly by both TRα and TRβ. Mice with either isoform or both isoforms knocked out have decreased expression of the "fast twitch" major histocompatibility complex (MHC) II gene and increased expression of the "slow twitch" MHC I gene *(132)*.

Hepatic response to TH is reported to be accentuated in TRα-knockout mice *(27,133)* and diminished in TRβ-knockout mice, suggesting that the liver is primarily a site of TRβ-mediated TH activity.

TR-β serves the primary role of mediating TH action in the cochlea and the retina. TRβ-knockout mice have decreased auditory evoked potentials, cochlear function, and susceptibility to audiogenic seizure *(47,130,134)*. TRα-knockout mice have no apparent cochlear defects *(47)*. TRα/TRβ double-knockout mice have decreased hearing *(50)*. TRβ-2-knockout mice have decreased numbers of M-cones in the retina and increased numbers of S-opsin cones *(135)*.

Transgenic Animal Models and RTH

From mouse TR-gene-knockout studies alone, it might be expected that RTH would strike TRβ-mediated tissues more profoundly and leave TRα-mediated tissues more sensitive to TH. Although this is partly true, RTH phenotypes are made more complex by the impact of

RTH-associated mutations on relationships with transcription cofactors and gene-promoter regions *(136)*. In vitro studies also suggest that TRβ mutations may exert dominant negative activity on the TRα isoform in some circumstances *(137)*.

Several investigative groups have overexpressed RTH-associated mutant DNA in transgenic mice. As with natural RTH, mutant TRs in transgenic mice exert dominant negative effects on targeted tissues. Thus, transgenic mice generated with the G345R TRβ mutation targeted to their pituitary glands have decreased cholesterol levels, in accord with increased TH levels *(138)*.

Mice generated with the 337-deletion mutation directed to their pituitary glands have increased levels of TSH, decreased TRH mRNA expression, and an increased T_4 response to TRH stimulation *(10)*. When the 337-deletion mutation is directed to myocardium, the resulting transgenic mice have alterations in the expression of several myocardial genes *(139)*. More global expression of the 337-deletion mutation results in brain development defects including decreased cerebellar development and decreased expression of certain hippocampal genes *(140)*.

Transgenic mice generated with the PV mutation have a number of RTH features including decreased weight and increased T_4 levels *(141–144)*. When the PV mutation is placed in a TRα isoform, transgenic mice have diminished brain glucose utilization and Srg 1 gene expression *(145)*. In the TRβ isoform, the PV mutant transgenic mice do not differ from wild-type mice in this regard.

Correlation Between Molecular and Clinical Defects

Attempts to correlate phenotypes of RTH with specific RTH-associated mutations have been limited by small study sizes and difficulties in rigorous clinical assessment of TH action. One of two larger series includes data from 124 subjects with 18 mutations *(146)*. Good correlation was observed between thyrotroph resistance to T_3 and two in vitro findings: (1) T_3 binding impairment, and (2) dominant negative activity. In the second series, data from 104 patients from 42 kindreds were compared with those for their 114 unaffected relatives *(29)*. The study reported good correlation between specific mutations and the following characteristics: goiter, attention-deficit–hyperactivity disorder, low intelligence quotient, speech impediment, short stature, upper respiratory infection rate, low body weight, hearing loss, and cardiac abnormality. A higher incidence of speech problems has been reported in patients with mutations in the second RTH hot spot relative to those with mutations in the third hot spot *(29,147)*.

Two molecular explanations for isolated CRTH have been reported. In one study, the CRTH-associated R338L, R338W, and R429Q mutations were found to have strong dominant negative activity as TRβ-2 mutations, while failing to have significant dominant negative activity as TRβ-1 mutations *(105)*. As already noted, knockout-mouse studies confirm that central action of TH is predominantly mediated by TRβ-2 *(127,128)*. In another study, the R383H mutation was found to block TH action on the TSH and TRH gene promoters while having little effect on promoters of peripherally expressed genes *(107)*. Thus, some RTH-associated mutations may exert their effects selectively in the hypothalamus and pituitary.

The observation of variable serum T_4 levels among individuals with identical TRβ mutations makes clear that additional factors contribute to the phenotype of RTH *(74,148,149)*. To date, none of these factors have been identified.

TREATMENT OF RTH

The management of RTH differs from that of other thyroid disease. Most patients with RTH are clinically euthyroid because the peripheral receptor defect is overcome by high circulating TH levels mediated by the parallel central receptor defect. Therapeutic maneuvers to reduce elevated serum TH levels would precipitate some relative tissue hypothyroidism and could cause irreversible damage in early life.

In the past, one third of patients with RTH either underwent ablative therapy or received antithyroid drugs *(28,31,120)* and developed overt hypothyroidism. Greater use of reliable TSH assays in general medical practice should minimize such erroneous treatment in the future.

Treatment with TH may be appropriate in patients for whom the increase in endogenous TH is insufficient. This may be especially true in infants with GRTH *(150)*. Although general criteria for treatment of patients with GRTH are lacking, young children with growth and/or mental retardation may benefit from TH treatment to overcome the resistance in some tissues *(150)*. As mentioned earlier, exogenous TH does not treat ADHD in general, but may alleviate ADHD-like symptoms in RTH patients *(42)*. Such therapy needs careful monitoring, including attention to TSH levels and indices of peripheral TH action.

No treatment superior to oral levothyroxine has been demonstrated. If tachycardia is present, cardioselective β-blockers, such as atenolol, may be indicated. There is one report of an unsuccessful attempt to minimize refractoriness to TH through chronic administration of high-dose vitamin A *(151)*.

Although the diagnosis of RTH has been reported *in utero (152)* and at birth *(21,153)*, the indications for treatment of fetuses and newborns are not clear.

A reduction in TH levels may benefit patients with CRTH. Unfortunately, reducing serum TH levels with antithyroid drugs may result in dramatically increased TSH levels and possibly in pituitary hyperplasia *(64,67)*. Thus, the only consensus treatment is to relieve symptoms through beta-blockade.

For CRTH, the literature reports failed treatment trials with a number of agents, including corticosteroids, bromocriptine, octreotide, and triiodothyronine *(154–159)*. Mixed results have been reported with trials of triiodothyroacetic acid (TRIAC) and dextrothyroxine *(157,160–168)*. Investigators reporting successful use of TRIAC suggest that it may have greater action on centrally expressed TR isoforms than TR isoforms expressed peripherally.

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11 Evaluation and Management of the Euthyroid Nodular and Diffuse Goiter

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CONTENTS

INTRODUCTION

The Solitary Thyroid Nodule

Palpable thyroid nodules among adult individuals living in the United States are very common, with a prevalence of 4–7% *(1)*. By contrast, the prevalence of nodules incidentally detected at autopsy, or by the use of high-resolution ultrasonography, has ranged from 20–65% in individuals without a prior history of thyroid disease *(2–8)*. Thyroid nodules are more common in women, estimates varying from a female-to-male ratio of 1.2:1 *(2,9)* to 4.3:1 *(10)*, and increase in prevalence with advancing age *(11,12)*. The likelihood of a single palpable thyroid nodule being malignant is less than 10% and possibly closer to 5% *(1,12,13)*. Nevertheless, because of the possibility of cancer, some clinicians, especially those in the surgical subspecialties, recommend that all nodules be removed. Other physicians, especially endocrinologists, recommend a more conservative approach in order to avoid unnecessary surgery. Thus, there is an ongoing debate regarding the appropriate evaluation and management of individuals with thyroid nodules. The main purpose of this chapter is to address these issues and to provide a clinically appropriate and cost-effective approach to the evaluation and management of nodular thyroid disease. There are also several excellent reviews, to which the reader is directed *(1,11,14–17)*.

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CLINICAL EVALUATION

History

The history is neither sensitive nor specific in detecting thyroid malignancy, but may provide some clues about risk factors suggestive of malignancy. A history of recent thyroid growth, pressure symptoms, difficulty in swallowing, and the development of hoarseness may suggest malignancy. On the other hand, nodules that have been present for many years may be malignant, and recent thyroid growth may simply result from fluid accumulation in a benign cyst *(1,11)*. Hoarseness is present in only 50% of patients with thyroid malignancy, whereas it has been reported that 17–50% of individuals who complain of hoarseness have benign thyroid disease (18–20). Most patients with thyroid malignancy do not have symptoms.

A history of childhood or adolescent irradiation to the head and neck area for conditions such as thymic or tonsillar enlargement, or facial acne, significantly increases the likelihood of cancer in a nodule, with well-differentiated thyroid cancer (90% papillary) being present in one-third of individuals with palpable nodules *(21,22)*. The patient with a nodule who has a family history of medullary thyroid cancer also has a greater likelihood of harboring a thyroid malignancy *(23)*. Familial nonmedullary thyroid cancer has also been reported, with 90% being papillary cancer *(24)*.

Another factor to be considered is that a thyroid nodule in a child is perhaps twice as likely to be malignant as a nodule in an adult *(25–27)*. Also, a single nodule in an individual over 60 yr old, especially a male, is much more likely to be cancerous than a nodule in a female patient of younger age *(13,28)*.

Sudden onset of neck pain suggests a benign process, usually hemorrhage into a benign cyst or a degenerating adenoma, or subacute (granulomatous) thyroiditis *(29)*. Hemorrhage causing pain may occur in thyroid cancer, although it is uncommon. A family history of benign nodular goiter increases the likelihood of benignity. Symptoms of hyperthyroidism suggest a benign, autonomously functioning thyroid adenoma, and symptoms of hypothyroidism would suggest chronic autoimmune (Hashimoto's) thyroiditis *(30)*.

Physical Examination

Thyroid cancer is usually firm to hard, although benign lesions, especially calcified adenomas, may also be firm or hard. A smooth, mobile nodule suggests benignity, as does the presence of tenderness. A hard, irregular, nontender nodule is suggestive of malignancy, especially papillary cancer, although Hashimoto's thyroiditis (HT) may also be hard and irregular. Regional lymphadenopathy, especially in the cervical or supraclavicular areas, suggests papillary thyroid cancer *(31,32)*. Phenotypic features of multiple endocrine neoplasia-type 2 syndrome suggest a diagnosis of medullary thyroid cancer *(33)*.

Although some studies indicate that the prevalence of thyroid cancer is similar in multinodular and uninodular glands *(13,34,35)*, others have reported a lower prevalence of cancer in multinodular thyroid glands (18,36). Clinically, a multinodular gland in which the nodules all have the same consistency on palpation is likely benign. A nodule in which recent growth has been demonstrated or that has a firmer or more irregular consistency than other nodules in the gland may be considered more suspicious for malignancy. Table 1 1ists risk factors for malignancy in thyroid nodules.

Table 1
Thyroid-Nodule Risk Factors Associated with Malignancy

History
Age <20 or >60 yr
History of irradiation to the neck or face
Male sex
Family history of medullary thyroid cancer
Growth of nodule during observation
Hoarseness
Physical-examination findings
Firm to hard, nontender nodule
Regional lymphadenopathy
Fixation to adjacent tissue
Vocal-cord paralysis

Adapted from Singer PA. Evaluation and management of the solitary thyroid nodule. Otolaryngol Clin North Am 1996;29:577–592.

Laboratory Evaluation

Blood Tests

The majority of patients with thyroid nodules are euthyroid. Exceptions may occur with single, autonomously functioning thyroid adenomas or toxic multinodular goiter, in which hyperthyroidism may be present, or when a nodule occurs as a result of HT, in which hypothyroidism may exist *(37)*. Obtaining a serum thyroid-stimulating hormone (TSH) measurement in an assay sensitive enough to differentiate between hyperthyroidism and euthyroidism is helpful, since a suppressed TSH, with or without an elevated free thyroxine (T_4) or triiodothyronine (T_3) level, is suggestive of a benign autonomous nodule, and an elevated TSH suggests underlying HT (but would not necessarily exclude cancer). Measurement of antithyroid antibodies is extremely useful in the diagnosis of HT, but their presence would not necessarily exclude malignancy *(38)*.

There is little or no utility in initially obtaining a serum thyroglobulin (Tg) measurement in the evaluation of nodular disease, since it is usually elevated in both benign thyroid disorders and well-differentiated thyroid cancer. It is an important adjunct, however, in the follow-up management of patients with well-differentiated thyroid cancer *(39,40)*.

Serum calcitonin is a marker for diagnosis and follow up of patients with medullary thyroid cancer *(41)*. Because of the relative infrequency of medullary thyroid cancer, however (5–7% of all thyroid cancers), a serum calcitonin measurement probably should not be obtained in the patient with a thyroid nodule unless there is a family history of medullary thyroid cancer or multiple endocrine neoplasia syndrome-type 2. Several groups have suggested routine calcitonin measurements in patients with nodular goiter *(42,43–46)*. The economic cost of routine calcitonin screening seems prohibitive *(43,47)*. Nevertheless, the likelihood of diagnosis of medullary thyroid cancer with routine calcitonin measurement varies between 0.5% and 1.4% *(44,46)*.

Imaging Procedures

The use of imaging studies in evaluating patients with solitary palpable thyroid nodules is perhaps the most contentious issue in the workup of such individuals. Many if not most of the patients referred to endocrinologists for consultation have already undergone radionuclide scans and/or thyroid ultrasound examinations. Usually such studies are unnecessary, and not only do they add expense to the evaluation, but they usually provide little additional information in establishing a correct diagnosis *(48,49)*.

Radionuclide Scans. For many years, the radioiodine (RAI) scan was the mainstay in evaluation of the thyroid nodule, with a cold thyroid nodule being considered suspicious for carcinoma *(50)*. It must be pointed out, however, that most thyroid nodules are cold, including cysts, colloid nodules, benign follicular lesions, hyperplastic nodules, and HT. The overall prevalence of carcinoma in cold thyroid nodules is reported to vary from 5–16% *(13,51–53)*. As mentioned, when there is a history of childhood irradiation, however, the likelihood of malignancy in a cold nodule is significantly greater. Nodules that appear warm (without suppression of RAI uptake in surrounding tissue) or isofunctioning may also be malignant *(51)*. Therefore, the concept that only cold thyroid nodules are malignant is not valid. Thus, with the exception of autonomously functioning (hot) nodules, which are virtually always benign, the thyroid scan does not help differentiate between benign and malignant lesions.

There are situations in which the thyroid scan may be useful, however, such as: (1) determining the function of a nodule palpated in a patient with Graves' disease (GD), since the presence of a cold nodule might prompt consideration for biopsy and subtotal thyroidectomy rather than radioiodine ablation or antithyroid drug therapy for the hyperthyroidism; (2) establishing the functional status of a nodule that has been shown by fine-needle aspiration (FNA) biopsy to be a follicular neoplasm; (3) differentiating the functional status of nodules in a multinodular goiter; and (4) helping to characterize the presence of nodules, especially if there is uncertainty about multinodularity or substernal extension. Iodine-123 (^{123}I) is generally the preferred isotope for thyroid scanning, although some specialists prefer technetium pertechnetate (^{99m}Tc). Both ^{123}I and ^{99m}Tc are useful imagining agents, although occasionally, nodules that are found to be functioning with ^{99m}Tc are cold when scanned with ^{123}I. Therefore, nodules that appear to be functioning or "hot" with ^{99m}Tc should be rescanned with ^{123}I. Interestingly, a recent survey of thyroid specialists revealed that 23% of American Thyroid Association respondents and 66% of European Thyroid Association members routinely perform thyroid scintigraphy in the evaluation of patients with nodules *(54)*.

Thyroid Ultrasonography. Despite its lack of specificity, many endocrinologists are using ultrasound in the evaluation of nodules. In a survey of thyroid specialists, 34% of respondents belonging to the American Thyroid Association and 80% of European Thyroid Association members said they routinely use thyroid ultrasound in the workup of patients with nodules *(54)*. There may be some merit to the use of ultrasound, however, it allows for more careful measurement of nodule size. Moreover, the presence of microcalcifications on ultrasound may increase the risk of malignancy to 29%, although the majority of nodules with microcalcifications are benign *(55)*.

The use of ultrasound in patients with single palpable nodules does illustrate the imprecision of palpation, since one review of such ultrasound studies showed that from 20–49% had additional nodules *(56)*. Another study of patients referred to a thyroid clinic found that 24% of 114 patients who were referred for solitary palpable nodules had additional nodules detected on ultrasound examination *(7)*.

The significance of nodules detected on ultrasound (so-called "thyroid incidentalomas") or with other imaging modalities, such as carotid duplex scanning or computed tomography (CT) or magnetic resonance imaging (MRI) for other conditions is still unclear, but has certainly led to increased use of ultrasound-guided FNA biopsy. Some workers feel that incidentally detected nodules of less than 1.5 cm without a history of thyroid cancer risk factors such as childhood irradiation, can be monitored *(3)*, while other workers are more aggressive. One study of ultrasound guided FNA of incidentalomas of >1 cm yielded cancer in 7 of 119 patients *(57)*, while differentiated cancer was detected in 5% of 450 nodules detected on ultrasound, of which 315 were less than 1.5 cm in size *(58)*.

Although multinodularity is thought by many thyroid specialists to be indicative of benign disease, others feel that the risk for malignancy is independent of the number of nodules, and emphasize the utility of ultrasound in the evaluation of patients with single palpable nodules in order to detect nodules that should undergo FNA *(58,22)*. Some workers advocate ultrasound-guided FNA of incidentially detected nodules >1 cm *(7)*, while others use 1.5 cm as the cutoff *(56)*, which in the author's opinion is a more reasonable approach.

Other Imaging Modalities. CT and MRI have no role in the initial evaluation of solitary thyroid nodules. If there are symptoms of tracheoesophageal pressure however, CT or MRI may help to define both the size of the nodular growth, as well as possible substernal extension and airway compression *(59)*. If significant tracheal narrowing is noted, especially if associated with symptoms of stridor, pulmonary function testing, with particular attention to the upper airway, is indicated. CT or MRI does not help in differentiating benign from malignant lesions.

Proton MRI has been reported as helpful in differentiating benign from malignant tyroid-follicular lesions, but enough overlap exists between benign and cancerous tissue to limit its clinical utility *(60)*.

FNA Biopsy

FNA biopsy is the mainstay in the laboratory evaluation of single thyroid nodules and of dominant nodules in multinodular goiters (*see* next subheading). Among many clinicians, including most endocrinologists, it is the most important, if not the only, test necessary in the initial evaluation of nodules *(1,14,49)*. FNA biopsy has been available in the United States since the mid-1970s, and as endocrinologists and cytopathologists have gained more experience with biopsy technique and cytologic interpretation, the sensitivity and specificity of the procedure have increased, resulting in better selection of patients for surgery. Since the introduction of FNA biopsy, the number of thyroid surgeries has declined by 50% in the United States, and the yield of detected cancer in patients undergoing thyroid surgery has increased from about 10–15% to 20–50% *(12,48,49,61)*. It is estimated that the use of FNA biopsy has resulted in a reduction of diagnostic and treatment costs for thyroid nodules by 25% *(4,62,63)*.

If done properly and with appropriate patient selection, FNA biopsy should have a false-negative rate of no greater than 5–10% and a false-positive rate of 1–5% *(48,64–66)*. A review of 18,183 FNA biopsy results from seven different medical centers indicated an overall false-negative rate of 0.5–2% and a false-positive rate of 2.9%. The sensitivity and specificity of the technique for the diagnosis of thyroid cancer were reported to be 83% and 92%, respectively *(64)*.

Technique. FNA biopsy may be performed using a needle as small as 25 gage, although I use a 22-gage needle, since very firm lesions may require a larger bore in order to obtain an adequate yield of cellular material. Although some clinicians do not use local anesthetic,

since the needle used is so thin, I do not think this attitude is justifiable, especially if multiple punctures are (and should be) performed.

I use a pistol-grip device with a 20-mL syringe, although many physicians simply use a syringe to obtain adequate material. Slides are then immediately fixed in Papanicolaou fixative and stained, while additional slides may be air dried for other stains. Material is also obtained for a cell button. Some clinicians use a rapid hematoxylin and eosin (H&E) stain and examine (or have the pathologist examine) the slides at the time of biopsy to ensure adequacy of the sample.

After the aspiration is completed, local pressure is applied and the patient is observed for 10–15 min for the development of local swelling.

Diagnosis of Lesions by FNA Biopsy (Fig. 1). The cytologic diagnosis of thyroid nodules can be subdivided into the following classifications: (1) malignant (or suspicious for malignancy); (2) benign; (3) indeterminate (or suspicious for follicular or Hürthle-cell neoplasm); and (4) insufficient for diagnosis. In two reviews of over 24,000 biopsies from 14 medical centers, cytologic diagnoses were benign in 70% (53–90%); indeterminate or suspicious in 10% (5–23%); malignant in 4% (1–18%); and insufficient for diagnosis in 17% (2–21%) *(48,65)*.

1. Malignant lesions. Papillary thyroid cancer constitutes approx 70% of thyroid cancers and is readily diagnosed by FNA biopsy because of its characteristic cytologic features (Fig. 1). It is helpful to have at least two of the cytologic features of papillary cancer present in order to be sure of the diagnosis, since individual features suggestive of malignancy may occasionally be present in benign lesions, including benign goiter, HT, or follicular lesions *(67)*. In my experience all papillary cancers diagnosed by FNA biopsy were confirmed surgically (Table 2). Two additional patients with cytologic features of a microfollicular tumor had surgically proven follicular variant of papillary thyroid cancer. Other malignant lesions that have typical cytologic features include medullary thyroid cancer, poorly differentiated (anaplastic) cancer, and primary thyroid lymphoma. Each of these tumors constitutes approx 5% of thyroid malignancies. None of them has cytologic features that allow a definitive diagnosis, although when medullary cancer is suspected, a positive immunocytochemical stain for calcitonin is diagnostic. If thyroid lymphoma is suspected, B-cell immunotyping may be performed on the aspirate. This is especially important to keep in mind, since a low-grade primary thyroid lymphoma may be difficult to differentiate from HT based on cytologic features alone *(68)*.
2. Benign lesions. Colloid nodules represent 40% of thyroid nodules and can be readily diagnosed by FNA biopsy. Between 15 and 25% of single thyroid nodules have cystic components on aspiration, and contain both fluid and solid elements. Most cystic lesions are degenerating benign adenomas or cystic colloid nodules, although malignancy may be present (*see* "Pitfalls of FNA Biopsy," item 2, following) *(12)*. Aspiration of a pure simple cyst (which constitutes a minority of lesions) will result in its collapse, and the fluid is generally straw yellow or slightly turbid. If crystal-clear fluid is obtained, the presence of a parathyroid cyst is likely, and fluid may be assayed for parathyroid hormone, which will be elevated *(69)*. (I have encountered only one parathyroid cyst among the more than 1500 biopsies I have performed.) When multinodular goiter is present, aspiration of a dominant or clinically suspicious nodule is indicated. Some workers suggest performing FNA biopsy on all palpable nodules in patients with multinodular goiter who have previously been exposed to childhood irradiation to the face or neck *(59)*.

Pitfalls of FNA Biopsy.

1. The problem of the indeterminate lesion. Between 5 and 20% of solitary thyroid nodules are classified by FNA biopsy as indeterminate or suspicious, rendering differentiation between

malignant and benign lesions problematic *(1,48,64,70,71)*. Lesions in this group appear as follicular or Hürthle-cell neoplasms. The diagnosis of such lesions can only be made histologically, with malignant lesions demonstrating either capsular and/or vascular invasion. Most centers report that approx 10–30% of lesions with indeterminate cytologic findings prove to be malignant at surgery *(66,71–74)*. This is in agreement with my experience, in which 30% of patients with follicular lesions that I have biopsied had surgically proven malignancy (*see* Table 2).

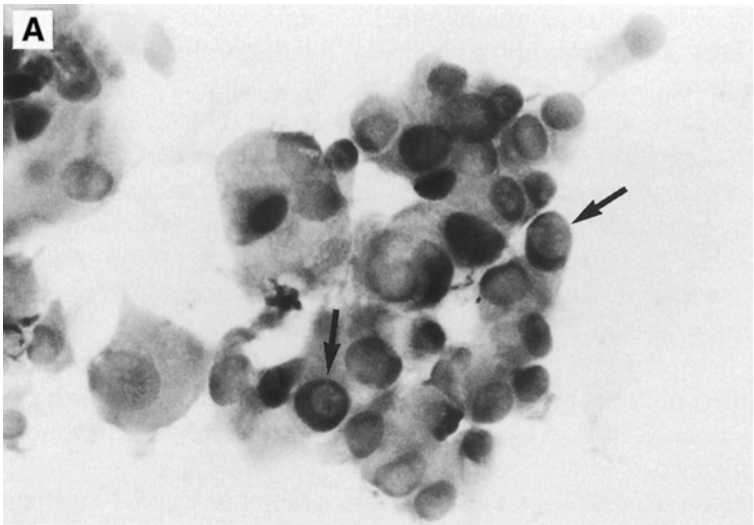
Because of uncertainty with differentiating malignant from benign follicular lesions cytologically, specific cellular markers have recently been used in an effort to make this distinction. In one recent study, thyroid peroxidase (TPO) immunostaining of cytologic samples was absent in 14 of 14 follicular cancers and in only 1 of 15 (7%) of follicular adenomas *(75)*.

In another study, the cell-surface protein galactin-3 was detected by immunohistochemical methods in 28 of 28 patients with histologically proven papillary or follicular cancers, and in only 2 of 39 (5%) of patients with surgically proven follicular adenomas *(76)*. Another study employing galactin-3 yielded similar results, with this marker being detected in cytology specimens from 5 of 5 follicular cancers and in 0 of 12 follicular adenomas *(77)*. Thus, molecular markers may show promise in resolving uncertainty of cytologically indeterminate nodules.

2. False-negative results. False-negative results with FNA biopsy have been reported for 1–11% of lesions referred for surgery *(48,65,78,79)*. Nodules of >3 cm are more likely than smaller lesions to give false-negative results. This problem can be minimized by taking at least several aspirates, and with lesions of >3 cm, even five or six (or more) aspirates, in order to ensure an adequate and representative sample *(66)*. This is particularly important with partly cystic nodules, in which cells are more likely to be diluted and dispersed by fluid. When fluid is encountered, it is helpful to aspirate as much as possible for cytologic examination, and to then biopsy remaining solid elements.

   Up to 14% of lesions that are partly cystic have been reported to harbor malignancy, especially if the fluid is bloody *(1,12,80)*. Therefore, benign results for such lesions must be interpreted with caution.

   Cystic nodules that do not reaccumulate fluid after aspiration are unlikely to contain cancer. In one study of 124 partly cystic nodules in 113 patients, ultrasound-guided FNA biopsy yielded satisfactory samples in 94% of nodules, suggesting that ultrasound is helpful in patients with such lesions, especially when nondiagnostic results have been obtained *(81)*.
3. Insufficient cellular material. Insufficient material for diagnosis is reported to occur in 5–25% of biopsies *(65)*. The reasons for this vary and may include inexperience on the part of the clinician doing the procedure, performance of an inadequate number of aspirations, or the presence of a cystic lesion. In other than pure simple cysts, an FNA biopsy yielding an inadequate sample must be considered nondiagnostic, and a repeat aspiration should be performed. Up to 50% of nodules that are reaspirated because of insufficient material may yield satisfactory results *(66,82,83)*. It cannot be overemphasized that material sufficient to establish a benign cytologic diagnosis must be obtained. Some cytopathologists believe that there must be at least six clusters of benign cells on each of at least two slides in order to diagnose a lesion as benign *(67)*. A diagnosis of malignancy can be made with fewer cells as long as characteristic cytologic features of cancer are present.
4. Consult with the cytopathologist. Thyroid cytology is considered by cytopathologists to be difficult and challenging. It is essential that the clinician who performs the FNA biopsy work closely with a cytopathologist who is both experienced and interested in this area of pathology. Also, it is important for the clinician to review the slides with the cytopathologist. Not only will this enhance the clinician's skills but will also provide the pathologist with important clinical information.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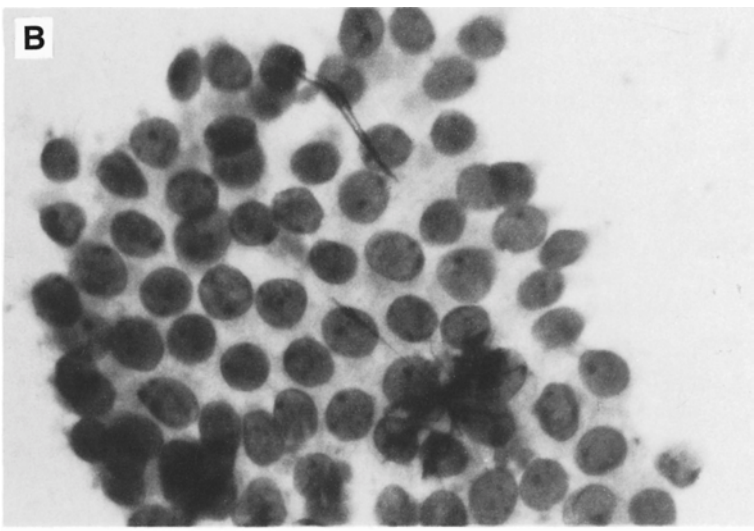
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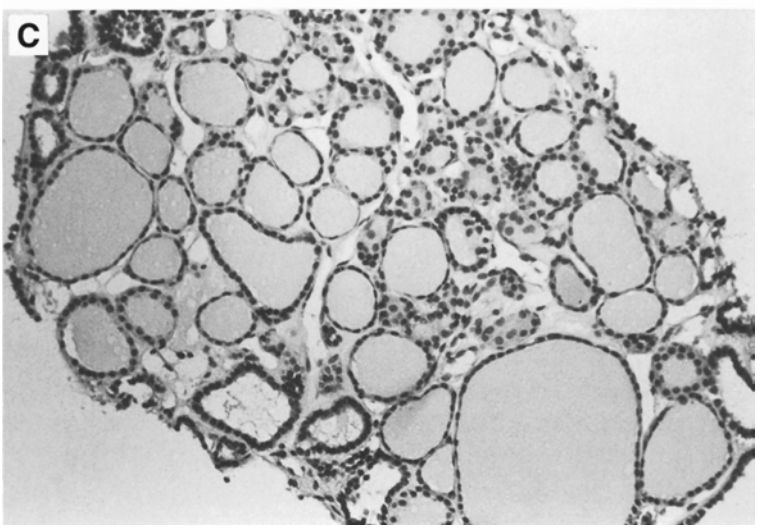
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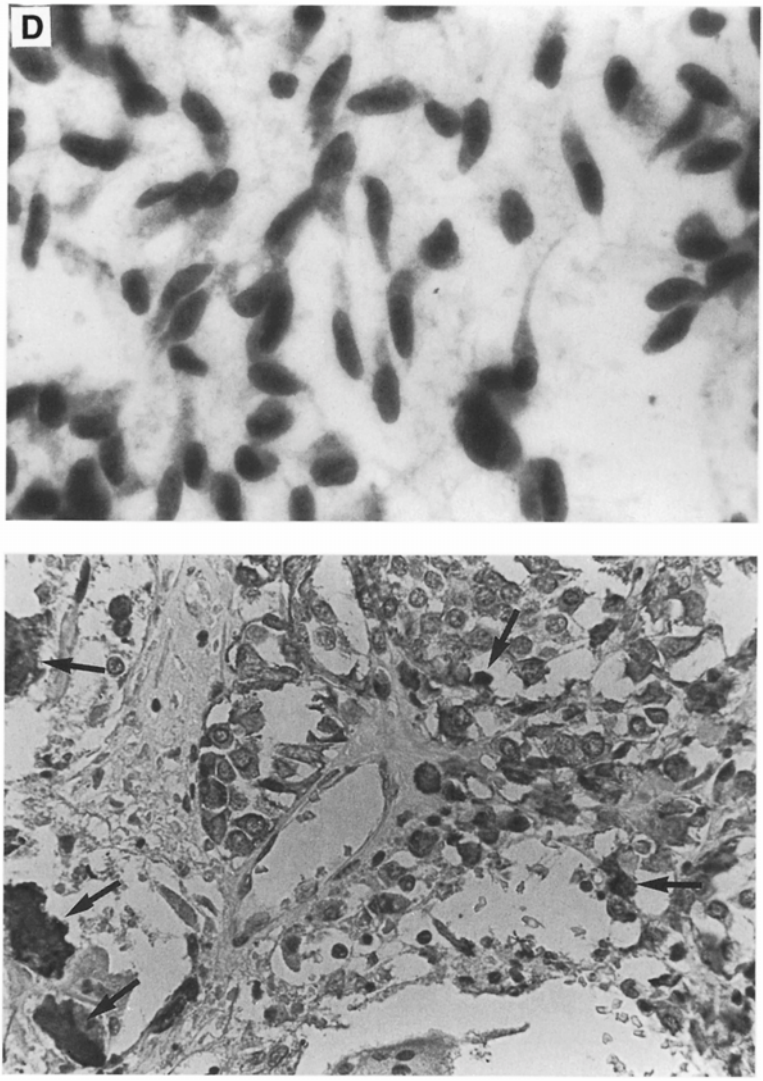

Fig. 1. Cytologic findings in various thyroid lesions. **(A)** Papillary cancer. Characteristic are intranuclear inclusions (arrows). **(B)** Follicular lesions. Note the group of follicular cells that are cytologically bland and relatively uniform. **(C)** Colloid nodule. Shown are colloid-filled follicles of varying sizes. **(D)** Medullary thyroid cancer. Spindle-type cells with eccentric nuclei are typical, but not diagnostic (top panel). The calcitonin stains (representative areas marked with arrows) on the bottom panel of the aspirate confirm the diagnosis. All slides shown are Papanicolaou preparations, except for the calcitonin stain in D. Adapted with permission from Singer PA. Evaluation and management of the solitary thyroid nodule. Otolaryngol Clin North Am 1996;29:577–591.

In some institutions the cytopathologist performs the FNA biopsy. Although the skill of the pathologist in performing the aspiration may be satisfactory, I believe that the biopsy of thyroid nodules should be done by an endocrinologist, since it is the clinician's responsibility to integrate the patient's clinical findings and biopsy results in order to develop an appropriate management plan for the patient.

Complications of FNA Biopsy. FNA biopsy is a safe procedure when performed by an experienced physician. Complications of FNA biopsy are, with few exceptions minor, transi-

Table 2
Frequency of Malignancy in Lesions Suspicious for Thyroid Cancer

FNA biopsy results	*Surgical prevalence of thyroid cancer*	*%*
Papillary thyroid cancer	114/114	100
Follicular, Hürthle-cell neoplasm[a]	41/123	33
Other, diagnostic or highly suspicious for thyroid cancer[b]	21/25	84
Total	186/262	71

[a]Three nodules interpreted as follicular neoplasms had follicular variant of papillary thyroid cancer.
[b]FNAB done for suspected medullary or anaplastic thyroid cancer, or for dominant nodule in multinodular goiter.
Adapted from Singer PA. Evaluation and management of the solitary thyroid nodule. Otolaryngol Clin North Am 1996;29:577–591.

tory, and relate mainly to local discomfort or minor hematoma formation. Among the more than 1500 biopsies that I have performed, only two patients developed significant swelling owing to hemorrhage, one of whom developed transient symptoms of tracheoesophageal pressure. In both patients the swelling gradually resolved without sequelae.

Other complications of FNA biopsy include entry into the trachea during aspiration, or puncture of the carotid artery or jugular vein. None of these complications are serious. Some clinicians (and patients) are concerned about the potential seeding of tumor, if present, along the needle track. While this has been reported, it is extraordinarily rare and of no concern *(84)*.

MANAGEMENT STRATEGY FOR THE SOLITARY THYROID NODULE

Surgery Versus Observation

Figure 2 outlines a suggested strategy for the management of thyroid nodules, based primarily on FNA biopsy. This strategy will not only result in savings in economic terms, but will also reduce the number of surgeries performed *(64,85)*. The schema also is compatible with a management approach suggested by another group, in which direct referrals to endocrinologists proved to be more clinically relevant and cost-effective than when patients with nodules were evaluated by physicians in other specialties *(86)*.

Although surgery is clearly indicated for malignant lesions, a frequently debated issue is what the preferred approach should be when there is a nodule with indeterminate cytologic findings. Most clinicians recommend surgery, because of the relatively high prevalence of malignancy in such lesions *(1,11,13,64,65)*. Before referring the patient for surgery, however, a ^{123}I thyroid scan should be done if the serum TSH is suppressed, in order to rule out an autonomously functioning nodule, since the cytologic features of follicular lesions may not be distinguishable from those of benign autonomously functioning lesions.

The extent of surgery recommended for patients with indeterminate findings is also a matter of debate. Since most nodules within the indeterminate category are benign, lobectomy is a reasonable choice. If the lesion proves to be malignant on histologic examination, completion thyroidectomy should then be done. The possibility of the need for a second operation must be discussed with the patient beforehand, since some patients will choose near-total thyroidectomy in order to avoid the possibility of a second procedure.

I generally advise lobectomy in younger or otherwise healthy individuals who have indeterminate cytologic findings. Advantages of a more limited operation include a much

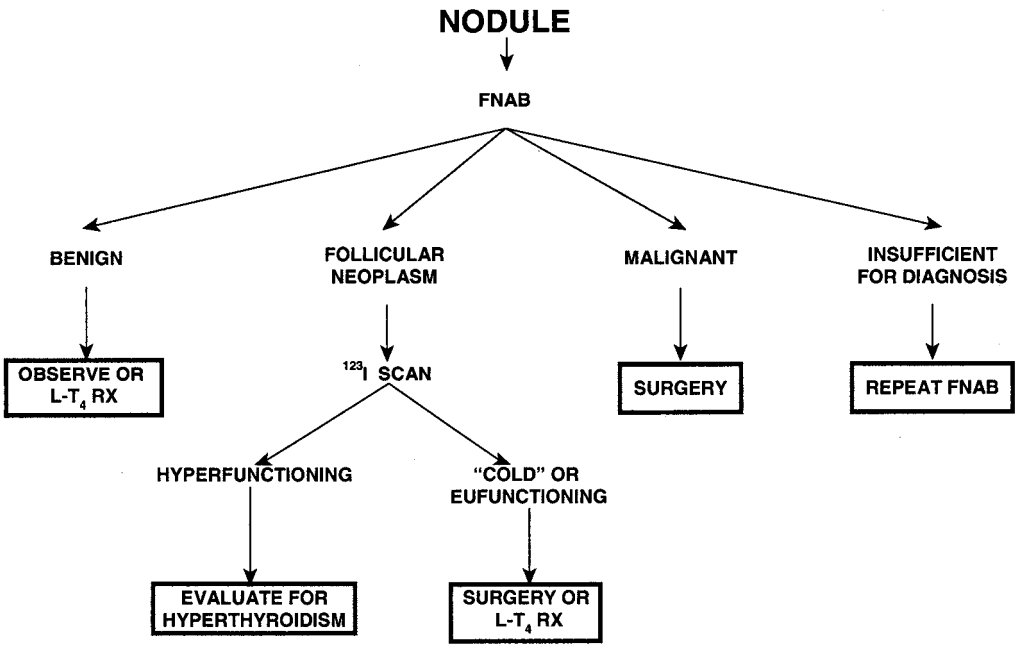

Fig. 2. Suggested algorithm for management of the solitary thyroid nodule. Reproduced with permission from Singer PA. Evaluation and management of the solitary thyroid nodule. Otolaryngol Clin North Am 1996;29:577–591.

greater likelihood of preserving parathyroid-gland function, minimizing risk to the recurrent laryngeal nerve, and probably avoiding the need for thyroid hormone replacement. In older patients or in patients with comorbidity, near-total thyroidectomy may be preferable in order to avoid the possible complications of a second procedure.

Some clinicians prescribe levothyroxine after lobectomy in order to prevent new nodule growth in the remaining lobe. Current evidence, however, suggests that levothyroxine does not prevent new nodule formation, *(87–90)*. On the other hand, administration of levothyroxine to patients undergoing partial thyroidectomy who have a prior history of head or neck irradiation does appear to prevent new nodule formation in the opposite lobe *(91)*.

Deferring surgery for patients with follicular lesions, either because they decline surgery or may not be good surgical candidates, may be an acceptable strategy, especially with nodules of <3 cm. This may be especially relevant for older or ill patients, in whom the risk of surgery may not justify an operation. The patient may be followed clinically, and if there is an increase in nodule size, surgery may then be performed. A reasonable delay of perhaps 6–12 mo for nodules that later prove to be malignant does not appear to result in increased morbidity or mortality. Surgery is generally recommended for lesions of 3 cm or larger; or if there has been evidence of recent growth; or if there is a history of childhood or adolescent irradiation.

THE ROLE OF LEVOTHYROXINE SUPPRESSION

The use of levothyroxine suppression for benign thyroid nodules remains controversial, from the point of view both of effectiveness and potential risks to the patient. In deciding whether or not levothyroxine therapy is appropriate for a particular patient, factors to

consider are: (1) the presence or absence of symptoms of pressure; (2) cosmetic concerns; (3) the nodule's growth pattern; (4) the patient's desire to avoid surgery; (5) the degree of certainty, by FNA biopsy, that the lesion is benign; and (6) the presence of risk factors that might predispose the patient to potential complications of levothyroxine therapy *(88)*. Levothyroxine administration, with doses sufficient to suppress serum TSH levels, appears to result in an increase in bone loss, especially in postmenopausal women *(92–94)*. Other studies, however, suggest that levothyroxine therapy does not increase the risk for postmenopausal osteoporosis *(95,96)*, and estrogen replacement therapy has been shown to ameliorate levothyroxine-associated bone loss in some patients *(97,98)*. Nevertheless, much evidence does support the notion that excess thyroid hormone leads to an increase in bone loss, and long-term TSH suppression should be avoided in postmenopausal women unless it is clinically essential.

In addition to potential adverse effects on bone, chronic TSH suppression may be associated with an increased heart rate and premature atrial contractions *(99)*, an increase in left ventricular septal and posterior wall thickness *(100)*, and, in older individuals, a greater risk for the development of atrial fibrillation *(99,101)*. The clinical significance of changes other than atrial fibrillation are unknown, and they appear to be reversible.

The debate over the efficacy of thyroid hormone in decreasing nodule size suggests that an inadequate number of well-controlled, double-blinded studies have been done on the effects of thyroid hormone on nodule size. Although a few randomized studies have failed to document the efficacy of levothyroxine suppression *(51,102,103)*, others have shown that levothyroxine is effective in causing nodule shrinkage, especially for lesions of <3 cm *(104–107)*. Despite the lack of agreement about the efficacy of levothyroxine therapy, a trial of levothyroxine may be justifiable if malignancy has been excluded, or if there are pressure symptoms or cosmetic concerns, *(90–91)*. Its use may also provide some diagnostic advantage, since malignant nodules would probably continue to grow despite such therapy, although in one series 13% of patients whose nodules shrank with levothyroxine and who subsequently underwent surgery proved to have papillary cancer *(108)*. Therefore, growth of a nodule during levothyroxine administration would be a strong indication for surgery *(51)*.

If levothyroxine therapy is used, it is unclear for how long it should be used. Some endocrinologists feel that a trial of levothyroxine for 6–12 mo, in order to determine whether or not nodule shrinkage will occur, is adequate *(88)*. This approach seems reasonable, and if there is shrinkage, determined either by palpation or ultrasound, the medication may be discontinued and the lesion reexamined in 3–6 mo. If there is regrowth levothyroxine may be resumed, although the absence of pressure symptoms or cosmetic concerns may obviate its need. Reaspiration of such lesions, as well as those that have not shrunk with levothyroxine therapy, may be indicated, in order to be certain of benignity. Routine re-biopsy of nodules generally is recommended only if there has been an increase in size. In a recent study of 45 patients who underwent repeat FNA biopsy, half of whom had nodule growth, no cancers were noted *(109)*. There is no benefit of additional levothyroxine therapy for benign nodules that do not decrease in size.

Cystic nodules and autonomously functioning thyroid adenomas do not shrink with levothyroxine therapy, and its use should be avoided in such cases, especially in patients with autonomous nodules, in whom levothyroxine administration would result in thyrotoxicosis.

The level to which the serum TSH should be suppressed with levothyroxine is unclear. Some clinicians believe that the TSH concentration should not be suppressed to the lower limits of detectability (employing a TSH assay with a functional sensitivity of 0.01 mIU/L), but rather to a range of 0.1–1.0 mIU/L *(17,88)*. It is thought that avoiding maximum TSH

suppression may minimize the potential for adverse skeletal and cardiac effects of chronic levothyroxine administration *(88,97)*.

MANAGEMENT OF EUTHYROID MULTINODULAR GOITER

The clinical and laboratory evaluation outlined above for patients with single thyroid nodules also applies to patients with euthyroid multinodular goiter. As previously mentioned, the presence of a dominant nodule or recent growth of a nodule raises the concern of malignancy, and FNA biopsy is indicated. The frequency of cancer in cold nodules in individuals from nonendemic goiter areas who have multinodular goiter was recently reported to be the same as in patients with single cold nodules (4.9% for multinodular goiter vs 5.4% for single nodules) *(13)*.

Patients with multinodular goiter are more likely than individuals with single nodules to have compressive symptoms, especially if the goiter is large or if there is substernal extension (Fig. 3). Surgery is the preferred treatment for patients with large multinodular goiters who have pressure symptoms or who are concerned with cosmesis. Since multinodular goiter increases in incidence with advancing age *(110,111)*, the benefits of surgery must be carefully weighed against possible risks of operation, especially in the elderly, in whom there may be significant comorbidity, and in whom substernal or intrathoracic goiter may predispose to a more complicated operation *(112)*. If surgery is indicated, subtotal thyroidectomy is recommended, with removal of all obvious nodular tissue. Nevertheless, recurrent growth does occur, and has been reported to occur as infrequently as in 3.4% and as often as in 50% of cases *(113)*. Postoperative thyroid hormone administration may not inhibit new nodule formation, but most clinicians advocate its use, largely to prevent the development of hypothyroidism *(114)*.

In general, individuals with asymptomatic euthyroid multinodular goiter, in whom malignancy has been excluded, may be followed yearly with careful palpation and a serum TSH measurement. If there is uncertainty about findings on palpation, ultrasonography may be indicated. Nodules that appear to be growing require FNA biopsy.

Medical management of euthyroid multinodular goiter with thyroid hormone is controversial. Although thyroid hormone therapy is commonly used in areas of iodine deficiency, and may result in a decrease in goiter size, its utility is less certain in areas of iodine sufficiency. Some authors have shown little if any benefit of thyroid hormone therepy, whereas others have shown a reduction of thyroid volume by 25% in more than half of treated individuals *(115,116)*.

Since the goal of levothyroxine therapy is to inhibit TSH secretion, its use does have potential disadvantages, including the aforementioned effects on bone and cardiac function. Since a large proportion of patients with multinodular goiter are older, potentially adverse effects are probably more significant in this population. In addition, up to 22% of individuals with nontoxic multinodular goiter may have significant functional autonomy, rendering thyroid hormone therapy more problematic in terms of risk for osteoporosis and cardiac function *(110,116)*. Thyroid hormone therapy should thus be avoided in patients with low serum TSH levels. Indeed, the value of thyroid hormone therapy may be questionable given only modest reductions in goiter size reported with its use, and the fact that most goiters regrow after discontinuance *(115,117)*.

Radioactive iodine (RAI) therapy has been used in some patients with euthyroid multinodular goiters, especially in those for whom surgery was believed to be contraindicated. Results have varied, although reduction in thyroid volume of up to 60% has been observed,

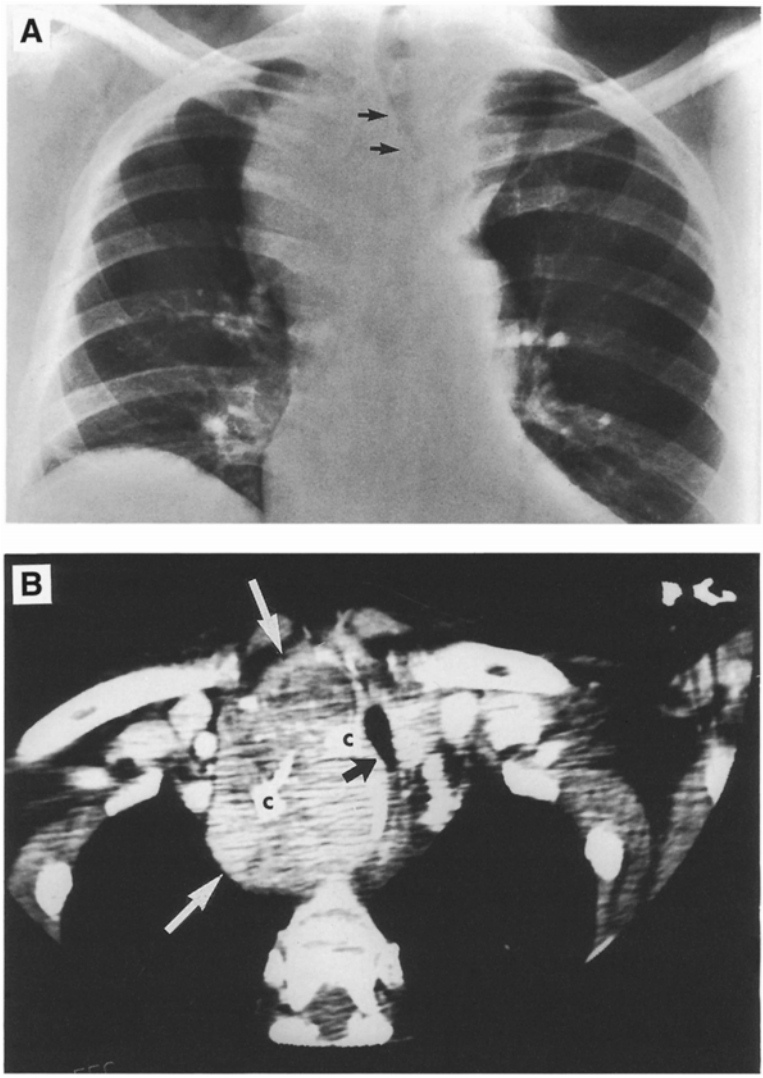

Fig. 3. Multinodular goiter in a 62-yr-old patient with symptoms of stridor. **(A)** Chest radiograph showing a large substernal mass with tracheal deviation and narrowing. **(B)** Computerized tomography at the level of the thoracic inlet, demonstrating the goiter (white arrows), tracheal narrowing (black arrows), and calcium deposits (c) within the goiter.

along with a decrease in compression symptoms *(118–121)*. A recent study compared the use of ^{131}I and levothyroxine therapy in patients with benign nontoxic multinodular goiter. Of 29 patients treated with ^{131}I, thyroid volume decreased by 38% at 1 yr and by 44% at 2 yr after ^{131}I administration, while of 28 patients treated with levothyroxine, thyroid volume decreased by 7% at 1 yr and by 1% at 2 yr. Not only was ^{131}I therapy effective, but this study also clearly demonstrates its superiority over levothyroxine therapy (122). Moreover, for levothyroxine to be effective, serum TSH needs to be suppressed, with its risks, especially in postmenopausal women and other older patients. Because of the relatively low uptake of RAI, large doses and/or repetitive treatment may be required. However, recombinant human TSH, which may be available in the future, may be useful in stimulating RAI uptake in low-uptake nodular goiters *(123)*.

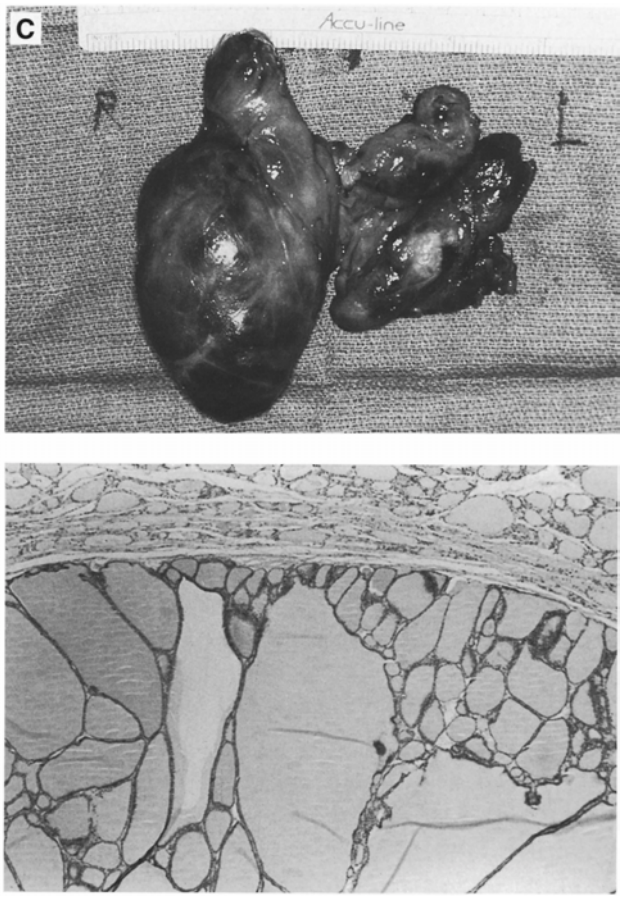

Fig. 3C. Surgical specimen showing both the gross specimen *(top)* and the histologic appearance *(bottom)*. Note the colloid-filled follicles.

SPECIAL CONSIDERATIONS

The Irradiated Thyroid

As mentioned under "Clinical Evaluation", individuals with a history of childhood radiation exposure have a significant likelihood of harboring a well-differentiated thyroid cancer. In addition, there is an increased incidence of benign nodules in irradiated patients. Patients with a history of irradiation and palpable thyroid nodules should undergo FNA biopsy of palpable thyroid nodules, and if the cytologic interpretation is malignant or indeterminate, surgery should be performed. Some physicians would opt for surgery irrespective of the FNA biopsy result, given the high risk for malignancy in irradiated thyroid glands *(124)*.

Patients with a history of irradiation without any palpable thyroid abnormalities present more of a clinical challenge, since many have nodules that are detectable only by ultrasound. An ultrasound study of 54 adults with exposure to external radiation as children, most of whom had no palpable abnormalities, revealed that 7 of the patients had a total of 157 nodules, 11 of which were greater than 1.5 cm; of these 11 patients, only 5 had palpable lesions *(125)*. Ultrasound then, would appear to be indicated in the initial evaluation of patients with a history of childhood radiation. Lesions 1 cm or greater should undergo FNA biopsy *(126,127)*. Although there are no data regarding serial ultrasound follow-up of patients

with a history of irradiation, an acceptable strategy would be to perform ultrasonography at 2- to 3-yr intervals, both to evaluate for growth of previously detected nodules and to detect new nodules because of the continuing risk of malignancy in previously irradiated individuals *(124,128–130)*.

Routine administration of thyroid hormone to suppress TSH in irradiated individuals who do not have palpable or ultrasonically detected abnormalities is probably not effective in preventing nodule formation, perhaps because radiation-induced abnormalities occurred years before detection. Thyroid hormone does reduce the recurrence rate for new benign nodule growth if used postoperatively in irradiated individuals, however (90).

Thyroid Nodules in Pregnancy

Thyroid nodules detected during pregnancy are generally managed as in the nonpregnant state. Because radioisotopes are contraindicated during pregnancy, radionuclide scans cannot be performed. Thyroid nodules clinically suspicious for malignancy and detected during the first or second trimester can be aspirated, and if cytologic results are positive or suspicious for malignancy, surgery can be safely performed during the second trimester *(130)*. An alternative approach is to defer FNA biopsy until the postpartum period and use to levothyroxine suppression during the pregnancy.

For lesions diagnosed in the third trimester of pregnancy, clinical observation with or without levothyroxine suppression may be more preferable than doing an FNA biopsy, since surgery should be avoided during the latter part of gestation in order to avoid induction of premature labor *(131)*.

It should be emphasized that since most thyroid nodules are benign, and since well-differentiated thyroid cancers usually grow slowly, a nodule detected during pregnancy, especially <2 cm, can probably be safely observed during the gestation. In a recent study, only 1 of 18 patients with a thyroid nodule detected during pregnancy had cancer in a lesion of <2 cm, suggesting that smaller lesions may be followed without intervention *(132)*. On the other hand, the prevalence of thyroid cancer in nodules of >2 cm may be higher than in the nonpregnant state, with one group reporting a 39% prevalence of cancer in single nodules of this size, while another study reported a 43% prevalence of cancer *(131,132)*, perhaps in relation to the elevated levels of HCG, a weak thyroid stimulator, during pregnancy.

The decision on how to proceed with evaluation and treatment of the pregnant patient with a thyroid nodule must be made only after she has been fully informed about potential risks of anesthesia and surgery to both the fetus (albeit minor) and herself, as opposed to deferring evaluation and treatment until after delivery. There is no apparent effect of pregnancy on accelerating the growth of thyroid cancer *(133)*.

MANAGEMENT OF EUTHYROID DIFFUSE GOITER

Euthyroid diffuse goiter may be subdivided into endemic and nonendemic types. Endemic goiter generally occurs in geographic areas of iodine deficiency. Thus, in the United States, endemic goiter is encountered almost exclusively in individuals who were raised in iodine-deficient areas.

Euthyroid diffuse goiter has a prevalence one-tenth that of nodular goiter *(115)*, and both diffuse and nodular goiter probably share the same underlying pathogenesis. Prolonged stimulation by TSH is considered an important factor for thyroid enlargement, especially with endemic goiter *(133)*. Stimulation by epidermal growth factor, insulin-like growth factor, and/or thyroid growth-promoting immunoglobulins has also been proposed as a possible etiologic factor in goiter formation *(134,135)*. It is hypothesized that diffuse goiter

develops over a relatively short period, and evolves into nodular goiter after more prolonged stimulation *(136)*. Evidence supporting this hypothesis was recently provided in a cross-sectional survey of 102 patients from a single geographic area of iodine sufficiency who had either euthyroid diffuse or nodular goiter. Patients with multinodular goiter tended to be older and had larger thyroid volumes as measured by thyroid ultrasonography than did individuals with diffuse or uninodular goiter *(110)*.

Patients with euthyroid diffuse goiter are usually asymptomatic and generally have only cosmetic concerns. Symptoms of pressure are uncommon. The clinical evaluation of such patients is relatively simple, with palpation being the most important element of the examination. The laboratory evaluation should consist of documenting euthyroidism with a serum TSH measurement; a serum anti-TPO antibody measurement is helpful in order to rule out chronic autoimmune (Hashimoto's) thyroiditis. Imaging studies, such as radionuclide scans or thyroid ultrasonography, add little to the evaluation. Moreover, since diffuse goiter is a benign disorder, FNA biopsy is unnecessary.

Treatment of patients with euthyroid diffuse goiter, when deemed to be indicated, is medical rather than surgical. Whether or not early treatment of diffuse goiter can inhibit the developmant of nodular goiter is unclear. If treatment is selected, then levothyroxine administration (in doses adequate to inhibit or reduce TSH secretion) may be employed, although reduction of goiter size by more than 20–30% after 1 yr of treatment is unusual *(136)*. Not unexpectedly, many thyroid experts are unenthusiastic about treating diffuse goiter, although others advocate the use of levothyroxine, subscribing to the notion that early treatment may prevent later development of nodular goiter. As with any issue in clinical medicine, the benefits of such therapy must be weighed against the potential risks of TSH suppression.

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12 Radiation and Thyroid Cancer

Lessons from a Half Century of Study

Arthur B. Schneider, MD, PhD, and Elaine Ron, PhD

Contents

INTRODUCTION

Once considered a "radioresistant" organ because large doses of radiation are needed to ablate it, the thyroid gland is now known to be one of the most sensitive organs to the long-term, neoplastic effects of radiation. Once thought to be a relatively rare and largely resolved problem, radiation-induced thyroid cancer is now known to be a common and persistent problem.

In 1950, Duffy and Fitzgerald first reported that young patients with thyroid cancer often had been exposed to radiation in the neck area many years earlier *(1)*. Subsequently, their observations on a small series of patients have been confirmed and expanded in large prospective studies using the methods of epidemiology, clinical investigation, and laboratory investigation, including molecular biology. The goal of this review is to synthesize current knowledge so that physicians can apply it in the clinical setting and investigators can have a sense of the direction of this multidisciplinary field.

LESSONS FROM EPIDEMIOLOGY

Evidence of an Association Between Radiation and Thyroid Cancer

Numerous epidemiologic studies have clearly demonstrated that acute, external radiation exposure increases the frequency of thyroid neoplasia in humans, particularly when

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the exposure occurs during childhood. Less is known about the carcinogenic effects of protracted external exposure and internal exposure to the adult thyroid, and the lifetime risks. Knowledge about radiation-associated thyroid tumors comes from a variety of studies of medical, military, environmental, and occupational exposures. Until the 1960s, radiotherapy was often used to treat a wide variety of benign diseases. Although the radiation treatment usually was successful, thyroid neoplasms developed more often than expected as a delayed effect when the treatment was received during childhood. Studies of patients treated for tinea capitis of the scalp, enlarged thymus gland, pertussis, enlarged tonsils, acne, and hemangiomas provide evidence that low and medium doses of external radiation can cause thyroid malignancies *(2–8)*. Data from patients given radiotherapy for malignancies such as Hodgkin's disease *(9–13)* and various childhood cancers *(14–16)* have demonstrated that thyroid cancer also can develop following high-dose radiation. Indeed, thyroid cancer is one of the more common radiation-associated second cancers observed.

The carcinogenic effects of fractionated or protracted radiation are less clear, yet these types of exposure are most relevant for protection against radiation. A major source of radiation exposure is diagnostic radiography. Two Swedish case–control studies have reported an association, based on personal recall, between diagnostic X-rays and thyroid cancer *(17,18)*. To overcome the problem of bias from personal recall, another Swedish case–control study searched for diagnostic radiology records for cases and controls in all Swedish hospitals. The frequency of any diagnostic X-ray exposure, high-dose diagnostic X-ray exposure, and exposure to diagnostic X-rays during childhood was similar for cases and controls, and no dose-response relation was observed *(19)*. In a smaller Swedish study using similar methods, the mean thyroid radiation dose from diagnostic X-rays was extremely small (about 7 mGy) and did not differ for cases and controls *(20)*. Although neither of these studies found an association between thyroid cancer and exposure to diagnostic X-rays, their results primarily refer to exposure in adults.

Thyroid cancer was the first solid tumor to be linked to radiation exposure from the atomic bombings in Hiroshima and Nagasaki. By the late 1950s, an apparent association between thyroid cancer and radiation was reported among the atomic-bomb survivors included in the Life Span Study (LSS)*(21)*. The LSS evaluates several major health outcomes for approximately 90,000 people exposed to the atomic bombings for whom individual organ doses of radiation could be estimated. Most recently, thyroid-cancer incidence was assessed from 1958–1987, and a strong association with radiation was demonstrated *(22)*.

Other environmental exposures occurred as a result of nuclear bomb testing during the Cold War, accidents at nuclear power plants, and high background radiation. People living in the Marshall Islands were accidentally exposed to radioactive fallout in 1954 when the wind changed unexpectedly during a nuclear weapons test. In early studies, an excess of thyroid carcinoma and nodules was observed among people receiving relatively high doses of radioiodines (RAIs) *(23,24)*. Later investigations reported that the prevalence of thyroid nodules increased with decreasing distance from Bikini Island, the site of the bomb detonation, but no clear dose-response was observed *(25,26)*.

Early studies of several thousand children exposed to fallout from nuclear weapons tests conducted at the Nevada test site over a 10-yr period did not find an excess of thyroid cancer *(27)*. A subsequent follow-up, which included a comprehensive dose reconstruction, reported a significantly increased incidence of all thyroid neoplasms combined, but based on only eight malignant tumors, the cancer risk did not reach significance *(28)*. An ecologic study of thyroid cancer and fallout from the Nevada weapons testing related age–calendar-year–sex and county-specific thyroid cancer mortality and incidence rates in the United States to

^{131}I dose estimates. Neither the cumulative dose nor the dose received between 1 and 15 yr of age was linked to thyroid cancer incidence or mortality, but an association was suggested for the dose received before age 1 yr *(29)*.

The prevalence of thyroid disease and radiation dose to the thyroid gland was determined for almost 3500 people who, as children, lived near the Washington State Hanford nuclear facility, during the years of atmospheric emissions of radioiodine. Preliminary results provide no evidence of an association between thyroid nodules or other thyroid diseases and RAI exposure (30).

The first reports of a dramatic increase in childhood incidence of thyroid cancer in Belarus and Ukraine 4 yr after the Chernobyl disaster suggested a connection with the accident, but did not show an association with radiation dose *(31–34)*. Furthermore, the role of better ascertainment through intense screening was not clarified *(35)*. Subsequently, a correlation between pediatric thyroid cancer incidence and residence in areas of increasing RAI contamination in Belarus, northern Ukraine, and the Bryansk region of Russia has been reported *(36–39)*. Further evidence for the role of RAIs comes from a case-control study of childhood thyroid cancer in Belarus *(40)*. The huge number of childhood thyroid cancers and the latency pattern provide additional evidence of an association with the accident *(41,42)*.

Studies of the incidence of thyroid cancer among children and young adults in several countries outside of the former Soviet Union have reported increasing rates since Chernobyl *(43–45)*. Whether the increase is related to Chernobyl, other risk factors, or better diagnosis is not known *(46)*.

Some of the limitations of our knowledge about effects of radiation, even for the highly studied thyroid gland, became apparent after the Chernobyl disaster. The experience gained from studies of the Chernobyl accident has already helped to clarify some issues, although many others remain. The early notion that there is a 10-yr postirradiation latency for all solid tumors has been modified by the <5-yr latency observed for thyroid cancers. How to compare the tumorigenic effects of external and internal radiation exposure remains an important question. Although the nature of the exposure and the existence of possible confounding environmental and other factors are complex issues, the Chernobyl experience emphasizes that internal RAI exposure cannot be ignored.

Persons living at extremely high altitudes are exposed to larger than usual doses of natural radiation. Studies conducted to date have not linked thyroid-disease prevalence or thyroid-cancer mortality with cumulative background exposure *(47–49)*. The relative small number of cases, the low doses, and the extremely narrow dose range, however, has limited the interpretation of these studies.

Studies of occupational exposures to radiation are of special interest because they involve low-dose fractionated exposures. Unfortunately, such studies generally have not been very informative in relation to thyroid cancer because most occupational cohorts include few, if any, women or children; yet, thyroid cancer is two to three times more common among women than among men, and children are the most highly susceptible to radiation damage to the thyroid. In addition, although thyroid cancer is seldom fatal, mortality is often the endpoint in occupational studies. Given these limitations, it is not unexpected that many worker studies have been negative. In the largest study, nine thyroid cancer deaths were observed among 95,000 British nuclear workers *(50)*. The risk for dying from thyroid cancer was significantly elevated, but the risk did not increase with increasing radiation dose, and the excess risk diminished somewhat with further follow-up *(51)*. Three other studies provide weak evidence for a possible association between radiation and thyroid

cancer *(52–54)*. Future follow-up of the radiologic technologist cohort in the United States *(54–56)* should be particularly informative, since 75% of the cohort is female and cancer incidence will be ascertained.

The Dose–Response Relationship Between Radiation and Thyroid Cancer

A pooled analysis of seven studies of persons exposed to acute, external radiation examined in detail the dose-response relationship with thyroid cancer *(57)*. The actual data sets were analyzed using common definitions, statistical methods, and assumptions. Five of the studies were prospective and were the basis for most of the analyses. The populations in these studies were: atomic bomb survivors *(22)*; infants irradiated for enlarged thymus gland in Rochester, New York *(6)*; children treated for tinea capitis in Israel *(5)*; children irradiated for benign head and neck conditions, mainly enlarged tonsils, at Michael Reese Hospital in Chicago *(2)*; and children receiving radiation for enlarged tonsils at the Children's Hospital Medical Center in Boston *(4)*. Two nested case-control series were included in the analyses when appropriate: thyroid-cancer cases and controls selected from a study of women receiving radiotherapy for cervical cancer *(58)*, and from participants in the late-effects study group of childhood-cancer patients *(14)*. The studies were conducted in several countries, and when combined include almost 120,000 people, nearly 700 thyroid cancers, and 3-million person-yr (PY) of follow-up.

The risk of developing thyroid cancer increased with increasing radiation dose in a linear fashion in each of the cohorts of persons irradiated before age 15 yr. The excess relative risk per Gray unit (ERR/Gy) ranged from a low of 1.1 (childhood cancer study) to 32 (Israel tinea capitis study), but the point estimates generally fell within the confidence intervals (CIs) of the other studies. Childhood-cancer survivors received extremely high thyroid doses, with the mean dose being nine to a hundred times greater than in other studies. The low-risk estimate for this study was influenced by the leveling of risk at high doses, probably due to cell killing. A reanalysis of the tinea capitis data, taking uncertainties in the estimated thyroid radiation doses into account indicates that dose error had little effect on the results *(59)*. Thus, methodologic, ethnic, lifestyle, and/or medical-system differences may explain the high risk. Combining all of the childhood exposure data, the pooled ERR/Gy was 7.7 (95%CI = 2.1, 28.7) and the excess absolute risk per 10,000 person-yr Gy (EAR/10^4 PY Gy) was 4.4 (95%CI = 1.9, 10.1). The dose–response relationship was linear, but appeared to somewhat underestimate the risk at low doses and overestimate the risk at high doses. Shore and Xue *(60)* combined the results from four patient populations irradiated during childhood, but not included in the pooled analysis, and found that the risks were compatible with those found in the pooled analysis, although somewhat lower (ERR/Gy = 5.3; 95%CI = 1.6, 9.0; EAR/10^4 PY Gy = 1.1).

Factors Influencing the Dose–Response Relationship

Age Effects

One of the characteristics unique to the thyroid gland is its extreme sensitivity to radiation during early childhood and its relative lack of sensitivity at older ages. No other type of cancer exhibits such a radical difference in sensitivity with age. Among the atomic-bomb survivors, the only prospective study that has a complete range of ages, a strong age-at-exposure effect can be seen. In an evaluation of age at the time of the bombings, the ERR/Gy was 9.46, 3.02, 0.34, and –0.23 for persons aged 0–9 yr, 10–19 yr, 20–39 yr, and 40+ yr, respectively *(22)*. In their review of radiation-induced thyroid cancer, Shore and Xue *(60)* compared the risks for adult and childhood exposure. They estimated the weighted mean

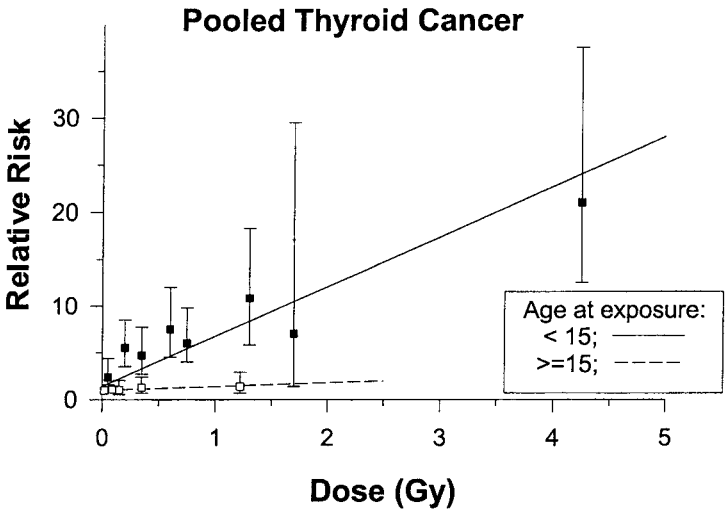

Fig. 1. Pooled, fitted dose–response relationship of risk for developing thyroid cancer following external radiation. The solid line represents the dose–response for persons exposed before age 15 yr, and the dotted line is for persons exposed at age 15 yr or older. Reproduced from Ron E. Thyroid cancer. In: Schottenfeld D, Fraumeni JF, Jr, eds. Cancer Epidemiology and Prevention. Oxford University Press, New York, 1996, pp. 1000–1021.

ERR/Gy to be 0.6 (95%CI = 0.1,1.0) for adult exposure, which is about nine times lower than the risk they estimated for childhood exposure. Although doses were not available, childhood radiotherapy also conferred a much higher risk than did treatment as an adult in two thyroid cancer case-controlled studies *(61,62)*, and the risk of developing thyroid cancer after high-dose radiotherapy for childhood Hodgkin's disease is substantially larger than after radiotherapy for adult Hodgkin's disease *(12,63)*. Data from the pooled analysis of external radiation exposure described earlier demonstrate a dramatic difference in the dose–response relationship for childhood and adult exposures (Fig. 1) *(64)*.

Even within the limited range of childhood exposure, the risk of developing thyroid cancer decreases significantly with increasing age at exposure. In the pooled analysis *(57)*, the risk for persons exposed to radiation before age 5 yr was five times greater than when exposure occurred at the ages of 10–14 yr. Besides the atomic-bomb survivors, only cervical cancer patients provided data on adult exposure. Although the estimate of the excess relative risk was very high (ERR/Gy = 35) for adult cervical-cancer patients, it was not statistically significant; the CI was huge (-2.2, ∞); and almost all of the risk was among patients treated between the ages of 30 and 39 yr. Data from Chernobyl also indicate that children are more susceptible to radiogenic thyroid cancer than are adults, and that there is a steep gradient in risk with decreasing age at exposure even among children *(65)*.

Gender

Thyroid cancer is one of the few cancers that is more common in women than in men. The difference is striking and is seen all over the world, but is not consistent throughout life. For children <10 yr of age, the difference is small *(66)*, but from adolescence to close to the age of menopause, the female-to-male ratio is about 3:1. After menopause, the female excess declines. The effect of gender on radiation risk is not consistent. In a recent summary of risk data, it was concluded that the ERR/Gy was similar for females and males, but that the absolute risk was greater for women *(67)*. In the pooled analysis *(57)*, the ERR/Gy for

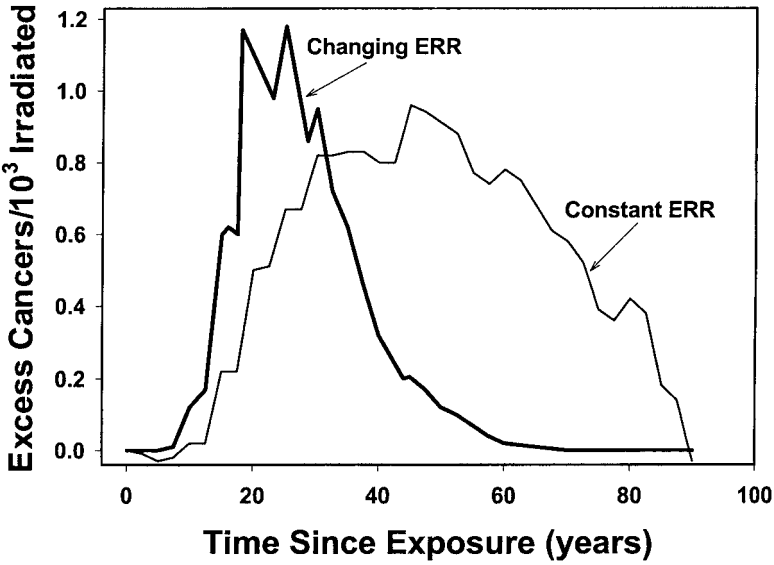

Fig. 2. Excess risk of developing thyroid cancer for white women exposed to 100 cGy at age two. The figure shows that the choice of statistical model, in this case one with constant excess relative risk (ERR, thin line) compared to one with varying ERR (thick line), has a major influence on risk projections. (Adapted from Shore RE, Xue X. Comparative thyroid cancer risk of childhood and adult radiation exposure and estimation of lifetime risk. In: Thomas G, Karaoglou A, Williams ED, eds. Radiation and Thyroid Cancer. World Scientific, Singapore, 1999, pp. 491–498.)

females was two times that for males, but the difference was not statistically significant ($p = 0.07$), and the direction of the sex difference varied across studies.

Time Since Exposure

Because no large cohort of radiation-exposed individuals has been followed for life, it is still too early to determine how long the risk of radiation-induced thyroid cancer will continue. It is known that radiation-associated thyroid cancer begins to occur about 5 yr after irradiation and continues to at least 40 yr after exposure. In several studies, an elevated risk was observed more than 40 yr after radiation exposure *(22,62,68,69)*. In the pooled analysis *(57)*, the risk was highest from 15–29 yr after exposure, but at 40 or more yr after exposure the risk was equal to that observed at 10–14 yr after exposure. Among infants irradiated for an enlarged thymus gland, the ERR/Gy began to decrease at about 20 yr after exposure, but the trend was not statistically significant. Clear time trends were not seen in the other cohorts. When a formal assessment of time since exposure was conducted in the pooled analysis, statistical models that allowed for variation in risk over time fit the data somewhat better than did models that assumed that risk remained constant over time. As seen in Fig. 2, a model that allows risk to vary over time shows that the excess risk of developing radiogenic thyroid cancer begins to decline about 25 yr after exposure and ends after about 65 yr, whereas a model with constant risk shows a much more prolonged excess risk *(60)*. This difference shows that the choice of statistical model has a major influence on risk projections.

Since the use of radiation therapy for benign diseases has been reduced dramatically, it is reasonable to predict that there will be fewer radiation-induced thyroid cancers in the future *(70)*. In a birth-cohort analysis, Pottern et al. *(71)* showed that thyroid cancer time trends in the United States are consistent with the wide use of radiation treatment for benign conditions between 1930 and 1960. It is too early, however, to conclude that the

radiation "epidemic" is over, and additional follow-up of the atomic-bomb survivors and other exposed populations is crucial for fully understanding the lifetime risks of thyroid cancer from radiation. Since the long-term survival of childhood cancer patients has increased substantially, clinicians should be aware that these patients have an enhanced risk of developing thyroid cancer.

Fractionation of Dosing

In animals, the effects of fractionation of radiation dosing have been studied for several malignancies, and one day between treatments appears to be sufficient to diminish the carcinogenic effects of radiation. The effects of fractionated radiation exposure on the thyroid gland have not been clearly established in either humans or animals. Based on three studies in the pooled analysis, there was a suggestion that spreading a dose over time (from a few days to > 1 yr) may lower risk *(57)*.

Histology

Well-differentiated papillary adenocarcinoma is most often associated with exposure to radiation *(2,3,5,61,62,68,72–74)*. Almost 90% of the nearly 700 thyroid cancers studied in the pooled analysis were papillary carcinomas *(57)*. An increased risk of radiation-induced follicular thyroid cancer has also been reported *(61,62)*, but overall the data indicate that the level of risk may be lower for follicular than for papillary carcinoma *(75)*. Although there have been case reports of anaplastic thyroid cancer developing after radiation *(76)*, there is currently little evidence linking radiation with anaplastic cancers. However, most of the study subjects are still relatively young, and only as they reach the natural ages for developing anaplastic thyroid cancers will we know whether these cancers are caused by radiation to the same extent as is papillary carcinoma. If there is an increase in anaplastic carcinomas, the clinical impact could be serious, since anaplastic thyroid cancer is extremely lethal *(77)*. Furthermore, survival is inversely correlated with age, even when adjusted for histology *(77,78)*.

Comparison of External and Internal Exposure

The extreme variation in the sensitivity of the thyroid to radiation at different ages directly influences comparisons between exposure to ^{131}I and external radiation. Children have rarely been exposed to ^{131}I for medical purposes, received radiotherapy with X-rays for benign and malignant diseases, and been subjected to ^{131}I exposure from the environment. In contrast, adults continue to have exposure to medical ^{131}I and have rarely been treated with X-rays for benign head and neck conditions.

The largest epidemiologic study of diagnostic ^{131}I was carried out in Sweden *(79,80)*. The mean dose to the thyroid from ^{131}I uptakes and/or scans was 1.1 Gy, and nearly all of the 35,000 patients were adults at the time of first examination (only 310 patients were <10 yr of age at exposure). Thyroid cancers were identified through the Swedish Cancer Registry. The first 5 yr of follow-up were excluded, to reduce the effect of underlying disease among the many patients who were examined because of suspected thyroid tumors. The risk increased with increasing dose, but most of the excess risk occurred between 5 and 10 yr after exposure. Between 10 and 19 yr—the years when the risk of thyroid cancer should be greatest—the number of observed cases was not significantly higher than expected, and there was no evidence of a dose-response relationship. Even without the first 5 yr of follow-up, the overall elevated risk appeared to have come from patients examined for suspected thyroid tumors. No excess risk was seen in patients referred for other reasons. Among the 1764 patients <20 yr of age at first examination and not referred for suspicion of thyroid

tumor, two thyroid cancers were diagnosed. Assuming a mean dose of approximately 1.5 Gy, the ERR/Gy was 0.25, which is considerably lower than the pooled estimate for persons less than 15 yr at exposure to external radiation *(57)*, or for atomic-bomb survivors <20 yr at the time of the bombings *(22)*. A recent small screening study of thyroid disease among German patients receiving diagnostic ^{131}I examinations and patients receiving nonradiologic diagnostic thyroid examinations before age 18 yr reported no substantial difference in thyroid-disease prevalence (relative risk [RR] = 0.9; 95%CI = 0.1, 5.1) *(81)*. Because participation rates were very low (35% and 41% for exposed and nonexposed groups, respectively), and the number of children exposed before age 10 yr was extremely small, the results from this study are far from conclusive.

In a study of over 35,000 adult hyperthyroid patients in the United States, thyroid cancer incidence was not significantly greater among the 23,000 patients treated with ^{131}I than among patients treated with surgery or antithyroid drugs. Almost all patients (91%) had Graves' disease (GD), and the mean administered ^{131}I activity was 10.4 mCi *(82)*. A longer follow-up of this group of patients found a small increase in thyroid-cancer mortality *(83)*. Additionally, a group of l0,552 adult hyperthyroid patients treated with ^{131}I was evaluated in Sweden *(84,85)*. The mean administered activity was 13.7 mCi, but was 19.6 mCi for the 42% who had toxic nodular goiter. Compared with age- and sex-standardized Swedish population rates, no significant excess thyroid cancer risk or thyroid cancer-related mortality was observed among GD patients. Although the cancer risk was higher in toxic nodular goiter patients, the lower confidence boundary was still below unity. Time since exposure did not influence this risk. Thyroid cancer incidence and mortality were also evaluated in over 7,000 hyperthyroid patients in Britain *(86)*. Compared to age- and sex-specific population rates, the risks of both thyroid-cancer incidence (standardized incidence ratio = 3.25; 95%CI = 1.69, 6.25) and mortality (standardized mortality ratio = 2.78; 95% CI = 1.16, 6.67) were significantly elevated.

Data on childhood medical ^{131}I exposure are extremely limited. In a study of 3500 patients < 20 yr of age exposed to a mean dose of somewhat more than 0.5 Gy from diagnostic ^{131}I examinations, four thyroid cancers were observed *(87,88)*. Based on a mean dose of 0.5 Gy, the crude ERR/Gy would be 3.9. In the study of children living near the Nevada test site, eight thyroid cancers were found, yielding an ERR/Gy of 7.9 *(28)*. Although the risks were not statistically significant, neither study had adequate statistical power to detect effects.

The data on childhood ^{131}I exposure have not shown a difference between childhood external radiation and ^{131}I *(89,90)*. The Chernobyl experience suggests that the carcinogenic effects of ^{131}I may be more similar to those of external radiation than originally thought *(37,91,92)*. Although the comparison between the risk estimates derived from the Chernobyl cases and from the pooled analysis *(57)* depends on the model used (excess absolute risk or excess RR), and the confidence intervals are broad, no statistical differences have been found. Following adult exposure, differences in risk between ^{131}I and external radiation are not detectable because no appreciable risk for developing thyroid cancer following either type of radiation has been observed.

Results from high-dose animal studies conducted years ago *(93)* suggested that the biologic effectiveness of ^{131}I is between two- and 20-fold lower than that of external radiation. However, a study of 3000 rats, conducted by Lee and Chiacchierini *(94)*, shows little difference in thyroid tumor outcome by radiation type, especially at doses below 400 cGy. The results from this study are particularly relevant because the rats were prepubescent, and it is for this age that human data are lacking. Brenner *(95)* compared X-radiation and ^{131}I in terms of chromosome aberrations, hypoxanthine phosphoribosyl transferase (HPRT)

gene mutations, and in vitro oncogenic transformation, and found the biologic effectiveness of ^{131}I to be about 60% that of X-radiation.

How Much of Thyroid Cancer is Attributable to Radiation?

Although it is uncertain how many thyroid cancers detected over the past few decades are related to prior radiotherapy in childhood, in areas of the United States where radiation treatment was common, the figure may be close to 10%. In a population-based case-control study of all persons who developed thyroid cancer in Connecticut between 1978 and 1980, approximately 9% of such cancers could be attributed to prior radiotherapy in childhood *(62)*. The percent of thyroid cancers caused by radiation varied with birth cohort. For persons born before 1930, the figure was 16%, but it dropped to 3% for those born after 1949. Based on reports from various hospital series, about l0% of thyroid-cancer patients have a history of therapeutic head and neck irradiation *(70,72,96)*.

Among atomic-bomb survivors whose thyroids received 1 Gy, the attributable risk was 26%. For survivors exposed before age 20 yr, the attributable risk rose to over 50%, whereas for those over age 20 yr at the time of the bombings only 3% of thyroid cancers could be attributed to radiation exposure *(22)*. The pooled analysis indicated that among persons whose thyroids were exposed to 1 Gy before age 15 yr, about 88% of cancers were caused by radiation exposure *(57)*.

Between 1951 and 1958, the U.S. Government conducted more than 100 above-ground nuclear tests at the Nevada test sites. These tests resulted in deposition of ^{131}I all over the United States. Based on findings from studies of thyroid-cancer risk associated with external radiation exposure, the U.S. National Academy of Sciences concluded that the nuclear weapons testing program increased the lifetime thyroid cancer risk for the US population, particularly for people who were young children during the time of the testing and who drank large amounts of milk or unprocessed milk, but that the uncertainty in both the dose estimates and the thyroid cancer risk associated with the radiation exposure preclude estimating the number of excess thyroid cancers with precision *(97)*.

Potential Problems in Epidemiologic Studies of Radiation and Thyroid Cancer

Since populations cannot be divided into predetermined groups of persons who will or will not be exposed to radiation, the methods used in epidemiology are often not as clear-cut as those used in laboratory studies. Epidemiology has to contend with lifestyle behavior patterns that may be unknown, as well as with genetic variation, dissimilar levels of medical attention, diverse medical practices, and other environmental and occupational factors. However, the wide publicity surrounding the role of radiation in causing thyroid neoplasia has resulted in additional methodologic problems. Physicians conduct careful clinical evaluations, utilizing the most up-to-date diagnostic tools, for persons with a known history of radiation. Furthermore, people who know they were exposed to radiation often participate in special screening projects. In both situations, irradiated individuals have a greater chance of having a thyroid cancer diagnosed than do nonirradiated individuals. If irradiated individuals participate in screening programs or are evaluated by physicians with an equal amount of thoroughness regardless of their radiation dose, then the enhanced ascertainment of thyroid cancers will not influence the slope of the dose–response relationship. Two radiation cohorts illustrate these problems.

In 1974, the Michael Reese Hospital in Chicago tried to notify all of its patients who had received radiotherapy for benign head and neck conditions during childhood. The potential health hazards were described and patients were invited for a free screening examination

at the hospital. Patients were also advised to see their local physician and document their radiation history if they were unable to come to Michael Reese Hospital. In subsequent follow-up of these patients, the age- and sex-adjusted thyroid-cancer rate was found to be about sevenfold greater after the 1974 alert and screening programs than before *(2)*. Among atomic-bomb survivors who are members of a biennial clinical examination program, the rate of thyroid cancer was 2.5-fold greater than among those not in the program. However, the large difference in thyroid-cancer incidence did not significantly influence the slope of the dose–response curve in either the atomic-bomb survivors (test for homogeneity of dose-response: $p = 0.86$) or the Michael Reese Hospital patients (test for homogeneity of dose-response: $p = 0.39$) *(57)*. A similar consideration affects the risk estimate for thyroid cancer related to the Chernobyl accident because intense examination programs have increased the detection of childhood thyroid cancers. However, as in the Michael Reese and atomic bomb cohorts, the dose–response analyses for Chernobyl studies indicate that the dramatic increase in incidence of thyroid cancer since the accident is not attributable to increased surveillance alone.

LESSONS FROM THE EVALUATION OF RADIATION-EXPOSED PATIENTS

Evaluating Individual Risk

As summarized above, many factors associated with individual risk assessment for radiation-induced thyroid cancer have been identified, and estimates have been made of their magnitude. In most cases, however, the major limitation in making an accurate risk assessment is the difficulty in obtaining the necessary historical data for the individual patient.

Estimating the Dose to the Thyroid

For radiation exposure arising from medical treatments, it is necessary to know the primary treatment target, the size of the primary treatment field, whether and how shielding was used, the age at exposure, the number of treatment fractions, and the characteristics of the radiation. With respect to the type of radiation, it is necessary to distinguish between conventional therapy and brachytherapy, the latter referring to a source of radioactivity implanted into or placed close to the target of treatment. It is also necessary to distinguish between conventional radiation, very-low-energy radiation as used to treat some dermal conditions (Grenz rays), and ultraviolet radiation, which primarily affects the skin. Internal exposure, from RAI treatment, is discussed above.

A careful history, based on knowledge of how radiation treatments were used between the 1930s and 1960s, is often necessary, because it can be difficult to obtain the original radiation records. The age at exposure provides an important clue. Treatments to shrink an “enlarged” thymus were given within the first few months of life. Treatments to shrink “enlarged” tonsils and adenoids were given in the same age range as for surgical tonsillectomy. For this purpose, both conventional radiation and brachytherapy were used. For brachytherapy, a radiation-tipped rod was inserted through the nose and was left in the posterior pharynx for a predetermined time. This method was also used to treat submariners and aviators so that they could withstand the large pressure changes required by their jobs. The thyroid exposure from pharyngeal brachytherapy was very small and did not significantly increase the incidence of thyroid cancer *(98,99)*. Brachytherapy was also used to treat hemangiomas. The thyroid dose varied considerably, depending on the location of treatment, and a dose-related increase in thyroid cancer was observed *(8)*. Dermatologic uses of radiation included conventional X-irradiation, usually for cystic acne in adolescents, and Grenz rays and ultraviolet rays for other conditions.

For environmental exposures to radiation, the mixture of isotopes may be important. From a nuclear explosion (e.g., fallout over the Marshall Islands) or accident (e.g., Chernobyl), the isotopes, particularly the short-lived isotopes, differ from those derived from the release of stored isotopes (e.g., Hanford nuclear reactor). In the latter case, some of the short-lived isotopes have had time to decay prior to release. Also, whether the time-course of exposure is rapid or prolonged may be important.

Additional Factors Related to Risk

In addition to the absorbed dose, it would be helpful to be able to estimate the radiation responsiveness of a given individual. As discussed in the following subsections, it is reasonable to expect variations in susceptibility to radiation.

Demographic Factors. The data reviewed above show that there are thyroid-cancer risk factors in the general population, and that there are factors, in some cases the same ones, that modify the effects of radiation *(57)*. The most illustrative and relevant factor in the clinical setting is gender. It is well known that in the general population women develop thyroid cancer approximately two to three times more frequently than do men. With respect to the effects of radiation, there are two important gender-related aspects. First, radiation effects appear to fit a multiplicative-risk model somewhat better than an additive-risk model. Second, the slope of the dose–response relationship may be greater in women. Therefore, since more baseline cases and possibly more radiation-related cases occur in women, the clinical concern for them should be higher than for men.

Other Radiation-Related Head and Neck Neoplasms. Salivary gland *(100,101)*, parathyroid *(102–104)*, and neural tumors *(100,105)* have been identified as other radiation-related neoplasms. In the Michael Reese Hospital study, the parotid glands were the most commonly affected, in part because they received the highest doses. About one-third of the salivary tumors in that study were malignant *(100)*. The dose–response analyses for the benign tumors in this series, and for the malignant tumors in the survivors of the atomic bombs, confirm the relationship *(101,106)*. Hyperparathyroidism also has been associated with radiation exposure, although the data are less extensive *(102–104)*. The most common neural tumors reported after radiation exposure are schwannomas and meningiomas *(100,105)*. The former may occur in peripheral nerves, spinal nerves, and cranial nerves, particularly the eighth nerve (acoustic neuromas).

In reviewing the pattern of tumors in the Michael Reese Hospital study, it was observed that the presence of one radiation-associated tumor made it more likely that there was a second *(107)*. A more recent analysis has indicated that such clustering is attributable to treatment factors and length of follow-up *(108)*. However, whatever the reason, the presence of one radiation-associated tumor should intensify the evaluation for the presence of others.

Familial Factors. Since siblings were likely to be cared for by the same pediatrician and managed in similar ways, we were able to study thyroid outcomes in many irradiated sibling groups *(109)*. For all nodules (benign and malignant combined), the concordance between siblings was higher than expected, making the history of radiation-related neoplasms in

sensitive than palpation *(110)*. Even after this was established, there was reluctance to screen with scanning because it involved further radiation exposure and because the small abnormalities it detected were thought to be of little or no clinical significance. One approach that was advocated was to reserve thyroid imaging for patients at demonstrably high risk *(111)*.

Recently, with advances in technology and experience, it has become clear that thyroid ultrasound is the most sensitive screening tool since it is able to detect nodules of only a few millimeters in size and does not involve radiation. Consequently, more and more patients are having this procedure done. Surprisingly, many relatively large thyroid nodules are detected by ultrasonography that are not palpable on physical examinations, and some nodules detected by physical examination are not confirmed by ultrasonography *(112,113)*. However, thyroid ultrasound's strength is also its greatest weakness. Owing to its high sensitivity, it reveals that a large fraction of the general population (from one-third to one-half) has nodular abnormalities *(114)*. The prevalence is even higher in irradiated individuals, reaching nearly 90% in the Michael Reese Hospital cohort *(112)*.

Although the ability of screening to find small thyroid cancers has been well documented, the overall benefit has never been tested by a prospective controlled study *(115)*. Ultrasound screening for thyroid cancer has been subjected to quantitative evaluation projecting from the factors known about thyroid cancer *(116)*. This analysis highlights the problem that, in addition to detecting inapparent thyroid cancers, screening also leads to surgery for benign thyroid neoplasms, mainly follicular adenomas. A reasonable approach is to reserve thyroid ultrasonography for patients at high risk of thyroid malignancy. When a nonpalpable nodule larger than 1–l.5 cm is found, an ultrasound-guided fine-needle aspiration (FNA) should be performed. This will reduce unnecessary surgery, although not for those found to have follicular neoplasms.

Other Diagnostic Testing

Because hypothyroidism may follow exposure to high doses of external radiation or ^{131}I, the serum level of thyroid-stimulating hormone (TSH) should be measured routinely in these circumstances.

Screening measurements of serum thyroglobulin (Tg) has some utility. On average, patients with thyroid nodules have higher Tg levels *(117)*. Also, patients with normal thyroid findings but elevated Tg levels have an increased chance of developing a thyroid nodule *(118)*. Therefore, although measuring Tg in radiation-exposed patients is neither sensitive (many patients with nodules have normal Tg levels) nor specific (there is no distinction between benign and malignant nodules), an elevated Tg level indicates the need for a more thorough evaluation, possibly including thyroid imaging.

Other thyroid tests are not indicated on a routine basis, but should be obtained when they would help evaluate other findings. For example, hypothyroidism may indicate Hashimoto's thyroiditis (HT), a condition that can mimic nodular thyroid disease. Antibodies to Tg and thyroid peroxidase (TPO) are reportedly more common in radiation-exposed children in Belarus *(119)*, but there is insufficient information to recommend screening in other areas. Periodic serum calcium measurements are a part of routine health screening and are sufficient for screening for hyperparathyroidism. Tests related to salivary and neural tumors are indicated when there are symptoms (e.g., unilateral hearing loss) or signs (e.g., a mass in the parotid gland) indicating a need for them.

LESSONS FROM THE COURSE AND TREATMENT OF PATIENTS WITH RADIATION-RELATED THYROID NODULES AND CANCER

Course of Benign and Malignant Radiation-Related Thyroid Nodules

The behavior of radiation-related thyroid cancer in the Michael Reese Hospital series was similar to the behavior of thyroid neoplasms in the general population *(120)*. Using recurrence as a measure of clinical behavior, the same predictive factors are found for cases related to and not related to radiation *(121,122)*. For small (<1.0 cm) radiation-related thyroid cancers, recurrences were seen in 11 of 149 cases from 1–22 years after surgery, so they cannot be ignored *(115)*. A short latency from radiation exposure to surgery was the main risk factor for these recurrences. The findings suggest that for external radiation exposure the same principles that apply to thyroid cancer in general should be applied to radiation-related cancers.

The situation may be different for the Chernobyl area cases of childhood thyroid cancer. They presented with clinical and histologic features associated with more aggressive malignant behavior *(123,124)*. In 330 childhood cases, Ukraine 93.1% had the solid variant of papillary thyroid cancer, regional lymph nodes were involved in 57.3%, and 14.5% had distant metastases. Similar aggressive features were present in cases in Belarus *(125)*. These more aggressive features are not typical of all childhood thyroid cancers *(126)*. However, some of these features may be a result of other factors in the region. These factors are iodine deficiency *(127)*, possible prior exposure to low-level radiation, and possible exposure to other agents (pollutants) in the environment. The childhood cases of thyroid cancer in Ukraine appear to respond less well to RAI treatment *(128)*. It is too soon to know how new cases in the area will present.

The behavior of radiation-related benign thyroid nodules in the Michael Reese Hospital series also was similar to the behavior of thyroid nodules in the general population, as recurrences were very common. However, in irradiated individuals, it is possible for a recurrence to be malignant. Also, in contrast to nonradiation-related benign nodules, in which its benefit has been difficult to prove, thyroid hormone (TH) treatment reduces the incidence of benign recurrences *(129)*.

A review of the literature relating to thyroid cancer after radiation treatment for Hodgkin's disease presented evidence for a more aggressive behavior of such cancer *(130)*. This observation may arise from a tendency of anecdotal reports to describe more severe cases.

FNA of Thyroid Nodules

Although FNA of thyroid nodules has gained wide acceptance, there are two reasons to be cautious about its application to radiation-related nodules. First, it is possible that the effects of radiation produce misleading results. So far, however, it appears that FNA has about the same diagnostic performance for nodules in irradiated as in nonirradiated patients *(131)*. Second, it is feared that multinodularity is so common in irradiated patients that FNA provides samples only of some nodules and leaves other, potentially malignant areas undiagnosed. In fact, small thyroid malignancies are undoubtedly present in some patients, as can be inferred from the many small thyroid nodules found by ultrasound mentioned earlier and as shown by the characteristics of the thyroid cancers that have been removed already *(112,132)*. However, in practice, this is not a serious limitation. Sufficient clinical follow-up has been obtained to conclude that small radiation-related thyroid cancers are

rarely aggressive *(115)*. Also, the sensitivity of thyroid ultrasound allows for careful follow-up. Therefore, although an argument can be made for removing all large radiation-related thyroid nodules, FNA can play an important role, especially in identifying suspicious nodules best treated with surgery.

Surgery

In patients who have thyroid imaging done for screening, it is possible to detect small (<1.0 cm in largest dimension) nodules, often multiple ones, that cannot be palpated. Whereas it is likely that some of these small nodules in patients with a history of radiation are malignant, their presence is usually not sufficient to recommend surgery. This recommendation is based in part on the observation that the association with radiation does not alter the behavior of thyroid cancer, and that size is one of the most important factors in predicting the behavior of such cancer. In other words, it would be unusual for a small thyroid cancer to invade or spread before its enlargement was detected. Enlargement is a strong reason for performing FNA and considering surgery.

In most instances in which large nodules are present, the decision about whether or not to perform surgery depends on the results of FNA. However, this is not always as easy as it may seem. Sometimes there are multiple nodules larger than 1–1.5 cm and not all can be adequately aspirated. In a patient with no radiation exposure, this would be considered a nontoxic multinodular goiter with little concern for malignancy. In a patient with a history of radiation exposure, the chance of malignancy is increased sufficiently so that surgery is a reasonable option. Even more common is the case in which one or more larger nodules are aspirated and found to be benign, but additional small nodules are present. For the reasons outlined in the previous paragraph, the presence of one or more small nodules does not necessarily influence the decision to perform surgery or not.

TH Therapy

How much TH should be given to patients with radiation-related thyroid cancer, and are there any cases in which hormone therapy is not indicated? The desired TSH level dictates how much TH to give. Many schemes have been devised to estimate the risk of a given thyroid cancer. In none was it found necessary to include a factor for radiation exposure. Therefore, radiation-related thyroid cancers should be treated as other thyroid cancers, including determining whether to fully suppress or partly suppress TSH. The one possible exception may be TH therapy for patients with very small thyroid cancers diagnosed after a partial thyroidectomy. In these cases, the presence of a thyroid cancer, even a small one, may indicate an increased risk for developing subsequent radiation-related thyroid tumors. Since the risks attached to TH therapy are low, its use in such patients is prudent.

Among patients who have had surgery for radiation-related nodules and only benign nodules are found, a substantial risk of recurrence has been observed *(129)*. In these cases, TH should be given, irrespective of the extent of thyroidectomy. This is based on the observation that surgery and TH therapy independently reduced recurrences. Should TH be given to patients who develop FNA-demonstrated benign nodules? For nodules that are not related to radiation exposure, the efficacy of this treatment has been reevaluated using ultrasound to follow the nodules. Unfortunately, the matter remains unresolved, with data both in favor *(133)* and against *(134,135)* its effectiveness. However, for radiation-related cases, the rationale for the use of TH is greater; it does reduce postoperative recurrences in patients with benign nodules and, although unproved, it may be effective in preventing or controlling the small thyroid malignancies found in irradiated patients. Similarly, for patients

whose only abnormality is a scan-detected or ultrasound-detected nodule of <1.0–1.5 cm in largest dimension, the rationale for using TH is that it may reduce the chance that one or more of such small nodules will progress.

Should prophylactic TH therapy be recommended for patients without abnormal thyroid findings? Although the answer is usually no, prophylactic therapy may be reasonable for patients with an especially high risk profile (e.g., high-dose radiation therapy, young age at exposure, other radiation-related tumors, siblings with radiation-related tumors, being female, and high serum Tg level).

Follow-Up

Based on current evidence, it is best to assume that the risk for radiation-related thyroid tumors persists indefinitely. Therefore, the evaluation described in the previous subheadings should be repeated every 1–2 yr. The recommended frequency of thyroid imaging varies. If it is performed for screening purposes, then about every 5 yr is reasonable. In patients with specific findings, more frequent imaging is indicated, in some cases annually. Because the long-term survival of childhood-cancer patients has increased substantially, clinicians should be especially aware that these patients have an increased risk of developing thyroid cancer.

Prevention

What can be done to minimize any future radiation-related cases of thyroid cancer? In the medical setting, radiation is no longer used to treat benign childhood conditions, other exposures are minimized with appropriate shielding, and healthcare workers are monitored carefully. However, malignancies still require radiation therapy, and some diagnostic radiographic procedures are used widely *(136)*. In fact, the use of pediatric computed tomograpy is increasing rapidly. Monitoring is indicated if the thyroid is substantially exposed to radiation.

Unfortunately, it remains possible that RAI could be released from a nuclear power-generating facility, either as a result of an accident like that at Chernobyl or an attack. It is well-known that exposure of the thyroid to radiation can be greatly reduced by the prompt, ideally prior, ingestion of potassium iodine (KI) *(137,138)*. Public-health officials and others should be working on a response to RAI releases that includes protection of the food chain, evacuation, and the timely availability of KI.

FUTURE LESSONS: *RESEARCH DIRECTIONS*

Three research themes will continue in the area of radiation-induced thyroid and other neoplasms. First, several of the long-term studies cited above will continue. This is important, because lifetime follow-up is necessary to understand the full effects of radiation exposure and the natural history of radiation-related neoplasms. Radiation effects begin after a variable latency period and increase until they reach a peak. The effects then diminish, but they are long lasting and probably indefinite *(22,139)*. For example, after the atomic bombings of Japan in the Second World War, the excess risk of leukemia was highest several years following the bombings, but the risk of leukemia has remained elevated even until now, more than 50 yr later *(140)*. Thyroid cancer after childhood irradiation follows a similar pattern, but the peak occurs later *(2,57)*. Because sporadic thyroid cancer is more aggressive in older patients, the behavior of radiation-related thyroid cancer in older individuals is an important matter for study.

The importance of the massive radiation release in the Chernobyl accident, and of the belated admission of radiation exposures during the Cold War, cannot be underestimated.

The psychosocial impact of exposure is equivalent to, or even greater than, what is more traditionally considered "health effects." This is true in the areas surrounding nuclear facilities in the United States, the former Soviet Union, and elsewhere. Adding to this is the long delay before the governments involved acknowledged the levels of exposure, making epidemiologic studies, such as the one in the Hanford, Washington area, especially difficult. Credible risk estimates are needed to help deal with the anxiety of people who lived in exposed areas.

The second research theme will be studies on the genetic determinants of radiation susceptibility. Although important ethical issues are involved, it is conceivable that in the future it will be possible to identify genetic-susceptibility factors and susceptible individuals. Given the sensitivity of the thyroid, it may play a role in such studies.

The third research theme on radiation and thyroid cancer will be studies at the molecular level aimed at understanding the pathogenetic mechanisms and host defenses. The list of somatic genetic mutations in thyroid cancer is long and diverse *(141)*. It is likely that radiation, a potent mutagen, causes cancer by such mutations. However, it remains unclear whether radiation-induced cancers could be identified by the presence of specific mutations. If this were possible, it would have important implications for epidemiologic studies and, possibly, for the diagnosis and treatment of thyroid cancer. In epidemiologic studies, mutation-identification of cancers would permit assigning a probability to a given case as to whether or not it resulted from radiation exposure. In the clinical setting, mutation-identification of cancers would alert a physician to the need to look for other neoplasms associated with radiation.

Magnitude and Duration of Risk

Since the rate of thyroid cancer in the general (nonirradiated) population increases with age, it is more difficult to confirm a continuing radiation effect in older individuals. The conclusion that there is a continuing excess risk of thyroid cancer in persons who have been exposed to radiation is based on the epidemiologic investigations referred to above. An increased understanding of genetic mechanisms indicates that radiation may induce mutations in a set of cancer-producing genes and/or may induce mutations in genes that are important in maintaining genomic integrity. The latter may be responsible for the more prolonged effects of radiation.

As mentioned above, the clinical course of external-radiation-related thyroid cancer is generally the same as that of sporadic thyroid cancer. Thus, the more aggressive behavior observed among older patients with differentiated thyroid cancer may be seen in irradiated patients. How the cases in the Chernobyl area will behave requires continued attention.

It remains important to study the full-life experience leading to the late health effects of radiation exposure. Where risks have already been identified, additional information on dose–response relationships, time factors, and interactions will be useful. Additional sensitive sites and nonneoplastic effects also may be identified.

Identifying Susceptibility Factors

It is very likely that not all individuals are equally susceptible to the effects of radiation. An increasing awareness of familial genetic factors that predispose to cancer has led to the identification of several genes involved in malignancy. Germline mutations in the *p53* gene and the *ret* gene are associated with the Li–Fraumeni and the MEN2 syndromes, respectively. There is an extremely high susceptibility to ultraviolet-radiation-induced cancers in familial syndromes (e.g., xeroderma pigmentosa, which is associated with mutations in the genes

involved in the repair of DNA damage). Children with ataxia telangiectasia are sensitive to ionizing radiation. However, it is not proven that there are more subtle variations in individual sensitivity. The most suggestive data come from a study of presumed heterozygotes for the ataxia telangiectasia gene. Among first-degree relatives of patients with this disorder, an increase in radiation-related breast cancer has been reported *(142)*. The *ATM* gene is central to the cellular response to radiation damage *(143)*, making it a natural candidate for study. Other genes involved in radiation damage repair are also candidates *(144)*.

To prove the existence of susceptibility factors for thyroid cancer, the initial challenge is to identify the phenotype and familial patterns of the phenotype. This is difficult because radiation exposure cannot be used to identify a phenotype in vivo, although it is possible with in vitro methods. In the Michael Reese Hospital study, multiple radiation-related tumors occurred in many individuals *(107,108)*. Among siblings, an elevated rate of concordant thyroid neoplasms was observed *(109)*. However, these studies do not differentiate between genetic and other shared factors, and do not define a specific phenotype for genetic susceptibility. In the future, if a phenotype could be identified, it could be linked to an area of the genome and then to particular genes.

Pathogenesis: What is the Radiation-Induced Lesion?

Although much remains to be understood, it is increasingly clear that somatic mutations play a central role in the pathogenesis of neoplasms, including thyroid cancer. Frequent mutations in the *ras* and PPARgamma1 genes are found in follicular neoplasms, *ret*-gene rearrangements are common in papillary thyroid cancer, and *p53*-gene mutations are found in anaplastic thyroid cancer.

The *ret* gene encodes a transmembrane tyrosine kinase that forms part of the receptor for neurotrophic factors. In many papillary thyroid cancers the gene is activated by genomic translocation *(145)*. The tyrosine kinase domain of *ret* is translocated to one of three genes, also called translocation partners (forming *ret*/*PTC1*, *ret*/*PTC2*, or *ret*/*PTC3*, the first being most common), so that its expression is controlled by the promoters of these other genes. (Other rare translocation partners have been found, and several variants of *ret/PTC3* have been described.) As a result, the expression of *ret*, which is normally low in thyroid follicular cells, is increased. Also, since the transmembrane and extracellular domains of the resulting receptor are lost, the tyrosine kinase domain is free in the intracellular compartment. In some cases *ret* expression is increased without genomic translocation.

In radiation-related cancer, DNA double-strand breaks are thought to be the most important lesions *(144)*. Therefore, it is in accord with this concept that in radiation-induced thyroid cancer *ret* rearrangements play a central role. Much of what has been learned about this comes from studies of Chernobyl cases, among which papillary cancer is the predominant type of cancer and *ret* gene rearrangements are common. A pattern of findings is emerging from a large number of studies, and only a brief summary of conclusions is possible here. First, the frequency of *ret* rearrangement in the Chernobyl cases is far higher than would be expected from cases not related to radiation, as reviewed by Pacini and colleagues *(146)*. Second, *ret* rearrangements are associated with the solid variant of papillary thyroid cancer *(147)*. Third, the earliest cases contained *ret/PTC3* more frequently than *ret/PTC1*, in contrast to cases unrelated to radiation *(148,149)*. Fourth, over time the proportion of *ret/PTC3* is declining *(150)*. The last observation suggests a correlation between the type of *ret* rearrangement and the behavior of the cancer, an important observation that requires additional attention. It does not appear that any pattern of *ret* rearrangement, at the level of the expressed mRNA, will distinguish radiation-related cases.

The *ret* rearrangement involves DNA double-strand breaks at two chromosomal locations. The exact locations of the breakpoints have been determined in a limited number of *ret*/*PTC3* cases, but the interpretation is not yet resolved. Nikiforov and colleagues *(151)* proposed a spatial correlation between the break sites on the two genes that can be demonstrated by aligning them in an antiparallel orientation. Klugbauer and colleagues *(152)*, based on additional cases, could not show this, and instead found areas of microhomology near the recombination sites. It remains unclear how many radiation-produced double-strand breaks occur for the rearrangement. Radiation may produce one break, followed by homologous recombination, or it may produce two breaks, followed by nonhomologous end-joining. Nikiforova and colleagues *(153)* have made a particularly intriguing observation. They found that *in situ*, the gene for ret and the gene for the translocation partner used to form *PTC3 (ELE1)*, although they are 30 megabases apart, are situated close to each other in the nucleus. This may account for the selection, from a presumably unlimited number of possible partners, of the translocation partners utilized by *ret*. This observation may have wide implications for carcinogenesis, and could even have clinical implications, leading to methods of protection against radiation.

The *p53* tumor-suppressor gene appears to play a role in the genesis of anaplastic thyroid cancers, but in sporadic differentiated cancers, most studies have failed to find *p53* mutations. In contrast, two studies of radiation-related differentiated thyroid cancers have reported *p53* mutations *(154,155)*. In the Michael Reese Hospital study, 4 of 22 (18%) cases showed mutations *(155)*, whereas in the study of childhood Chernobyl cases, 6 of 26 (23 %) cases had *p53* mutations *(154)*.

SUMMARY

The problem of radiation-induced thyroid cancer remains. Even persons irradiated several decades ago are still at risk for thyroid cancer. Many people are becoming aware of previous radiation exposures. Radiation continues to be used in the medical setting, and the possibility of accidental exposure exists. Individuals exposed to head and neck radiation need to be evaluated to assess their risk of thyroid and other radiation-associated cancers. Understanding radiation-induced neoplasms at the molecular level is progressing and should lead to important insights.

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13 Management of Thyroid Cancer

Stephanie L. Lee, MD, PhD

Contents

INTRODUCTION

Thyroid carcinomas are malignancies arising from either follicular epithelial or parafollicular thyroid cells. Thyroid epithelial cancers are characterized as differentiated or anaplastic. The differentiated thyroid cancers, papillary and follicular carcinomas, constitute 90–95% of all thyroid cancer. Medullary thyroid carcinoma arising from parafollicular or C cells accounts for 3–5% of thyroid carcinomas. Primary thyroid lymphoma is also an uncommon thyroid malignancy, accounting for 3–5% of thyroid malignancies. Medullary thyroid carcinoma is a neuroendocrine tumor of parafollicular or C-cell origin, and accounts for 3–5% of thyroid carcinomas. Carcinomas metastatic to the thyroid include breast, colon, and renal carcinomas; melanoma is also metastatic to the thyroid.

Clinically significant thyroid cancer is an uncommon neoplasm. The estimated incidence of thyroid carcinoma in the United States has risen steadily from 12,200 in 1996 to 20,700 in 2002 *(1)*. The increase in papillary thyroid carcinoma is responsible for the rise. As shown in Fig. 1, most of the increase in cases has occurred in women, with a female to male gender ratio of 3:1. Thyroid carcinoma has risen in incidence from 1–2% of reported cancers in women over the past decade. Although it is believed that this increase in incidence is partly explained by increased surveillance and detection, there is some concern about a true increase from unknown environmental factors. Thyroid cancer is now the eighth most common carcinoma in American women. Variations in its incidence have been noted between ethnic groups *(2,3)* and among geographic regions *(3)*. A similar increase in the incidence of

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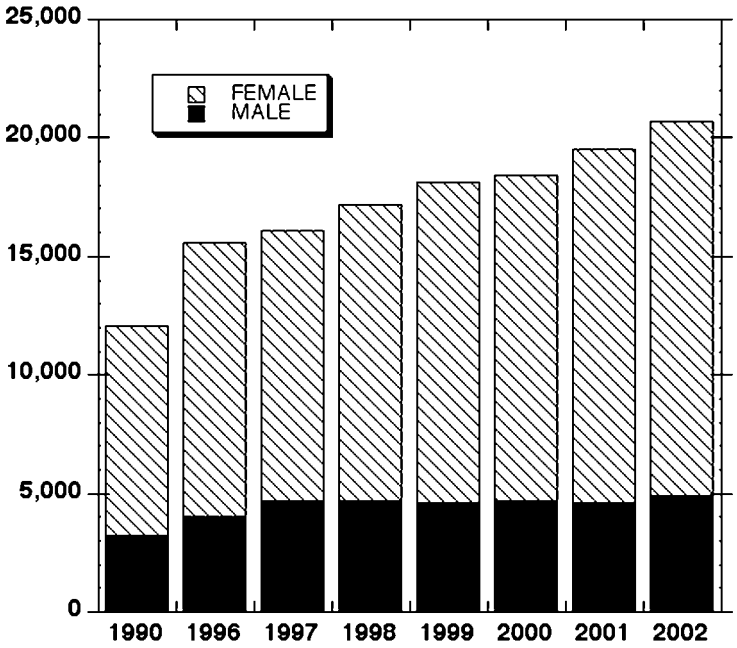

Fig. 1. Annual estimated incidence of thyroid carcinoma in the United States for males and females. Data obtained from the American Cancer Society ***(1)***.

thyroid cancer has been reported in Canada *(4)*. Although the incidence of this cancer is low, the low mortality from it results in its having a high prevalence rate *(5)*.

OCCULT THYROID CARCINOMA

Papillary microcarcinomas (≤1.0 cm in diameter) are common. These occult carcinomas have been reported in 6–37% of consecutive thyroid glands in autopsy series *(6–10)*. The difference in prevalence rates reported for these occult carcinomas is related to geographic differences, extent of radiation exposure, amount of resected thyroid tissue processed for histiologic assessment, number of sections examined per specimen, and differences in the assessment of nuclear features for diagnosis *(8,11)*. When the entire thyroid is sliced into very thin sections for histologic examination, the incidence of occult carcinoma increases into the 30–40% range (personal communication, Dr. Ronald DeLellis, Deparment of Pathology, Cornell University Medical School, New York City, NY).

Occult papillary carcinomas are frequent incidental findings in thyroid glands resected for other indications. They are not clinically relevant unless multicentric *(12,13)* or associated with metastases. The risk of mortality is 0.2%, and the recurrence rate is 6–8% after removal of a solitary micropapillary carcinoma without metastatic spread *(13)*. The excellent overall prognosis in cases of micropapillary carcinoma has led to the recommendation that lobectomy plus isthmusectomy constitute adequate surgery for a solitary micropapillary carcinoma of <1 cm without metastases. If the tumor is found to be multifocal, associated with adenopathy, or of a follicular histologic subtype, completion thyroidectomy is recommended. It should be noted that without completion thyroidectomy and ^{131}I ablation therapy, recurrence of thyroid cancer cannot be identified by measurement of serum thyroglobulin (Tg) levels or radioactive iodine (RAI) whole-body scans.

Detection of nonpalpable thyroid nodules by radiographic imaging studies is common, and occurs in >50% of adults older than 65 yr *(14)*. Most of these nodules are usually histologically benign and rarely consist of occult papillary microcarcinomas. It is not necessary to biopsy nonpalpable nodules <1.0 cm in diameter unless they grow or become palpable.

PATHOLOGY, PROGNOSTIC FEATURES, AND TREATMENT OF DIFFERENTIATED CANCERS

Differentiated Thyroid Carcinoma

Three major categories of carcinoma are derived from thyroid follicular cells: papillary, follicular, and anaplastic *(15)*. The most common thyroid carcinomas, papillary and follicular carcinomas, are grouped together and discussed in this section as differentiated thyroid cancers. It is thought that the prognosis is similar for the differentiated cancers when based on the extent or spread of disease. The biologic behavior of these carcinomas is different, however, and must be taken into account in their management. A number of algorithms for prognostic scoring have been proposed, based on the patient's age, the size of the primary tumor, and the presence of vascular invasion, extrathyroidal invasion, and local or distant metastases *(16–21)*. Appropriate classification of tumors as benign or malignant, and distinction among the major categories of carcinoma, can vary with the level of training and the personal bias and experience of the pathologist *(15)*. It is reasonable to obtain second opinions from experts in thyroid pathology at other institutions if there is a question of malignancy.

Papillary Thyroid Carcinomas

Papillary carcinomas constitute approx 85% of primary thyroid malignancies *(22,23)*. Papillary carcinoma is an unencapsulated tumor that occurs in a younger population than follicular thyroid carcinoma. One of the largest American cohorts of patients with thyroid cancer is followed at the Mayo Clinic. Most of these patients presented when 30–60 yr old *(24)*. The thyroid follicular epithelium in papillary thyroid carcinoma is often seen as folded, monolayered sheets, with a central fibrovascular core that has the appearance of fingerlike or papillary projections. Current diagnostic standards also require the demonstration of characteristic nuclear features, including overlapping nuclei with a ground-glass appearance, nuclear grooves, and infolding of cytoplasm or intranuclear inclusions. The histologic variants of papillary thyroid carcinoma, including the follicular variant and columnar-cell, clear-cell, and diffuse sclerosing variants have the distinctive nuclear characteristics described above. The usual papillary carcinomas including the follicular variant of papillary thyroid carcinoma and the mixed follicular-papillary carcinomas, constitute 90% of papillary carcinomas *(25)*. These tumors are generally slow growing, are multicentric in 26–32% of patients, and are bilateral in about 20–25% of patients *(13,24–26)*. The carcinoma spreads through lymphatics within the thyroid to the regional cervical (Fig. 2) and upper mediastinal (Fig. 3) lymph nodes in 38–43% of patients *(13,24–26)*. Distant spread, usually to the lung, is found in 2–4% of adult patients at the time of diagnosis (Fig. 3).

Some variants of papillary thyroid carcinoma—tall-cell *(27–29)*, columnar-cell *(30–32)*, sclerosing *(33)* and oxyphilic *(34,35)* variants have more aggressive growth characteristics and metastatic potential than the usual variants of papillary carcinoma, with a greater mortality.

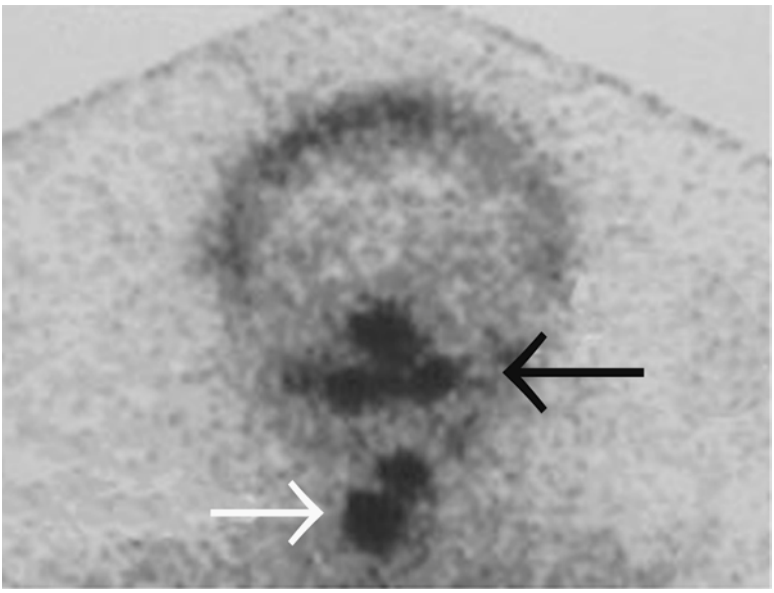

Fig. 2. ^{131}I whole-body scan of thyroid remnant and thyroid cancer metastatic to a cervical lymph node. Anterior view of head and neck was obtained 48 h after the administration of 2.0 mCi of ^{131}I. Image was obtained with a large-field-of-view γ-scintillation camera with a high-energy parallel-hole collimator. The patient was a 35-yr-old woman who had undergone near-total thyroidectomy for a 3-cm papillary thyroid carcinoma, with a thyroid remnant in the right thyroid bed. Black arrow shows normal physiologic secretion of RAI in the nasooropharynx. White arrow shows RAI uptake into the thyroid remnant (lower foci) and into cervical metastases of the patient's thyroid cancer (upper foci).

Follicular Thyroid Carcinomas

Follicular carcinoma typically occurs in an older population than does papillary thyroid carcinoma. Follicular carcinomas are encapsulated, with capsular and vascular invasion. Clinically, these tumors are characterized as being of minimal or high risk according to the degree of vascular and capsular invasion and presence of distant metastases. The tumors tend to be uninodular and have less lymph node involvement than papillary carcinoma, but a higher frequency of distant metastases to the lungs and bones (Fig. 4) from hematogenous spread *(36–38)*.

Follicular carcinomas generally have a more aggressive clinical course and higher mortality than the papillary thyroid carcinomas if the tumor at presentation shows extensive capsular *(36,39)* or vascular invasion and/or distant metastases *(36,40)*. Two variants of follicular thyroid carcinoma, Hürthle cell (oxyphilic) carcinoma *(41)* and insular carcinoma *(42,43)*, may be associated with poorer outcomes than the usual follicular carcinoma.

Prognostic Features of Differentiated Thyroid Carcinomas

The 10-yr survival of patients in whom differentiated thyroid carcinoma is diagnosed at age <40 yr is >95%, whereas for patients in whom it is diagnosed at 40–59 yr of age it is approx 80% *(13,21,44)*. Several prognostic scoring schemes have been proposed, based on retrospective case analyses, in an attempt to stratify prognostic risks *(16–20)*. DeGroot et al. *(22)* used five different prognostic scoring schemes to evaluate 269 patients, and all of these schemes predicted disease-specific mortality in the lower-risk groups of patients. This suggests that none of the prognostic scoring schemes accurately predicts mortality risk

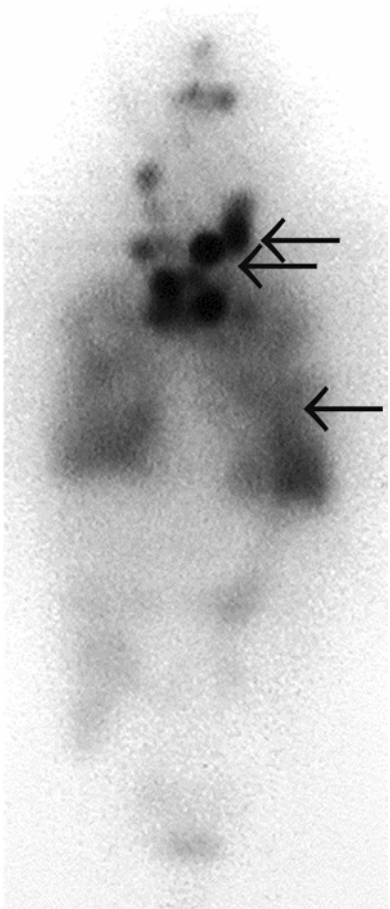

Fig. 3. ^{131}I whole-body scan of papillary thyroid cancer with regional adenopathy and diffuse pulmonary metastases. The patient was a 70-yr-old male presenting with right vocal-cord paralysis from a 5.0-cm primary papillary thyroid carcinoma invading the right laryngeal nerve. A ^{131}I whole-body scan was done 48 h after a dose of 2 mCi of ^{131}I. ^{131}I uptake is seen in multiple metastatic lymph nodes in the cervical neck and upper mediastinum, as well as in diffuse pulmonary metastases not seen on a CT scan. Single arrow: diffuse uptake of ^{131}I in the lungs, by micrometastases. Double arrow: regional metastases in the cervical neck and upper mediastinum.

for all patients. Therefore, clinicians should know the individual factors that contribute to prognostic risk in their patients with thyroid cancer.

Age at Diagnosis

Most studies of differentiated thyroid carcinomas have documented increased tumor-specific mortality in older patients. Most studies agree that the age at diagnosis at which the risk of mortality increases is between 45 and 50 yr old *(26,41,44)*. Older age is associated with more extensive local invasion, a higher frequency of distant metastases, and a greater tendency toward dedifferentiation. On the other hand, children under 18 yr old do not have a high mortality rate despite their higher rates of extrathyroidal invasion, lymphatic metastases, pulmonary metastases, and clinical relapse *(21,45,46,47)* compared to middle-aged adults. It is likely that these childhood cancers are more responsive to therapy with surgery and RAI than are thyroid cancers in adults.

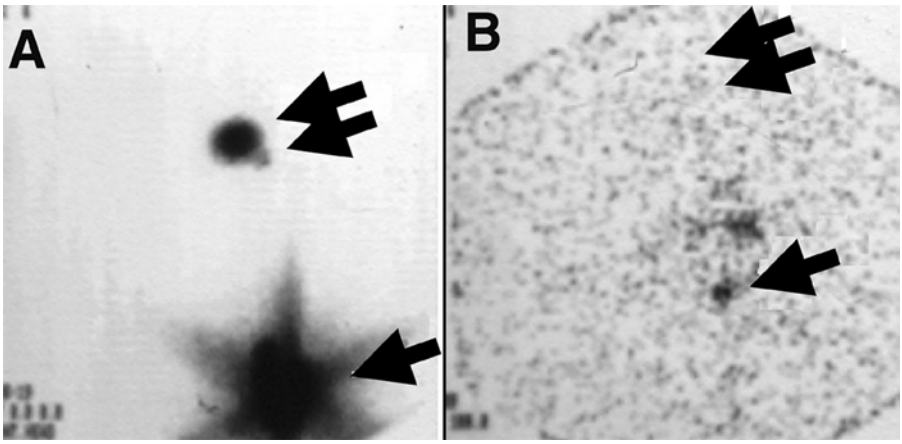

Fig. 4. ^{131}I whole-body scan of follicular thyroid cancer metastatic to the skull. After thyroidectomy and dosimetry, the patient was treated with a maximal dose of 350 mCi of ^{131}I with resolution of metastatic uptake in bone. A plain film of the skull was normal. Patient's Tg levels were subsequently unmeasurable with or without levothyroxine suppression. **(A)** ^{131}I uptake into skull metastasis and secretion into nasooropharynx. **(B)** Resolution of ^{131}I uptake into skull lesion. Single arrow: physiologic secretions of RAI into nasooropharynx. Double arrows: follicular carcinoma metastatic to skull.

Gender

Multivariate analyses suggest that male gender is an independent prognostic risk factor for mortality from thyroid cancer *(44,48,49)*. However, epidemiologic studies have not shown that reproductive status or exogenous estrogen therapy alters thyroid-cancer risk *(50,51)*.

Tumor Size

Multivariate analyses have verified the importance of the size of the tumor to the mortality risk. The analyses suggest that the risk increases when a tumor exceeds a threshold diameter. Although mortality risk is absent for papillary carcinomas of <1 cm in diameter *(13)*, follicular carcinomas do not have a minimum diameter associated with the absence of mortality. There is no "occult" follicular thyroid carcinoma. The small, <1.5-cm, follicular carcinoma should be examined in terms of other risk categories, such as extent of capsular and vascular invasion. Mortality from thyroid cancer increases with the size of the primary tumor. In a large retrospective study, the 20-yr mortality for papillary thyroid carcinoma was 0.8% for tumors <2.0 cm, 6% for tumors 2.0–3.9 cm, 16% for tumors 4.0–6.9 cm, and 50% for tumors >7 cm in diameter *(19)*. Likewise, an analysis for follicular thyroid carcinoma demonstrates a worse 20-yr mortality for larger primary tumors of approx 80% for tumors <2.9 cm compared to approx 70% for tumors >5 cm in diameter *(51,52)*.

Extrathyroidal Invasion

Invasion of differentiated thyroid carcinoma through the thyroid capsule and into extrathyroidal tissues, such as connective tissue, fat, skeletal muscle, esophagus, or tracheal cartilage, is a significant indicator of poor prognosis, reflecting aggressive tumor behavior with an increased recurrence rate and increased disease-specific mortality *(12,13,52–55)*.

Differentiated Thyroid Carcinoma and Metastases

The reported incidence of local lymph node metastases ranges from 37–65% in different studies of papillary thyroid carcinoma, and is 6% in follicular thyroid carcinoma *(13,23,52)*.

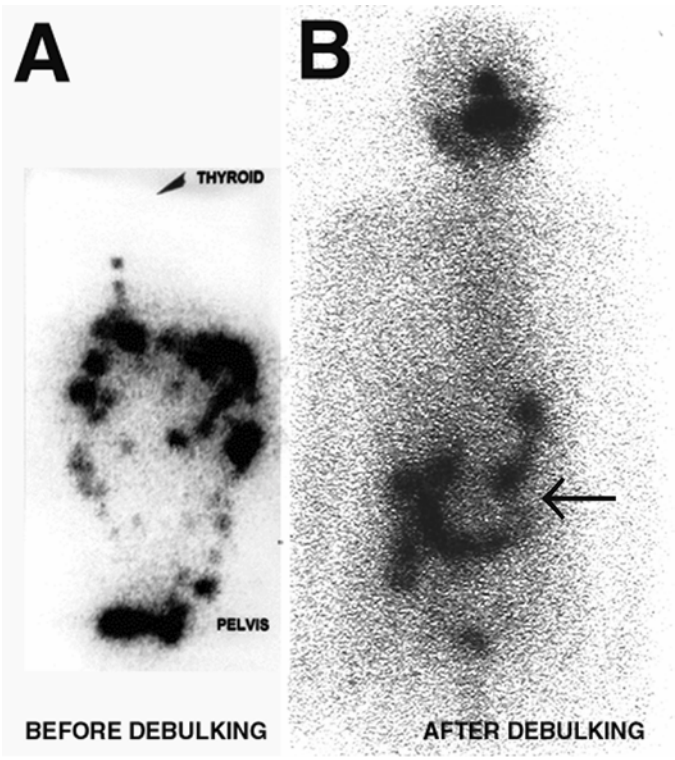

Fig. 5. ^{131}I whole-body scan of follicular thyroid cancer from a struma ovarii metastatic to the abdomen and pelvis. After a surgical procedure to debulk the metastatic tumor, and dosimetry, the patient was treated with a maximal dose of 300 mCi of ^{131}I with resolution of the abnormal ^{131}I uptake and increased Tg levels. **(A)** Before surgical debulking with multiple metastases in the abdomen and pelvis. **(B)** After surgical debulking and 300 mCi ^{131}I. Arrow: normal physiologic secretion of RAI in gastrointestinal tract and bladder.

The significance, if any, of unilateral cervical metastases (Fig. 2) of the differentiated thyroid cancers is minimal, while bilateral disease from papillary thyroid carcinoma may just reach statistical significance *(13,56)*. Distant metastases (Figs. 3–5) are the most significant indicator of a poor prognosis in differentiated thyroid carcinoma, with a tumor-specific mortality that rises from 36–47% at 5 yr to approx 70% at 15 yr *(13,18, 20,21,24,26,37,38,41,44,48,57)*.

TREATMENT OF DIFFERENTIATED THYROID CARCINOMAS

Overview

The treatment of thyroid cancer remains controversial *(58)*, in part because of the absence of large prospective multicenter studies evaluating the efficacy of the treatments outlined in the following subheadings. A prospective study has been difficult to design and implement because of the slow-growing nature of the differentiated thyroid cancers, which would require decades of clinical follow up to yield statistically significant endpoints. As a consequence, large clinical studies are retrospective and complicated by a multitude of different therapeutic approaches that have changed with time.

Initial Surgical Treatment

Surgical Management

The goal of surgery for differentiated thyroid carcinoma is to remove all cancer tissue in the neck, including the thyroid and cervical lymph nodes that contain metastases *(59,60)*. Appropriate surgery is a lobectomy and isthmusectomy for low-risk patients who have a unifocal, micropapillary thyroid cancer (≤1.0-cm diameter), no history of significant exposure to ionizing radiation, a clinically normal contralateral lobe and no ipsilateral adenopathy. Essentially all other thyroid carcinomas require total or near-total thyroidectomy leaving no more than 1–3 g of normal thyroid tissue. Total or near-total thyroidectomy reduces recurrence rates to a greater extent than does lobectomy for papillary thyroid carcinomas, because this tumor is often multifocal and bilateral. Removal of more thyroid tissue facilitates total ablation of the normal thyroid gland by RAI therapy.

If, after a thyroid lobectomy, a follicular neoplasm is determined to be a follicular carcinoma or a follicular variant of papillary thyroid carcinoma on the basis of a review of surgical sections, a completion thyroidectomy should be scheduled before significant scarring develops, usually within a few days or a week after surgery *(59,60)*.

The patient should have an intraoperative assessment for palpable adenopathy, especially if the primary tumor is a papillary thyroid cancer. Palpable adenopathy should be removed, since RAI therapy is unlikely to completely ablate macroscopic metastases. In the situation of ipsilateral adenopathy, many clinicians recommend a formal lymph-node dissection with papillary thyroid carcinoma, including the central compartment (paratracheal and tracheoesophageal regions), the ipsilateral supraclavicular area, and the lower third of the jugulocarotid chain *(61)*. Node sampling is frequently done because the more extensive dissection surgery has not been shown to reduce recurrence rate or improve survival. The argument for more extensive dissection is that 40–60% of adult patients will have adenopathy. Of these patients with metastases, 80% will have tumor within the central compartment *(62)*. A modified neck dissection and removal *en bloc* of lymph nodes is suggested for patients with extensive nodal disease and invasive primary tumors. Radical neck dissections are not necessary for the usual differentiated thyroid carcinoma *(60)*.

Morbidity of Thyroid Surgery

Morbidity from thyroidectomy is uncommon with an experienced thyroid surgeon. Complications associated with a thyroidectomy include damage to the recurrent laryngeal nerve, permanent hypoparathyroidism, and postoperative hematoma *(60,62)*. A prospective study of 500 consecutive thyroidectomies showed that 6.6% developed postoperative unilateral vocal-cord paralysis, with 1.0% of the patients having recognizable nerve damage during the operation *(63)*. Complete recovery of vocal-cord function was documented in 93% of these patients. No bilateral vocal-cord palsy or paralysis was reported. Unilateral vocal-cord paresis or paralysis may result in a breathy, hoarse voice. Bilateral paralysis will cause a median position of the vocal cords, resulting in a functional tracheal obstruction and need for intubation and tracheostomy to maintain a patent airway.

Often unrecognized is damage to the external branch of the superior laryngeal nerve, resulting in easy voice fatigue and a decreased pitch range, with inability to "raise the voice" *(64)*. Damage to the internal branch of the superior laryngeal nerve, which is involved in the sensory reflex that protects the airways during swallowing, results in choking *(65)*. Serious complications should not occur with lobectomy, because of the bilateral location of the recurrent laryngeal nerves and the parathyroid glands. The incidence of these complications is very low with surgery done by experienced thyroid surgeons.

Temporary postoperative hypocalcemia occurs in approximately 2% of patients who undergo thyroid surgery. The hypocalcemia is usually a consequence of surgical trauma to parathyroid glands or their blood supply, and resolves spontaneously after several days of oral calcium therapy *(62)*. Severe, symptomatic, or prolonged hypocalcemia occurs in approximately 0.5% of postoperative thyroidectomy patients. Mild hypocalcemia with total calcium levels 7.5 and 9 mg/dL requires oral calcium (1.5–3 g of elemental calcium) and supplemental vitamin D therapy, usually in the form of calcitriol (0.25–0.5 μg once or twice daily). The therapeutic target is an ionized calcium or total calcium corrected for serum albumin level in the slightly low to low normal range. If the serum calcium falls below 7.5 mg/dL, calcium should be given intravenously. Persistent postoperative hypoparathyroidism can be confirmed by a persistent increase in serum phosphorus levels because of the loss of the phosphaturic effect of parathyroid hormone.

RAI Scanning and Therapy

General Concepts of RAI Scans and Treatments

Approximately 80% of differentiated thyroid carcinomas retain the ability to take up RAI but at a much lower efficiency than normal thyroid follicular cells. RAI (iodine-131, ^{131}I) is administered after thyroid surgery, to destroy the thyroid remnant and metastatic disease, to allow the use of serum Tg as a marker for tumor persistence or recurrence, and to decrease the long-term risk of tumor recurrence and death. Ablation of the thyroid remnant is necessary to remove the large amount of iodine uptake by the normal thyroid-tissue remnant so as to make possible the detection by subsequent ^{131}I scintigraphy scans the much less intense RAI uptake by metastatic disease. The large amount of Tg made by normal thyroid tissue makes this test of little diagnostic importance as a tumor marker unless the patient has previously had both a near-total or total thyroidectomy and ablation therapy with ^{131}I *(66)*.

Diagnostic RAI Whole-Body Scan After Thyroidectomy

Radioactive decay of ^{131}I releases a β particle that travels only 1–3 mm. Along the path of its travel, the β particle collides with cells, causing damage and thyroid-cell destruction. The radioactive decay of ^{131}I also releases a γ ray that allows radionuclide scanning. For many years, postoperative diagnostic scans of the thyroid were done with ^{131}I. Over the course of time, larger doses of ^{131}I were given, to increase the sensitivity of the scan. As the ^{131}I doses increased from 1 mCi to 10 or more mCi, a phenomenon of "stunning," which results in a reduction of the expected uptake of ^{131}I in the therapeutic dose of ^{131}I, was observed *(67,68)*. A diagnostic scan dose of 3 or 5 mCi of ^{131}I resulted in a subsequent reduction of ^{131}I uptake, producing a reduction in therapeutic dose of ^{131}I in 40% and 67% of patients, respectively. The clinical importance of stunning has not been studied in a systematic manner to determine whether it affects long-term patient survival.

After the initial reports of stunning, diagnostic scans after thyroidectomy were performed with lower doses of ^{131}I, ranging from 1–3 mCi. Recent data suggest that postthyroidectomy scintigraphy scans with ^{123}I before ^{131}I ablation may be superior to ^{131}I scans because of superior quality of the images as a result of the difference in γ-ray energy emitted, and also because the typical dose of 1–5 mCi ^{123}I does not cause stunning. ^{123}I is a pure γ-ray emitter and does not release a β particle with radioactive decay. ^{123}I can be used for thyroid scintigraphy scanning, but cannot be used for ablation therapy. Whole body scans with ^{123}I (5 h and 24 h after a 1.5-mCi dose of ^{123}I) and with ^{131}I (48 h after a 3-mCi dose of ^{131}I) were compared to a posttreatment scan after ^{131}I ablation in the same hypothyroid

postthyroidectomy patient. Thirty-five foci of RAI uptake were seen on the posttherapy ^{131}I and ^{123}I scans, with excellent concordance in size and location of foci. Thirty-two of the 35 foci were seen on the pretherapy ^{131}I scan. One focus was seen in the ^{123}I and diagnostic ^{131}I scans, but not on the posttherapy scan, suggesting a phenomenon of stunning. The study investigators concluded that the 1.5-mCi ^{123}I scan had an improved quality of imaging without the potential for stunning, as compared with ^{131}I pretherapy scans *(69)*. The disadvantage of ^{123}I scanning is the increased cost and short half-life of the isotope as compared with ^{131}I.

^{131}I Treatment of Differentiated Thyroid Carcinoma

Postoperative ^{131}I therapy is an important part of the treatment of patients with significant differentiated thyroid carcinomas of >1.5 cm in diameter. The ^{131}I therapy will destroy microscopic carcinoma and therefore decrease the long-term risk for recurrent disease *(13,22,26,44,70)*. Using decision analysis based on multiple published studies, the recurrence rate of thyroid carcinoma has been calculated to be reduced by as much as 50% by ^{131}I therapy *(71)*. In a large American study of stage II or III tumors (cervical adenopathy or direct tissue invasion), the recurrence rate at 35 yr after surgery was reduced from approximately 35% to <8% *(13)*. A reduction in mortality has been shown in large retrospective studies if the primary tumors ranged in size from >1–1.5 cm, were multicentric, or showed soft-tissue invasion at diagnosis *(13,18)*. There is no evidence that ^{131}I therapy of low-risk tumors (<1 cm) benefits the patient.

Determining ^{131}I Therapy Doses

The three methods of determining the therapeutic dose of ^{131}I for differentiated thyroid cancer are empiric, single-lesion dosimetry, and maximal-tolerated-dose dosimetry. The most widely used method is empiric therapy, which is based on the extent of disease. An oral dose of ^{131}I of 29.9–100 mCi is given for remnant ablation, 150 mCI for cervical adenopathy, and 175–200 mCi for distant disease. "Single-lesion dosimetry," proposed by Maxon, uses measurements of the radiation dose delivered to a specific focus from a small diagnostic dose of ^{131}I in order to prescribe the minimum amount of ^{131}I needed for ablation. Maxon demonstrated that ^{131}I ablation of thyroid remnants requires a delivered dose of 30 Gy, and that regional nodal metastases are ablated when 8–10 Gy is delivered *(72,73)*. These results suggest that cervical metastases with very low or absent ^{131}I uptake cannot take up adequate amounts of ^{131}I to be ablated, and should not be treated. Maxon found a nearly equal effectiveness of empiric dosing and dosimetrically determined dosing of ^{131}I for the ablation of thyroid remnants (86% vs 84%) and for cervical metastases (68% vs 81%) *(74)*. The concept that a threshold delivered dose is important for adequate therapy was confirmed by studies showing that if >10 Gy was delivered, three of four cervical metastases were ablated, whereas if 3–4 Gy was delivered, only two of eight lymph nodes were treated *(75)*. If <3.5 Gy was delivered to treat cervical adenopathy, no response of lesions was observed. The dosimetrically determined doses of ^{131}I were often significantly lower than the empiric doses described in the literature, of 100 mCi of ^{131}I for thyroid remnants and 150 mCi of ^{131}I for local cervical metastases. The long time required of a nuclear technician and the need for a γ-ray camera for dosimetry studies makes this method of determining ^{131}I doses impractical for the typical patient with differentiated thyroid cancer and minimal disease or local cervical metastases.

No data are available about the effectiveness of single-lesion dosimetry and distant metastatic disease. Empiric dosing is successful in 50% of cases of pulmonary disease and 9% of cases of bone metastases *(37,70)*. The smaller the pulmonary metastases the more likely that RAI will be effective. Remission is seen in 83% of patients with microscopic pulmonary disease (not seen on chest X-ray), 53% of those with micronodules, and 14% of those with macronodules. Bone lesions are resistant to RAI therapy, but anecdotal reports of complete response have been described for microscopic disease that cannot be seen on plain bone films (Fig. 4).

The third method of determining an RAI dose is maximal-dose therapy. The currently used method for maximal-dose therapy is based on a combination of protocols from Beierwaltes *(76)*, Benua et al. *(77,78)*, Leeper and Shimaoha *(79)*, and Van Nostrand, Nevtze, and Atkins *(80)*. This method determines the maximally tolerated dose of ^{131}I that avoids marrow suppression and pulmonary fibrosis; it is used only for life-threatening, invasive local disease and distant metastases. Van Nostrand in a recent review discusses the rationale and techniques for the two major approaches for dosimetry-determined doses of radioiodine *(81)*.

Whole-Body Scan Following RAI

The therapeutic dose of ^{131}I used for thyroid cancer allows a posttherapy scan performed approximately 1 wk after the treatment. The posttherapy scan has an increased sensitivity to small foci of iodine-avid metastases, because of the higher administered dose of ^{131}I than that used for the diagnostic scan. Approximately 10% of patients will demonstrate additional metastatic disease on a posttherapy scan *(82)*. Another benefit of posttherapy scanning is detection of the poorly characterized phenomenon of stunning, which results in a reduction in ^{131}I uptake for an undefined time after a diagnostic dose of ^{131}I *(67,68)*.

Practical Guide for RAI Diagnostic Scan and Treatment of Differentiated Thyroid Cancer

The following recommendations are based on my personal experience and interpretation of the thyroid-cancer literature. There are many alternative views and other ways to manage patients with thyroid cancer. This division of opinions was demonstrated after analysis of questionnaires distributed at the premier American professional organization for thyroid disease, the American Thyroid Association, showed no consensus on how to manage a set of patient scenarios with thyroid cancer.

Hypothyroidism Before Diagnostic Whole-Body RAI Scan and Treatment

The efficacy of RAI therapy can be enhanced by increasing the delivery and uptake of RAI by making the patient moderate to severely hypothyroid and depleting body stores of stable iodine. TSH stimulation of thyroid follicular cells increases both the sodium/iodide symporter activity and the organification of iodine, increasing the delivery and retention of RAI by the tumor. Stable iodine competes with RAI for uptake by the sodium/iodide symporter into the thyroid follicular cells, reducing the delivery of RAI to tumor cells. This decrease in uptake can be avoided by depleting the body stores of stable iodine with a low-iodine diet.

Following near-total or total thyroidectomy, patients are allowed to become hypothyroid. This may be accomplished by not giving TH supplementation after surgery. Because of the severity of the hypothyroid symptoms after thyroidectomy, patients are often given liothyronine (L-T_3, at 37.5–50 μg in two or three divided doses twice daily) for 3 wk, which

is then discontinued for 2–3 additional weeks. This regimen reliably results in an increased level of thyrotropin (TSH >50 μIU/mL), stimulating RAI uptake by normal and malignant thyroid tissues *(83)*. An alternative way to minimize the symptoms of hypothyroidism is to reduce the dose of levothyroxine (L-T_4) by 50% for 4 wk, rather than stopping this treatment completely. The rise in TSH is usually 25–30 μIU/mL *(84)*. Historically, iodine uptake could be stimulated by exogenous bovine thyrotropin *(85)*, but this practice was abandoned because of its potential for inducing the development of anti-TSH antibodies and an allergic reaction during administration, and also because the preparation is unavailable. A small uncontrolled study suggests that thyroid-remnant ablations can be performed after two recombinant human thyrotropin injections and a high dose of ^{131}I (mean = 110 mCi) for ablation *(86)*. In a small randomized trial, stimulation with recombinant human thyrotropin was not sufficient to ablate thyroid remnants with 30 mCi ^{131}I *(87)*.

Verification of an appropriate increase in the level of thyrotropin, and measurement of serum Tg (and documentation of the absence of pregnancy in women) is made 2–2.5 wk after discontinuation of liothyronine, just before administration of the ^{131}I or ^{123}I tracer dose for whole-body scintigraphy. A minimum acceptable increase in the level of thyrotropin is >25 μIU/mL, but it is preferable for the level to be >50 μIU/mL.

Hypothyroidism can result in significant constipation. It is recommended that laxatives be used to avoid constipation during the hypothyroid period, with a dose given on the day of ^{131}I or ^{123}I administration to avoid prolonged retention of radioiodine in the gut. Generally, a non-iodine-containing laxative, such as milk of magnesia, can be used on a daily basis, with one-half to one bottle of magnesium citrate being given on the day of RAI administration. Patients should be warned of the possibility of loose stools after the laxative administration.

Stable Iodine Depletion Before Whole-Body RAI Scan and Treatment

Dietary iodine restriction is important to maximize RAI uptake in view of the excess intake of stable iodine in the diet of some American subpopulations. Extreme excess of stable iodine can block RAI uptake. It is also important to inquire whether the patient is consuming a diet of high iodine content, has had a radiology study with contrast medium in the previous month, or is taking an iodine-containing medication such as amiodarone. A low-iodine diet *(88,89)* increases the radiation dose delivered in whole-body RAI scanning for and treatment of thyroid cancer *(89,90)*. To confirm adequate preparation or to assure that radiocontrast medium has been eliminated, a 24-h urine sample can document excretion of iodine that generally equals dietary iodine intake. The optimal extent of iodine deficiency (mild <100 μg/d; moderate <50 μg/d, or severe <25 μg/d) or the duration of the deficiency has not been established for RAI scans or treatments. Iodine-deficient diets have been used from 1–12 wk before RAI therapy. Generally, I start patients on a low-iodine diet 1 wk before the tracer dose of ^{131}I is given and the diet continues until 3 d after the end of ^{131}I therapy. Other strategies to increase iodine depletion are not typically used for the average patient. Iodine depletion can be increased through the use of loop diuretics and mannnitol, but adequate hydration is necessary for the clearance of excess RAI by the kidneys.

Other Considerations before RAI Administration

^{131}I administered to a pregnant woman will expose her fetus to a significant amount of radiation and will potentially destroy the fetus' thyroid, causing neonatal hypothyroidism. It is extremely important that a negative test for pregnancy be documented in every woman who might be pregnant before ^{131}I is given. A negative test for pregnancy by assaying for the β subunit of human chorionic gonadotropin must be documented within 1 wk of RAI administration in every woman of childbearing age unless she has had a tubal ligation

or hysterectomy. Women must not be breastfeeding during whole-body RAI scanning or treatment, since RAI is excreted in breast milk. It is usually recommended that pregnancy be prevented for 6 mo after a therapeutic dose of ^{131}I.

Initial RAI Ablation after Thyroid Surgery

Initial ablation is the objective with the first postthyroidectomy dose of ^{131}I. This ^{131}I treatment of the thyroid remnant and metastatic disease was found to reduce the frequency of recurrence and mortality from thyroid cancer in several large retrospective studies in patients whose primary tumors were >1–1.5 cm in diameter or who had multicentric disease and had local metastatic adenopathy or soft-tissue invasion at diagnosis. Many classification studies have been proposed to identify prognostically low-risk groups of patients in which RAI therapy could be avoided, but with each of the classification systems used, patients in the lowest-risk groups continued to die of thyroid carcinoma *(18)*. For this reason, ^{131}I ablation is appropriate after total or near-total thyroidectomy for any patient with a differentiated thyroid follicular carcinoma >1–1.5 cm in diameter or multifocal disease, soft-tissue invasion, adenopathy, or distant disease. Without prospective randomized trials, there will continue to be disagreement about the utility of ^{131}I ablation in patients with low-risk disease (small, intrathyroidal tumors with no soft-tissue invasion and no distant disease).

At 6 wk after thyroidectomy, and after the preparation described above, a patient should have a ^{131}I or ^{123}I whole-body scan to determine extent of disease. A focal area of RAI uptake is considered positive if the count in that region is higher than the background count and this cannot be explained by normal physiologic processes, such as salivary or gastric RAI uptake, cardiac blood pooling, gastrointestinal RAI elimination, or urinary RAI excretion (Fig. 2 and Fig. 4B).

Empiric dosing with ^{131}I is used in most patients during their initial ablation therapy. Generally, if a patient has a primary tumor >1–1.5 cm, or multifocal disease, a single focus of uptake in the thyroid bed on the side of the known residual thyroid is considered to be remnant uptake and is treated with a 100-mCi dose of RAI. There is considerable controversy about low-dose (30 mCi) versus high-dose (100 mCi) ablation of thyroid remnants. A metaanalysis of 10 cohort studies and three randomized trials suggested that a single high dose of ^{131}I is more effective than low-dose treatment with 30 mCi *(91)*. Patients with evidence of metastases to regional (cervical and upper mediastinal) lymph nodes or invasion outside the thyroid capsule even without evidence of RAI uptake outside the thyroid bed in a whole-body scan should be treated with 150 mCi of ^{131}I. If unsuspected distant metastatic disease is found in the initial whole-body scan, then a larger, empirical therapeutic dose of 200 mCi is given. For patients who have extensive invasive primary tumors, especially with direct invasion of the trachea, blood vessels, or esophagus, or who are known to have macroscopic distant metastases before the RAI scan, a ^{131}I dosimetry study is performed concurrently with the diagnostic scan to determine the maximal safe dose of ^{131}I *(79–81)*. ^{131}I treatment with the largest safe dose determined by dosimetry is reasonable as palliative therapy, since there are few other therapeutic options, whereas local disease can be adequately treated with simple empirical doses of ^{131}I.

Radiation safety practices to protect the public are delineated either by the United States Nuclear Regulatory Commission and the current U.S. Code of Federal Regulations (CFR) part 35 guidelines, or by guidelines determined by those states that have assumed the responsibility for radiation protection. Doses of radiation for cancer therapy can be administered on an outpatient basis in many states with physical restrictions, depending on the patient's prescribed ^{131}I dose, the percent uptake of radioisotope, and the patient's

living conditions and contact with other family members, especially children and pregnant women. Patients are sent home with written instructions to sleep alone, drink fluids liberally, and avoid personal contact with family members for 5–7 d, and with emergency telephone contact numbers. In a study of the radiation exposure of family members of 30 patients who received RAI doses of 75–150 mCi as outpatients and instructions to sleep alone and avoid family members for just 2 d found that the resulting radiation doses to family members and pets in the household were very low *(92)*. With proper instructions and emotional support to ease the fear of radiation, I have found it uncommonly necessary to hospitalize patients for ^{131}I therapy (<1 patient/yr). If hospitalization is required, the patient is admitted to a radiation isolation room until the total-body content of ^{131}I is below 30 mCi or 8 mCi. If the patient is discharged with a ^{131}I content of <30 mCi and >8 mCi, physical restrictions are applied for depending on the home conditions, but if the patient is discharged with <8 mCi of retained ^{131}I, no physical restrictions are required. At discharge, the patient is returned to a regular diet and levothyroxine is initiated to suppress TSH levels (<0.01 μIU/mL). Rapid relief of hypothyroidism is aided, in stable patients without cardiac disease, by supplemental liothyronine (L-T_3) given in a tapering dosage over a 2-wk period (Cytomel, 25 μg twice daily for 5 d, 12.5 μg twice daily for 5 d, and 12.5 μg each morning for 5 d) or by doubling the dose of levothyroxine for 3–7 d. Posttreatment whole-body scans should be performed from 5–7 d after treatment to determine whether additional disease can be detected after the therapeutic dose of ^{131}I and whether all previous known sites of uptake have retained the therapeutic dose of ^{131}I.

RAI Treatment of Residual or Recurrent Metastatic Disease

Follow-up evaluation for recurrent or persistent disease in the use of whole-body RAI scanning, through the low- to moderate-risk patient, is performed after 12 mo or earlier if there is evidence of recurrent disease (e.g., rising Tg levels, adenopathy). New adenopathy may be identified as recurrent thyroid cancer through fine-needle aspiration (FNA) biopsy for histology and by measurement of Tg levels *(93,94)*. Patients at moderate and high risk (extensive adenopathy, extensive direct tissue invasion, distant disease) should be reevaluated after 6 mo with a hypothyroid scan as described above and with additional imaging such as a magnetic resonance imaging (MRI) scan of the affected region *(95)*. With increasing frequency, it is becoming accepted to perform the first posttreatment RAI scan of low-risk patients (small intrathyroidal tumors without invasion or adenopathy), with unmeasurable Tg levels on levothyroxine therapy, after two daily injections of human recombinant TSH (hrTSH) *(96,97,98)*. To achieve adequate sensitivity, evaluation after the administration of recombinant TSH must include both an RAI whole-body scan and measurement of the stimulated Tg level. Recently it has been suggested that a hrTSH-stimulated thyroglobulin <2 μg/L alone and without a RAI whole body scan is sufficient to screen for thyroid cancer recurrence. It should be noted that this is true only in patients with a prior negative RAI whole body scan and hypothyroid-stimulated thyroglobulin level of <2 μg/L (96).

Persistent or progressive cervical disease as demonstrated by abnormal RAI uptake on the whole-body scan and an elevated Tg should be treated with ^{131}I. Local cervical disease is treated with 150 mCi of ^{131}I. Microscopic distant disease is treated with 200 mCi of ^{131}I, but maximal-dose therapy with dosimetry should be considered for macroscopic pulmonary disease or bone metastases. Higher doses, determined by dosimetry, should be considered for patients who have persistent or recurrent uptake despite previous ^{131}I ablation therapy. Empiric therapy every 6 mo for macroscopic disease is generally unable to produce a complete response, but maximal-dose RAI therapy determined by dosimetry is also without

objective evidence of efficacy. The maximum tolerable dose of ^{131}I is determined with dosimetry such that the dose delivers no more than 200 cGy to the red marrow, and whole-body retention of radioactivity is less than 80 mCi (diffuse pulmonary disease) or 120 mCi (no pulmonary disease) at 48 h.

RAI scans and therapies are repeated every 6–12 mo until there is no further evidence of disease, with subsequent evaluations being done at 1 yr and 2 yr, and then at intervals of 2–5 yr. A recent and frequent change in practice, once a negative whole-body scan has been achieved, is to use recombinant TSH (0.9 mg intramuscularly daily for two doses)-stimulated Tg measurement alone *(96)* or with an RAI scan to detect thyroid-cancer recurrence. Prognostic characteristics of the presenting primary tumor should determine the frequency and intensity of follow-up studies *(95)*. Recurrence of differentiated thyroid cancers can take place over a period of many decades after diagnosis. Most recurrences take place within the first few yeas after diagnosis, but only 50% of recurrences occur within the first 5 yr and 75% of recurrences occur within the first 10 yr after diagnosis *(13)*. Recurrent disease has been documented more than 30 yr after the diagnosis of thyroid cancer, suggesting that although the interval between evaluations should increase with time after diagnosis, the patient should have lifelong surveillance for recurrent thyroid cancers.

Recurrent disease is suspected if the thyroid hormone (TH)-suppressed Tg rises to >5 ng/mL, the hypothyroid Tg rises above 10 ng/mL (21), or the recombinant-TSH-stimulated Tg rises above 2 ng/mL *(96–98)* with a negative anti-Tg antibody titer. The current practice is to administer RAI therapy with the patient in the hypothyroid state unless the patient's endogenous TSH cannot be increased (pituitary insufficiency) or the patient is medically unable to tolerate the hypothyroid state. Dosimetry studies show that after recombinant TSH, ^{131}I clearance is about twice as rapid as in a hypothyroid patient. This is thought to result from reduced renal clearance of RAI in the hypothyroid state *(97)*. The increased clearance led to the recommendation that a higher than usual diagnostic dose of 4 mCi of ^{131}I be used in whole-body scans after injections of recombinant TSH. Further, a higher dose of ^{131}I might be necessary if ^{131}I treatment is given after injection with recombinant TSH rather than after induction of hypothyroidism. No formal studies have been reported that compare the effectiveness of ^{131}I therapy with induced hypothyroidism and after recombinant TSH injection. Currently, there is no U.S. Food and Drug Administration-approved indication for recombinant TSH injections prior to RAI therapy.

Clinical judgment should be used in deferring treatment for persistent minimal thyroid-bed uptake of RAI or local cervical adenopathy after repeated therapeutic doses of ^{131}I if there is no clinical progression of disease and no mass lesion is associated with the uptake by ultrasound or MRI scanning. If a macroscopic mass is detected, it should be removed surgically. Single-lesion dosimetry predicts inadequate treatment with ^{131}I if the usual empiric doses are used for tumors with low uptake and diameters of >1–1.5 cm. I do not administer ^{131}I in patients with elevated thyroglobulin levels and no uptake on an RAI whole body scan, because there is no evidence that the resulting decrease, but not resolution of, the thyroglobulin levels is associated with a reduction in mortality.

Side Effects of RAI Treatment

Side effects of RAI therapy are generally minimal *(99,100)*, and include radiation thyroiditis, neck edema, sialoadenitis, and a sensory change in taste. The side effects of maximal-dose therapy are the same as those observed with empiric ^{131}I therapy. The most common problem is radiation sialoadenitis with tenderness and swelling of submandibular and parotid salivary glands *(100)*. It is usually recommended that patients increase the flow of saliva throughout

the first few days after RAI therapy to decrease the risk of sialoadenitis by sucking on sour candy such as lemon drops or on lemon slices or by direct salivary gland massage. It is also important for the patient to remain well-hydrated for good saliva flow. It is recommended that patients sip approximately 1 cup of water each hour while awake, but do not exceed 12–16 cups per day. It is rare for RAI therapy alone to cause symptomatic xerostomia. Repetitive treatments cause decrements in salivary function, resulting in recurrent, transient sialoadenitis. Xerostomia becomes a very important complication after ^{131}I therapy combined with external-beam radiation. Severe nausea requiring intravenous hydration is uncommon, except as a consequence of patient anxiety. Nausea is usually managed with oral prochlorperazine, but with severe symptoms, oral or intravenous ondansetron is recommended. There is no evidence of reduced fertility or, in subsequent pregnancies, of an increased risk for abnormal birth weight, congenital anomalies, or prematurity *(101,102)*. ^{131}I therapy has been associated with a theoretical risk of leukemia *(103)* and bladder cancer *(104)*, which is extremely rare. Bone-marrow failure and pulmonary fibrosis were observed with high-dose therapy (>500–1,000 mCi) before the dosimetry limits of maximal-dose therapy were established. Since the institution of the limits described in the protocols for high-dose therapy, these complications have not been observed *(81)*. Oligospermia occurs transiently with RAI therapy, but infertility is rare except after maximal-dose therapy.

Dosimetry Studies and Maximal-Dose ^{131}I Therapy

Benua et al., at the Memorial Sloan-Kettering Cancer Center, developed a method of quantitative ^{131}I dosimetry in the early 1960s *(78)*. The rationale for this method is based on the concept that the radiation dose delivered to thyroid cancer is proportional to the total administered dose of ^{131}I, and that maximal therapy can be administered if it is below a limit defined by Benua and colleagues' empiric criteria. The maximum tolerable dose is determined through dosimetry of a tracer dose of ^{131}I such that a therapeutic dose os ^{131}I delivers no more than 200 cGy of radiation to the red marrow, and whole-body retention is less than 80 mCi (diffuse pulmonary disease) or 120 mCi (no pulmonary disease) at 48 h. This method permitted Benua and Leeper *(79)* to safely administer single doses of ^{131}I of up to 654 mCi. The original method involved obtaining blood and urine samples over 5–8 d to calculate the radiation dose to blood *(105)*. Currently, most institutions calculate total-body retention from the external exposure rate of the patient, using a γ-ray probe having a fixed geometry with a diverging collimator *(106)*. Following the administration of a tracer dose of ^{131}I (2–5 mCi), γ-ray-probe counts of the patient (and background) are obtained immediately and at intervals of 2, 4, 6, 24, 48, 72, and 96 h. Heparinized blood samples are obtained at the same time-points and the radiation they emit is counted in a γ-counter to obtain the percentage of the administered dose per liter of blood. These values are integrated over time to calculate the rads per millicurie of β-emission energy to the blood and bone marrow. This method gives a measurement of radiation exposure to the blood and marrow for each mCi of ^{131}I dose administered.

Empirical data collected by Benua et al. *(78)* defined limits of safety with this method. The maximal tolerated dose of RAI must deliver less than 200 cGy of radiation (β plus γ emissions) to the blood and marrow, with a whole-body retention of ^{131}I of less than 120 mCi (no pulmonary metastases) or 80 mCi (diffuse pulmonary metastases) at 48 h. Pulmonary fibrosis is avoided when there is diffuse pulmonary metastases by limiting the whole-body retention of ^{131}I to less than 80 mCi at 48 h. The highest dose of ^{131}I that satisfies both of these conditions is the maximal permissible dose. A nadir of the platelet count, white-cell count, and reticulocyte count is seen approximately 4 wk after the dose,

with normalization of these parameters by 6–8 wk. Patients are monitored for decreases in peripheral blood counts for 2 mo and instructed to call the endocrinologist if fever occurs. A brief summary of these methods of dosimetry and recent modifications is discussed by Van Nostrand *(81)*.

Special Considerations for RAI Therapy

If rapid turnover of RAI is suspected or if tumor may be resistant to therapy (macroscopic masses, bone and lung metastases), lithium carbonate can be administered as an adjuvant to RAI. Lithium increases the retention of RAI in metastatic deposits, increasing the effective radiation delivered by the treatment dose *(107–108)*. In patients with normal renal function, lithium carbonate may be initiated 2 d before RAI therapy, with a 600 mg loading dose followed by 10 mg/kg in divided doses given twice daily for 1 wk. There is a narrow therapeutic index for side effects, and trough lithium levels should be monitored and maintained between 0.6 and 1.2 mmol/L. The trough level can usually be obtained on the morning before RAI therapy. Despite careful dosing, most patients note some degree of nausea and tremor and may require antiemetic therapy or a reduction in the lithium dose.

Bone metastases and bulky disease from differentiated thyroid carcinomas often respond to ^{131}I therapy despite avid uptake of RAI *(109–110)*. It has been suggested that a single focal bone lesion and noninvasive bulky disease should be surgically resected *(111)* and subsequently treated with ^{131}I (Fig. 5). Nonresectable focal bone lesions may also be treated with focal external radiation *(110)* or pamidronate *(112)*, with a significant reduction in pain.

Ultrasonically guided percutaneous ethanol injections have been used in a small series of 29 metastatic lymph nodes unresponsive to ^{131}I therapy in patients who were considered poor surgical candidates or who refused surgery. All treated lymph nodes decreased in volume, from a mean of 492 mm^3 to 76 mm^3 at 1 yr and 20 mm^3 at 2 yr. No major complications were noted. This therapy may be useful in patients with limited nodal metastases or focal tumors from papillary thyroid cancer who are not candidates for further surgical or RAI treatment *(113)*.

Levothyroxine Suppression Therapy of Differentiated Thyroid Carcinoma

After initial surgery and RAI therapy for thyroid carcinoma, all patients should receive levothyroxine to prevent hypothyroidism and to suppress TSH levels. TSH, a growth factor for thyroid follicular cells, stimulates the growth of thyroid-cancer cells. Retrospective analysis of a large cohort of differentiated thyroid-cancer patients suggests that levothyroxine therapy reduces disease recurrence and mortality *(13)*.

There is disagreement about the optimal degree of TSH suppression because of the potential for acceleration of bone loss in menopausal women *(114)*, and of atrial fibrillation *(115)*. The recent trend is away from full suppression of TSH *(116)*. One suggestion for TSH suppression is based on the extent of disease. TSH should be titrated to the bottom half of the normal range (0.5–2.0 μIU/mL) for uninodular tumors of <1.5 cm in diameter without invasion or adenopathy. TSH should be suppressed to below the normal range but should still be measurable with uninodular intrathyroidal tumors of 1.5–4.5 cm without invasion or adenopathy. TSH should be suppressed to between 0.05 and 0.1 μIU/mL for nodal metastases, and should be fully suppressed to <0.05 μIU/mL with distant metastatic disease. The extent of TSH suppression should be modified for patients with ischemic cardiac or skeletal disease. It is also suggested that the levothyroxine dose be decreased in patients who are disease free for 5 or 10 yr because of the reduction with time of thyroid cancer recurrence.

Uncommon Therapeutic Modalities

External-Beam Radiotherapy

External-beam radiation therapy is reserved for older patients with differentiated thyroid carcinoma and metastatic disease that is refractory to RAI and unresectable, and for patients whose tumors are not iodine-avid. External-beam radiation therapy has been beneficial in providing symptomatic relief of painful bone lesions, spinal-cord compression from vertebral metastases, and brain metastases. The usual dose for the neck is 50–60 Gy given in 20 fractions over a period of 4 wk, with the field between the hyoid bone to just below the suprasternal notch. The field is planned carefully to avoid high doses of radiation to the spinal cord. External radiotherapy delivers radiation doses of up to 60 Gy, and is limited by toxicity to nearby normal tissues, whereas RAI of ablation iodine-avid tissue can deliver doses exceeding 300 Gy *(72)* with minimal, if any, local or systemic toxicity. For patients in whom the primary tumor and macroscopic adenopathy from thyroid carcinoma is removed surgically, RAI therapy has been the standard of care for the ablation of microscopic residual disease. One study examined 96 patients with significant extrathyroidal invasion by primary thyroid tumors and incomplete resection, and who were treated with ^{131}I therapy. Half of the patients subsequently received 55 Gy of local external radiotherapy to the neck. The external radiation therapy reduced the local disease recurrence rate from 21 to 3% without changing survival *(117)*. Because of the high dose of ^{131}I delivered to iodine-avid tumors, it is reasonable to treat with ^{131}I in addition to external radiation *(118)*.

Radiation may be more effective when combined with adjuvant low-dose doxorubicin, a radiation sensitizer *(119)*. Complications of radiation include moderate skin erythema and desquamation; local mucositis of the esophagus, trachea and larynx; dysphagia; extreme xerostomia; and in rare circumstances esophageal or tracheal stenosis and dysfunction. In addition, late changes to the skin include telangiectasias and increased skin pigmentation.

Chemotherapy for Differentiated Thyroid Cancer Unresponsive to Surgery and ^{131}I Therapy

Chemotherapy is not an effective modality for the treatment of thyroid cancer. It may, however, be useful in patients with progressive disease unresponsive to RAI and external-beam therapy, and which is surgically unresectable. Partial responses have been reported for doxorubin in one-third of patients treated with 60–75 mg/m^2 every 3 wk. In a small number of individuals exhibiting partial responses, median survival can increase from 3–5 mo to 15–20 mo *(120)*, although with significant treatment toxicities. In one study that combined doxorubin and cisplatin at 60 mg/m^2, only 2 of 22 patients showed a partial remission, with significant toxicity *(121)*.

Differentiation Agents for RAI Uptake Negative Thyroid Cancer

Loss of differentiation may occur that results in differentiated thyroid cancer losing RAI uptake and adopting rapid growth characteristics. Redifferentiation agents, including retinoic acids, have been proposed and now have shown clinical efficacy in controlled studies.

Additional Diagnostic Tests for Differentiated Thyroid Carcinoma

Tg as a Tumor Marker for Differentiated Thyroid Carcinoma

Tg is synthesized only in thyroid follicular cells, making it a sensitive and specific tumor maker for thyroid cancer, but only after near-total or total thyroidectomy and RAI ablation therapy, and if the patient does not have anti-Tg autoantibodies *(66,94,95)*. Often,

dedifferentiated thyroid follicular tumors—anaplastic thyroid cancers—will continue to make Tg even when they have lost the ability to take up iodine.

The incidence of anti-Tg autoantibodies ranges from 15–30% in the thyroid-cancer population *(122–124)*, and can either falsely increase or falsely depress reported serum Tg values depending on the type of assay used. A measurement of anti-Tg antibody titer should be made at the same time as the serum Tg assay. Patients with anti-Tg autoantibodies cannot be followed for recurrent disease by monitoring Tg levels. These patients should instead be followed with imaging studies such as ultrasound examination. Anti-Tg autoantibodies tend to decrease with time after thyroidectomy and RAI ablation. Persistent or rising anti-Tg autoantibody titers has been associated with persistent or recurrent metastatic disease *(123)*. Although a method is not commercially available, Tg mRNA can be measured in the blood of patients with atni-Tg autoantibodies *(125)*.

After thyroidectomy and RAI ablation, serum Tg levels should be very low (<1–3 ng/mL) with or without levothyroxine therapy. Tg expression is TSH dependent. The sensitivity of serum Tg measurement for detecting thyroid-cancer recurrence increases when TSH levels are high either after stopping levothyroxine therapy *(66)* or after injections of recombinant TSH. If a patient has a Tg of >5 ng/mL during levothyroxine therapy, >10 ng/mL without levothyroxine therapy, and >2 ng/mL measured 4–5 d after injections of recombinant TSH (0.9 mg intramuscularly, given daily for 2 d), additional studies should be done for metastatic disease, such as a neck ultrasound scan and a hypothyroid RAI whole-body scan. Although the Tg levels given above are used to define recurrent disease, any detectable Tg level should be considered clinically significant, especially if hypothyroidism or injections of recombinant TSH further increase the Tg value.

Other Imaging Methods for Metastatic Thyroid Carcinoma

Besides the ^{123}I and ^{131}I whole-body scans described previously, other, nonspecific nuclear scans and other types of imaging modalities have some clinical application in thyroid cancer *(126)*. These scans can generally be performed without making the patient hypothyroid or depleting the patient of stable iodine. Thallium-201, technetium-99m-sestamibi (hexakis 2-methoxy isobutyl isonitrile) and ^{111}In Pentetreotide (Octreoscan) are useful in imaging metastases that have lost iodine-concentrating ability *(127,128)*. Recently, studies have shown that whole-body positron emission tomography (PET) with fluorine-18 fluorodeoxyglucose (^{18}F-FDG PET scanning) is useful in 80% of patients with tumors that do not take up RAI, but not for tumors that are still iodine-avid. PET scanning with FDG may become the first line imaging method for localizing metastatic disease in the patient with a thyroid tumor that has low ^{131}I avidity (^{131}I scan negative) but who is still positive for Tg *(129–131)*. ^{18}F-FDG-positive thyroid cancer may be associated with reduced survival. Gallium-67 may be useful in anaplastic carcinoma or thyroid lymphoma *(132)*.

MRI is an excellent method for finding metastatic foci of thyroid cancer in the neck *(133)* in patients who have tumor that does not take up RAI. Pulmonary lesions should be followed with high-resolution computed tomographic (CT) scanning *(134)*. Of note, radiocontrast agents are not necessary for pulmonary parenchymal lesions. Ultrasound examination of the neck is the best low-cost, radiation-free method for evaluating a patient for local cervical adenopathy.

TREATMENT OF ANAPLASTIC THYROID CARCINOMA

Anaplastic thyroid carcinomas are very rare and are derived from the follicular cells of the thyroid, but because of dedifferentiation grow quickly. The tumor growth is independent of

TSH. Anaplastic thyroid carcinomas do not take up iodine. These tumors are very aggressive solid tumors. The tumor presents as a rapidly growing and usually painless mass in the neck. Anaplastic carcinomas often appear to arise from a multinodular goiter or thyroid nodule. These tumors have a high prevalence of mutations in *p53 (135)*, suggesting that they represent a dedifferentiation of a well-differentiated neoplasm. Anaplastic carcinomas are large-cell tumors that can be subcategorized into spindle-, giant-, and squamoid-cell types. The "small-cell" tumors described in the older literature are actually lymphomas, medullary carcinomas, or insular carcinomas *(136)*.

The median survival with anaplastic carcinoma was only 4 mo and the 5-yr survival was 0% in one series, despite vigorous therapeutic efforts *(135,136)*. There does not seem to be a survival advantage of any single treatment or combination of treatments, including partial or total surgical resection, radiotherapy, or chemotherapy *(137)*. Although these treatments decrease local disease, nearly all patients in two studies died of distant metastases *(138,139)*. A reasonable plan is to resect enough tissue to confirm the diagnosis and to protect the airway, sometimes with a tracheostomy *(140)*. Multiple chemotherapeutic agents have been tested singly and in combination against anaplastic thyroid cancer, with only paclitaxel showing even partial activity *(141)*. A study of 20 patients treated with intravenous paclitaxel at 120–140 mg/m^2 over a 96-h period every 3 wk found a 53% response rate, with an increase in median survival from 10 wk to 32 wk in responders, and all patients eventually dying of anaplastic thyroid cancer.

TREATMENT OF MEDULLARY THYROID CARCINOMA

Presentation of Medullary Thyroid Carcinoma

Medullary thyroid carcinoma is a neuroendocrine tumor of the parafollicular C-cells that secrete calcitonin. Medullary thyroid carcinomas constitute 3–5% of thyroid malignancies. Eighty percent of medullary thyroid carcinomas are sporadic. Sporadic medullary carcinoma presents as a unifocal tumor in the upper portion of the thyroid gland in the 5th to 6th decades of life. Fifty percent of these patients will have detectable cervical adenopathy, 15% have compressive symptoms such as dysphagia or hoarseness, and about 5% are accompanied by distant disease with increased levels of calcitonin and sometimes of carcinoembryonic antigen (CEA). The remaining 20% of medullary carcinomas are familial, as part of the multiple endocrine neoplasia type-2 (MEN-2) syndrome inherited as an autosomal dominant trait with nearly complete penetrance. Some affected families show only medullary carcinomas, while the medullary thyroid carcinomas in others are associated with the MEN-2a or -2b syndrome. Inherited medullary carcinomas are usually multicentric and bilateral *(142)*. Nodular hyperplasia of parafollicular C cells is seen outside the tumor masses *(143)*. Familial MEN-2 associated medullary cancers usually develops in early childhood *(142)*.

Medullary thyroid carcinomas are hormonally active, and systemic symptoms of diarrhea or facial flushing may result from the high levels of calcitonin, calcitonin-gene-related peptide, or other secreted peptides. Rarely, these tumors will make adrenocorticotropic hormone and cause Cushing's syndrome.

Medullary Thyroid Carcinoma and MEN-2a

Patients with medullary thyroid carcinoma may also have parathyroid hyperplasia and pheochromocytoma. Approximately 20% of patients have four-gland parathyroid hyperplasia. The treatment in such cases is removal of three and one-half glands with forearm reimplantation of half of a gland and cryopreservation, if available, of the remainder of the glands. Pheochromocytomas are present in 10–50% of cases of medullary thyroid carcinoma,

and are usually bilateral. They require biochemical diagnosis with measurement of urinary catecholamines, metanephrines, and vanillylmandelic acid. Although pheochromocytoma is frequently bilateral, it is recommended that a unilateral adrenalectomy be performed for this tumor if imaging studies show only a unilateral mass. If a contralateral pheochromocytoma occurs, it should also be removed. Delay in removal of both adrenal glands prevents the onset of adrenal insufficiency. Occasional kindreds may have associated cutaneous lichen amyloidosis *(144)*.

Medullary Thyroid Carcinoma and MEN-2b

The clinical course of medullary thyroid carcinoma is more aggressive in kindreds with MEN-2b. This syndrome is similar to MEN-2a, except that parathyroid disease is not present and patients have a marfanoid habitus, gastrointestinal ganglioneuromatosis, and mucosal neuromas.

Genetics of Familial Medullary Thyroid Carcinoma and MEN-2

Relation Between Mutations in the RET Protooncogene and MEN-2a and -2b and Familial Medullary Thyroid Carcinoma

The genetic cause of the MEN-2 syndromes was first reported in 1993 to be mutations in the RET protooncogene at the pericentromeric region of chromosome 10 *(145,146)*. This gene encodes a tyrosine-kinase receptor, and identified point mutations couple expression of the gene with a constitutive promoter, causing continuous activation of tyrosine kinase. These germline mutations can be identified in 95% of kindreds with the MEN-2 sydromes, are similar to the sporadic mutations described in some cases of papillary carcinoma, and are related to inactivating mutations of the RET protooncogene associated with Hirschsprung's disease.

Genetic Testing and MEN II A and B and Familial MTC

Until recently, biochemical screening, with yearly pentagastrin-stimulated assessment of circulating calcitonin levels, was the only available method for identifying affected family members within known kindreds having inherited medullary carcinoma. This method was associated with both false-positive results, resulting in unnecessary thyroidectomies, and false-negative results, with failure to prevent metastatic carcinoma *(146,147)*. Specialized commercial laboratories can identify RET protooncogene mutations with blood or buccal smears from patients. Once such mutations are identified, screening of other family members is simplified. This approach allows prophylactic thyroidectomy to prevent otherwise fatal cancers *(142,144,147–150)*. It has been suggested that genetic testing be done on all cases of medullary thyroid carcinoma, since it is not always possible to distinguish familial cases through family history and tumor morphology *(151)*.

Management of Medullary Thyroid Carcinoma

The treatment of medullary thyroid carcinoma is surgical. External radiation therapy is not recommended because it is ineffective and the resulting postradiation scarring makes reoperation to remove local recurrent metastases difficult *(152)*. Chemotherapy is not effective against medullary thyroid carcinoma. The tumor metastasizes locally and distantly. The patient should have a total thyroidectomy with a central-node dissection *(142,148,149)*. Frequently, tumor remains in tiny local lymph nodes, resulting in persistent disease. It has been suggested in some studies that exhaustive dissection to remove all nodes may lead to a complete remission in some patients. Few surgeons have the expertise to do this type of technically demanding surgery *(142,153,154)*. Recurrence and persistent disease are

common in medullary thyroid carcinoma. Patients require lifelong screening of calcitonin to detect recurrences. Despite this, many patients survive for years with massive tumor burdens. Increased calcitonin levels necessitate a careful search for resectable disease. Somatostatin-receptor imaging, using indium-111 pentetreotide, has proven able to detect metastatic foci of medullary thyroid carcinoma *(155–156)*. In addition, intraoperative localization, using a nuclear γ-ray probe after indium-111 pentetreotide injection *(157)*, may assist in the surgical removal of metastases. It has been reported that some metastases may be localized and treated with ^{131}I-metaiodobenzylguanidine (MIBG) *(158)*.

TREATMENT OF THYROID LYMPHOMA

Primary non-Hodgkin's lymphoma of the thyroid is very uncommon, being seen in less than 3–5% of patients with thyroid neoplasms, and is often associated with preexisting autoimmune thyroid disease in an elderly woman *(159,160)*. This cancer must be considered in the context of a rapidly growing goiter in an elderly patient, and should be clearly distinguished from anaplastic carcinomas. One series demonstrated the frequency of several subtypes of primary thyroid lymphoma as diffuse large B-cell lymphoma, 50%; mucosa-associated lymphoid tissue (MALT) 23%; follicular lymphoma, 12%; Hodgkin's disease, 7%; small lymphoma, 4%; and Burkitt's lymphoma, 4%. The initial diagnostic procedure, an FNA biopsy, can be complemented by B-cell immunotyping with flow cytometry *(161,162)*. Although diffuse B-cell lymphoma is easily recognized by FNA biopsy, the MALT lymphomas often required immunotyping for diagnosis *(161)*. Sometimes a surgical core biopsy is necessary. The role of total thyroidectomy in thyroid lymphoma is diminishing *(160)*, although some clinicians continue to advocate thyroid surgery. Careful staging should include CT scanning or MRI of the neck, chest, and abdomen, and F(18)-FDG PET scanning. Combined-modality therapy with external radiation and chemotherapy using cyclophosphamide, doxorubicin, vincristine, and prednisolone (CHOP) *(163–165)* is the recommended treatment for thyroid lymphoma. In three studies, this resulted in an 8-yr survival of nearly 100% *(159,163,164)*.

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14 Thyroid Dysfunction During Pregnancy and After Delivery

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Contents

INTRODUCTION

Thyroid dysfunction during pregnancy may affect the mother as well as the fetus. Before considering the clinical entities occurring during and after pregnancy, it is useful to briefly review thyroid physiology and immunology in relation to pregnancy

PREGNANCY AND THYROID FUNCTION

Pregnancy has an appreciable effect on thyroid economy *(1,2)*. There are significant changes in iodine metabolism characterized by increased excretion of iodine in the urine, accounting for the increase in thyroid volume during pregnancy even in areas of moderate dietary iodine intake *(3)*. Although thyroid size in pregnancy increases in areas of iodine deficiency, it does not do so in those regions that are iodine-sufficient *(4)*. There is ample evidence that iodine deficiency during pregnancy is associated with maternal goiter and a reduced maternal T_4 (thyroxine) level, which is seen in areas of endemic cretinism *(5)*. Even in moderately iodine-deficient regions, urinary iodine excretion is higher in all trimesters than in nonpregnant women, and may be causative of maternal goiter formation as assessed by ultrasound examination *(3)*. Thyroid hormone (TH)-transport proteins, particularly thyroxine-binding globulin (TBG), increase during pregnancy because of enhanced hepatic synthesis and a reduced degradation rate resulting from oligosaccharide modification.

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Table 1
Physiologic Changes in Pregnancy That Influence Thyroid Function Tests

Physiologic change	*Thyroid-function-test change*
↑Thyroxine binding globulin	↑Serum total T_4 and T_3 concentration
First trimester HCG elevation	↑Free T_4 and ↓TSH
Second and third trimester	↓Free T_4 and free T_3 (within normal range)
↑Plasma volume	↑T_4 and T_3 pool size
↑Type III 5-deiodinase (inner ring deiodination) owing to increased placental mass	↑T_4 and T_3 degradation resulting in requirement for increased hormone production
Thyroid enlargement (in some women)	↑Serum thyroglobulin
↑Iodine clearance	↓Hormone production in iodine deficient areas

HCG, human chorionic gonadotropin; TSH, thyroid-stimulating hormone.

The serum concentrations of TH have been reported by different groups to be decreased, increased, or unchanged during gestation depending on the assays used. However, there is general consensus for both a transient rise. in FT_4 in the first trimester owing to the relatively high circulating human chorionic gonadotropin (HCG) concentration, and a decrease in free thyroxine (FT_4) in the second and third trimesters, albeit within the normal reference ranges. Changes in the free triiodothyronine (FT_3) concentration also broadly parallel those of FT_4, again within the normal range. The precise reason for the decline in free TH is not clear, but the interaction of thyroid-stimulating hormone TSH, estrogen, and thyroid binding proteins is of importance *(2)*. In iodine-deficient areas (including many continental European countries where marginal iodine deficiency is seen) the pregnant woman may become significantly hypothyroxinemic, with preferential T_3 secretion. The thyroidal 'stress' is also evidenced by a rise in the median TSH and serum thyroglobulin (Tg) concentrations. The increase in thyroid volume already referred to is substantially greater in iodine-deficient areas *(6)*. This gestational goitrogenesis is preventable by iodine supplementation not only in areas of severe iodine deficiency (24-h urinary iodine <50 μg), but also in areas such as Belgium and Denmark, where trials have shown clear beneficial effects of iodine supplementation on maternal thyroid size *(7)*. The aim of these studies is to increase the iodine supply to pregnant and lactating women to at least 200 μg/d.

Thus, pregnancy is associated with significant but reversible changes in thyroid function (Table1). The findings associated with the hypermetabolic state of normal pregnancy can overlap with the clinical signs and symptoms of thyroid disease *(8)*.

HCG

The placenta secretes HCG, a glycoprotein hormone sharing a common α subunit with TSH but having a unique β subunit, which confers specificity. Evidence derived both from in vitro studies of thyroid tissue and of eukaryotic cells stably expressing the human TSH receptor (TR), suggests that HCG or a molecular variant of it is able to act as a TSH agonist *(9)*, although this is controversial *(10)*. A recent screening study of more than 23,000 pregnant women has shown an incidence of gestational transient hyperthyroxinaemia of 0.285%, which has been ascribed to elevated HCG levels *(11)*. No therapy is necessary for this condition. There is good evidence that hyperemesis gravidarum (HG), which sometimes

requires hospitalization because of the development of dehydration and ketosis, may be associated with hyperthyroidism caused by excess HCG stimulation *(12)*. Goodwin et al. *(13)* established a correlation between the severity of HG (as evidenced by dehydration, vomiting, and ketosis) and TH concentrations, and suggested that HCG plays a causal role in the hyperthyroidism. The situation is complicated by the fact that modification of the carbohydrate structure attached to HCG may alter the biologic activities of the molecule; for example, asialo-HCG plays a critical role in hyperthyroidism during pregnancy, and exhibits increased thyrotropic effects *(14)*. The structure and thyrotropic activity of HCG preparations is variable. This relates to heterogeneity in several properties, including charge, sialic acid content (decreased sialic acid content enhances HCG bioactivity in both gestational trophoblastic disease [GTD] and gestational thyrotoxicosis [GT]), interchain nicks at positions β47-48 or β44-45, and possibly the expression of as many as six β-subunit HCG genes *(15,16)*. The precise relationship between HCG structure and bioactivity is not known, not only because of the number and complexity of HCG isoforms, but also because bioactivity is difficult to measure in a way meaningful to human physiology. An important problem is the poor correlation between bioactivity as measured by in vitro and in vivo systems.

Despite these reservations, it is noteworthy that the HCG produced by hydatiform-mole tissue, as well as that produced in the first trimester of normal gestation, is reported to have high specific thyroid-stimulating activity *(16,17)*. Therefore, the development of thyrotoxicosis in GTD and GT may be related not only to the high serum HCG concentrations in these conditions, but also to the nature of the HCG isoforms that are produced in these settings. The sensitivity of the TSH receptor to HCG has also been demonstrated in a unique case report of familial gestational hyperthyroidism caused by a mutant TSH receptor *(18)*. This report described both a woman and her mother who had recurrent gestational hyperthyroidism and normal serum HCG concentrations. Both the mother and daughter were heterozygous for a missense mutation in the extracellular domain of the TSH receptor which was more sensitive to HCG than was the wild-type receptor. Whether HCG has any effect on antithyroid antibody expression during pregnancy is unknown.

Immunologic Changes

Pregnancy has a profound effect on the immune system *(19)* in order to maintain the fetal-maternal allograft, which is not rejected despite displaying paternal histocompatability antigens *(20)*. Although there is no overall immunosupression during pregnancy, dramatic clinical improvement usually occurs in patients with immunologic disorders such as rheumatoid arthritis (RA) when they become pregnant. T-cell-subset studies in pregnancy are discrepant, with peripheral blood $CD4^+$ and $CD8^+$ cell levels having been variously reported to decline, remain unchanged, and increase *(21,22)*. Although the distinction between TH1 and TH2 responses in humans remains less clear than in the mouse *(23)*, the general agreement is that in pregnancy there is a bias toward a TH2 response *(24–26)*. This seems to be achieved by the fetal/ placental unit producing TH2 cytokines, which inhibit TH1 *(27)*. TH1 cytokines are potentially harmful to the fetus since interferon (IFN)-α is a known abortifacient *(28)*.

The high concentrations of estrogen produced in normal pregnancy almost certainly contribute to the decrease in antithyroid autoantibody (ATA) levels observed in pregnant patients with autoimmune thyroid disease (AITD) *(29)*. Despite the decrease in autoantibodies, no significant changes are reported in the number of B cells in the circulation in normal human pregnancy. Although progesterone may favor TH2 cells, evidence has indicated that estrogen delivers a negative signal to B-cell function during pregnancy, which shows a

slow reversal in the postpartum period. In keeping with these observations, autoantibody titers and inflammation decrease throughout pregnancy, as observed in all autoimmune diseases investigated *(29)*. However, after most pregnancies there is a marked increase in many different types of autoantibody secretion and an exacerbation of diseases in the months after delivery, not just of AITD but, for example, also of multiple sclerosis or rheumatoid arthritis *(30)*.

INFLUENCE OF ANTITHYROID ANTIBODIES ON FERTILITY AND FETAL LOSS

ATAs present during pregnancy may be a marker for an increased risk of miscarriage, as shown by two large studies *(31,32)*. A significantly higher incidence of ATAs (36%) in women with recurrent abortions than in controls (9%) was noted in two studies *(33,34)*. In contrast, another study *(35)* found that the incidence of ATAs in women with recurrent (three or more) abortions was not significantly increased over that in a random control population, but that the frequency of ATAs was greater than that of non-organ-specific antibodies. However, Esplin et al. *(36)* concluded that women with a history of recurrent pregnancy loss do not have an increased incidence of ATAs, although an association was found between the presence of anti-thyroid peroxidase (anti-TPO) antibodies before pregnancy and miscarriage in women without a history of recurrent abortion *(37)*. It appears that ATAs may be a marker for autoimmune-mediated recurrent spontaneous abortion in some patients, although the mechanisms are uncertain. Geva et al. *(38)* suggested that the presence of ATAs in euthyroid women with unexplained infertility or tubal obstruction is greater than in controls. Vaquero et al *(39)* described a group of 42 women with anti-TPO and/or anti-Tg antibodies who had recurrent first-trimester abortions. They noted that treatment with TH was more effective than intravenous Igs (81% vs 55%) in producing live births, suggesting that mild degrees of thyroid failure, perhaps at the level of the genital tract, may be causal in relation to the association of ATAs and recurrent abortion. Overall, there is a clear association between the presence of ATAs and pregnancy loss. In addition, most data indicate a correlation between the presence of ATAs and recurrent abortion *(40)*.

HYPERTHYROIDISM AND PREGNANCY

Hyperthyroidism in pregnancy occurs in up to 0.2% of women. The most common cause is Graves' disease (GD) (85–90%), but the condition may have other causes, such as toxic multinodular goiter, toxic adenoma, and subacute thyroiditis. Rarer causes include struma ovarii, hyperemesis gravidarum, and hydatidiform mole. The clinical presentation of hyperthyroidism may not be obvious, since symptoms of tachycardia, sweating, dyspnea, and nervousness are seen in normal pregnancy, as are cardiac systolic-flow murmurs *(41)*. Maternal complications include miscarriage, abruptio placentae, and preterm delivery. Congestive heart failure and thyroid storm may also occur, and the risk of preeclampsia is significantly higher in women with poorly controlled hyperthyroidism *(41–43)*. Neonatal hyperthyroidism, prematurity, and intrauterine growth retardation may be observed. Hamburger *(44)*, in a retrospective survey, documented a 5.6% incidence of fetal death or stillbirth in 249 pregnancies of hyperthyroid mothers, and a further 5% incidence of fetal and neonatal abnormalities. There is therefore no doubt that hyperthyroidism should be treated to lessen this complication rate.

Gestational amelioration of GD is often associated with a reduction in titer of TRAb and a change from stimulatory to blocking-antibody activity *(45)*. A variety of TRAbs may arise

Table 2
Guidelines for Measurements of TRAbs[a] in a Pregnant Woman With Graves' Disease

Patient status	*Measurement*
Euthyroid, previous ATD only	Not necessary
Euthyroid/hypothyroid, +/- T_4 therapy, previous ^{131}I/surgery	Check in early pregnancy: if low or absent, no further testing if high,[b] check fetus and check antibodies in last trimester
Receiving ATD during pregnancy	Measure in last trimester

[a]TSH receptor antibody assay normally measured by competitive inhibition (i.e., not indicating whether the antibodies are stimulating the thyroid).
[b]High, ≥ 40 mIU/L.
ATD, antithyroid drugs; TRAbs, anti-thyroid-stimulating-hormone antibodies.

in pregnant patients with GD. Zakarija et al. *(46)*, for example, reported the presence of two species of stimulating antibody in a patient who gave birth to three children with transient neonatal hyperthyroidism. TSH-blocking antibodies have been shown to cause maternal hypothyroidism during gestation *(47)*. In addition, TSAbs can cross the placenta and be shown to be present in the fetus by cordocentesis *(48)*.

Management

Preconception

There is a good case for a preconception clinic for patients with Graves' hyperthyroidism who wish to become pregnant. First, such a clinic can provide education about the effects of the disease on maternal health and fetal well-being, to allay fears that are commonly present in these women. The patient's thyroid status should be checked frequently to minimize the risk of miscarriage should she be hyperthyroid at the time of conception. If treatment has been commenced with methimazole (MMI) or carbimazole, a change to propylthiouracil (PTU) may be considered to reduce the admittedly rare occurrence of aplasia cutis reported after the administration of the first two drugs *(49)*, although this is disputed *(41)*.

Previously Treated Patients with GD

Pregnant patients with GD may have received antithyroid drugs (ATDs), surgery or radioiodine (RAI) therapy and may be euthyroid or hypothyroid with or without levothyroxine therapy. The important concern is that neonatal hyperthyroidism may still occur. Recent guidelines state that if ATDs have been previously used, there is no need to measure TRAbs since as the mother's thyroid function gives a reliable estimate of fetal thyroid status, and the risk of neonatal hyperthyroidism is very low *(50)*. Titers of TRAbs should be measured in a euthyroid pregnant woman treated early in pregnancy with any of the modalities. If the TRAb level is high at this time, the fetus should be evaluated carefully during gestation and the antibodies measured again in the last trimester (Table 2).

Graves' Hyperthyroidism Inadvertently Treated With RAI in Early Gestation

The practical procedures surrounding the administration of RAI therapy for GD vary widely *(51)*. In many clinics, routine pregnancy testing is not performed before $^{131}1$ administration. Despite patient's denial of pregnancy, several reports of inappropriate RAI

Table 3
Management of Graves' Hyperthyroidism in Pregnancy

Confirm diagnosis
Start propylthiouracil or methimazole
Render patient euthyroid; continue with low-dose ATD up to and during labor
Monitor thyroid function regularly throughout gestation (at 4–6 wk intervals); adjust ATD if necessary
Check TSAb titer
Discuss treatment with patient
Effect on patient
Effect on fetus
Breast feeding
Inform obstetrician and pediatrician
Review postpartum; check for exacerbation

ATD, antithyroid drugs.

administration have highlighted the concern about the consequent fetal risk of irradiation *(52)*. The maternal thyroid uptake, gestational age of the fetus, and ability of the fetal thyroid gland to concentrate iodine are all vital in determining RAI exposure *in utero (53)*. The fetal thyroid concentrates iodide after 10 to 12 wk of gestation, and is relatively more avid for iodine than is the maternal thyroid. The fetal tissues are also more radiosensitive. Administration of up to 15 mCi of ^{131}I for hyperthyroidism in mothers of fetuses up to 10 wk gestation does not compromise fetal thyroid function, and the low attendant degree of fetal whole-body irradiation is not considered sufficient to justify termination of pregnancy *(54)*, although this is often done. Limited clinical data suggest that ^{131}I given after 10 to 12 wk of gestation results in biochemical hypothyroidism and even cretinism in the neonate *(55)*. In these circumstances, termination of pregnancy may be advised, but dosimetry studies should be performed. If the pregnancy continues to term, intrauterine hypothyroidism may be diagnosed by umbilical-cord sampling. The neonate should be evaluated at birth specifically for hypothyroidism and for malformations, which are more common with higher doses of radiation.

Patients Found to Have Hyperthyroidism During Pregnancy

Medical therapy for pregnant patients with hyperthyroidism is preferred by most clinicians, as RAI is contraindicated and surgery requires pretreatment with antithyroid drugs to render the patient euthyroid (Table 3).

PTU should be given in a dose of 100–150 mg three times daily until the patient becomes euthyroid, at which time the dose should be reduced to the lowest amount to maintain the euthyroid state with a serum T_4 concentration at the upper end of normal *(43)*, and this should possibly be continued up to and through labor if necessary. In terms of rapidity of action and potential for inducing fetal hypothyroidism, there is probably little reason to choose PTU over MMI which has the advantage of a single daily dose. *(56,57)*. The so-called "block and replace" regimen in which thyroxine is given with an ATD, should be used with caution because the dose of ATD might be too high and cause fetal goiter and hypothyroidism. Hashizume et al. *(58)* reported that administration of levothyroxine to pregnant women with Graves' hyperthyroidism during pregnancy and after delivery, together with MMI, was effective in reducing the incidence of postpartum recurrence of

hyperthyroidism (*see* below), but these results have not been confirmed. Rarely, an episode of infection or the development of preeclampsia may precipitate thyroid storm requiring the use of thionamides, iodides, beta-blockers, fluid replacement, and possibly steroid therapy and plasmapheresis *(59)*. PTU has a shorter half-life than MMI and is not present in as high a concentration in breast milk. Hence, women receiving PTU can breastfeed without significant risk to the neonate *(60,61)*. However, MMI can also be safely given to the nursing mother with no evidence of infant thyroid dysfunction or later changes in intellectual achievement (*61a,61b)*. Common complications of thionamide therapy include skin rash, arthralgia, and nausea in about 2% of patients. MMI (or carbimazole) may be used as an alternative in this situation with only a 33% chance of cross-reaction. Agranulocytosis is rare and is an indication for immediate withdrawal of the ATD drug and possible treatment with granulocyte colony-stimulating factor, although the results of this are not always satisfactory *(62)* and there is no experience with it in pregnancy. There is no benefit in routine monitoring of the white-blood-cell count, as the fall in the whole blood-cell count may be very rapid. The suggestion of a specific MMI-induced embryopathy in children exposed to this drug during the first trimester of pregnancy requires further study before being accepted *(63)*.

There is no significant effect of ATDs *in utero* on the long-term health of the neonate or child, assuming that the dose during gestation does not cause iatrogenic fetal hypothyroidism *(64,65)*. β-Adrenergic blocking agents such as propranolol may be used for a few weeks to ameliorate the peripheral sympathomimetic actions of excess TH, but their prolonged use can result in retarded fetal growth and an impaired response to anoxic stress, together with postnatal bradycardia and hypoglycaemia. β-Blocking drugs will need to be used in the uncommon instance of intolerance to both of the available thionamide drugs.

Subtotal thyroidectomy is indicated if the control of hyperthyroidism in a pregnant patient is poor because of poor compliance or inability to take drugs. Patients with a very large goiter may also require surgery because of pressure symptoms. Surgery is preferred in the second trimester, since there is a higher risk of associated abortion at an earlier stage of gestation. In general, surgery should be avoided if it is considered that medical therapy has a reasonable chance of success.

Postpartum GD

Patients with GD may develop Graves' hyperthyroidism followed immediately by transient hypothyroidism as a result of coexisting destructive thyroiditis during the early postpartum period despite increasing thyroid-stimulating antibody (TSAb) activity *(66)*. This may be important when considering postpartum relapse of the disease. In patients with GD, TRAbs have been shown to decrease during late gestation, with a significant rebound in the late postpartum period *(67)*. Screening for TSAb during pregnancy may reveal patients at risk of postpartum relapse of Graves' hypothyroidism *(68)*.

HYPOTHYROIDISM AND PREGNANCY

The incidence of hypothyroidism during pregnancy is approx 2.5% *(69)*. In areas of mild iodine deficiency the incidence of thyroid abnormalities is higher, and thyroid autoimmunity may particularly be associated with diminished thyroid reserve and an increase in spontaneous abortion *(70)*. Most patients with hypothyroidism during pregnancy are asymptomatic but have been found to have a high TSH on screening. Previous studies have documented the deleterious effects of hypothyroidism on maternal and fetal well-being, drawing attention to an increased incidence of abortion, obstetric complications, and fetal abnormalities in

untreated women *(71)*. Women already receiving levothyroxine for hypothyroidism require an increased dose during gestation *(72,73)*. This is critical to ensure adequate maternal thyroxine levels for delivery to the fetus, especially during the first trimester. As noted later in this chapter, low maternal thyroxine concentrations may lead to impaired neurodevelopment in the neonate and child. The dose should normally be increased by 50–100 μg/d.

Maternal Thyroid Disease in Pregnancy: Effect on Child Development

Recent work has raised concern that in an iodine-sufficient area, maternal thyroid dysfunction (hypothyroidism or subclinical hypothyroidism) during pregnancy results in neurointellectual impairment of the child. Two studies *(74,75)* have shown that low TH concentrations in early gestation can be associated with significant decrements in the intelligence quotient (IQ) of the children when tested at 7 yr of age and 10 mo, respectively. Pop et al. *(76)* have also shown a significant decrement in IQ in children aged 5 yr whose mothers were known to have circulating anti-TPO antibodies at 32 wk gestation and were biochemically euthyroid. Moreover, as shown recently *(77)*, the 7-yr-old children of hypothyroid women who were retrospectively diagnosed as having hypothyroidism on the basis of high TSH concentrations in second-trimester sera also showed impaired psychological development as compared to carefully matched control children. The neurodevelopmental impairment is similar to that seen in iodine-deficient areas, and implies that iodine status should be normalized in regions of deficiency.

However, much of the United States and parts of Europe are not iodine deficient, which raises the question of routine screening of thyroid function during early pregnancy or even before conception. Another reason for screening could be to focus on the risk for postpartum thyroiditis *(78)*. The following numerical values should be considered in relation to such a strategy: the incidence of an increased TSH in pregnancy is around 2.5%; the prevalence of anti-TPO antibodies is 10% as ascertained at a routine antenatal booking clinic; the incidence of thyroid dysfunction observed in anti-TPO-antibody-positive pregnancies ranges up to 15% *(79)*. Although these numbers are impressive, the question of whether there is any effective intervention must be addressed. To date, there are no randomized trials examining, for example, the effect of T_4 intervention therapy given to susceptible women on subsequent child development. These considerations emphasize that it is important to ensure an adequate TH supply to the developing fetus in all areas of the world, whether iodine-deficient or not. In addition, pregnant hypothyroid women need to optimize their replacement thyroxine therapy by increasing the dose by 50–100 μg/d. Further studies in this area are required to answer questions relating to screening of thyroid function before and during pregnancy.

NODULAR THYROID DISEASE

Thyroid nodules are claimed to be detected in up to 10% of pregnant women. Fine-needle-aspiration (FNA) biopsy is the first investigation of choice for such nodules, and in one report *(80)* yielded a malignancy/suspicious result in 35%. When malignancy is diagnosed it is usually a differentiated tumor that may be surgically resected in the second trimester, or in some cases safely left until the postpartum period before therapy is started. The impact of pregnancy on thyroid cancer seems to be minimal in that there is no difference in rates of metastases or recurrence from those in nonpregnant women with the same disease *(81,82)*. Whether women already treated for thyroid malignancy should become pregnant is of concern, but current evidence suggests that differentiated thyroid cancer should not inhibit an intended pregnancy *(83)*. Previous ^{131}I therapy does not result in demonstrable adverse events in subsequent pregnancies *(84)*.

POSTPARTUM THYROID DISEASE

H.E.W. Roberton, a general practitioner in New Zealand, described the occurrence of lassitude and other symptoms of hypothyroidism relating to the postpartum period *(85)*. These complaints were treated successfully with thyroid extract. The syndrome remained generally unrecognized until the 1970s, when reports from Japan *(86)* and Canada *(87)* rediscovered the existence of postpartum thyroid disease (PPTD) and recognized the immune nature of the condition. A number of reviews of the condition are available *(88–91)*.

Incidence

A variable incidence (from 3–17%) of PPTD has been reported worldwide because of wide variations in the number of women studied, the frequency of thyroid assessment postpartum, diagnostic criteria employed, and differences in hormone assay methodology *(92)*. However, there is a general consensus that PPTD occurs in 5–9% of unselected postpartum women *(90)*. Women with type 1 diabetes have a threefold greater incidence of PPT than do nondiabetic women *(91)*, and in these cases there is a strong association with ATAs. PPTD is also more likely to recur in a woman who has had a previous episode of postpartum thyroid dysfunction. (*see* next).

Clinical Features

PPTD is characterized by the development of transient hyperthyroidism, hypothyroidism, or both during the first 6 mo of the postpartum period. Hypothyroidism is permanent in up to 25–30% of women. The transient hyperthyroidism presents at about 14 wk postpartum, and is followed with transient hypothyroidism at a median of 19 wk, although the latter may occur as late as 36–40 wk. Very occasionally the hypothyroid state is seen before the hyperthyroidism. PPTD occurs in 50% of women positive for anti-TPO antibody (isolated anti-Tg antibody occurs only in about 5%), of whom 19% are hyperthyroid alone, 49% are hypothyroid alone, and the remaining 32% are hyper- followed by hypothyroid. An example is shown in Fig. 1. Some groups have described up to one third of patients with PPTD as being without antibodies or indeed any immune abnormality *(93,94)*. It seems intrinsically unlikely that such a high proportion of patients are to be found with no ATAs. Although the clinical manifestations of the hyperthyroid state are not usually severe, lack of energy and irritability are particularly prominent even in antibody-positive women who do not develop thyroid dysfunction. In contrast, the symptomatology of the hypothyroid phase may be profound. Many classic hypothyroid symptoms occur before the onset of TH reduction and persist even when recovery in hormone levels is seen *(95)*.

Association with Depression

Early accounts of PPTD noted an anecdotal association of depression with thyroid dysfunction in the postpartum period *(96)*. More recently, an increase in frequency of mild to moderate depression has been confirmed in antibody-positive women irrespective of thyroid status *(97,98)*. Some depressive symptomatology is seen as early as 6 wk postpartum, but there is no clear explanation for these findings. An attempt to prevent depressive symptomatology in women positive for anti-TPO antibody by administration of 0.1 mg thyroxine for 18 wk postpartum was unsuccessful *(99)*.

Diagnosis

The diagnosis of PPTD is made by standard thyroid function testing in women positive for anti-TPO antibody or from a classic presentation of transient thyroid dysfunction in

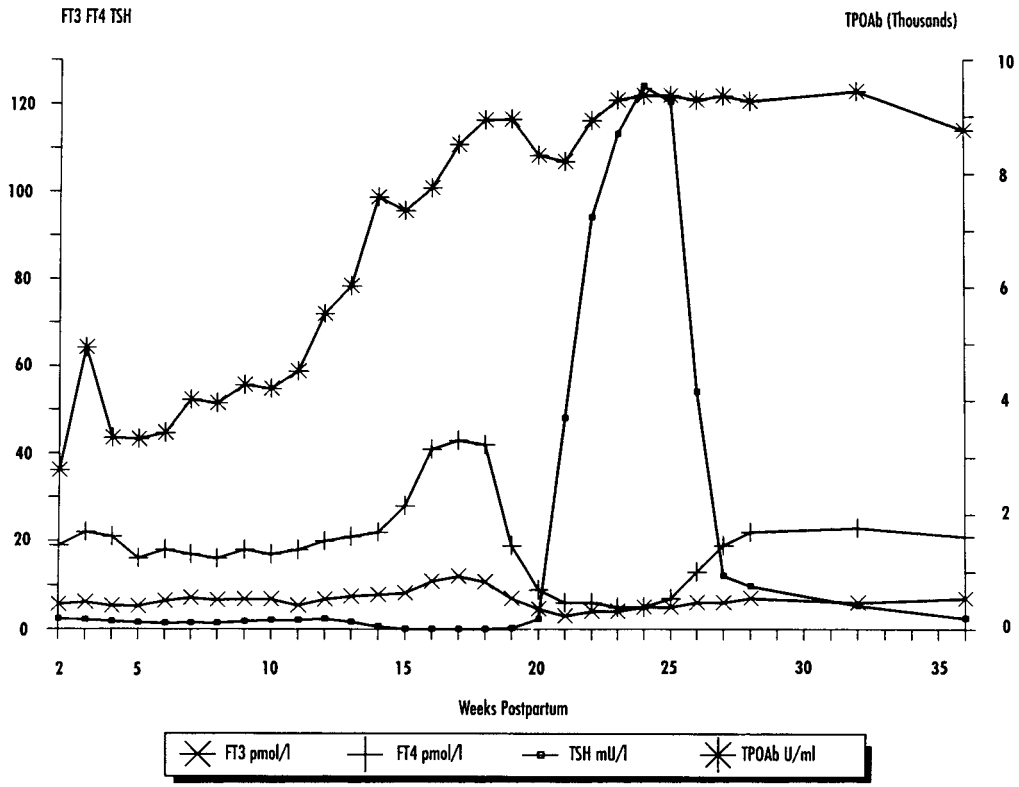

Fig. 1. Graph depicting changes in anti-antibody, FT_4, FT_3, and TSH in a postpartum woman studied for 36 wk postpartum. An episode of transient postpartum thyroiditis with hyperthyroidism (at about 17 wk postpartum) was followed by transient postpartum hypothyroidism at about 23 wk. Note the accompanying rise in anti-TPO antibody titer.

an antibody-negative woman. If possible, it is useful to determine normal ranges for TH during the postpartum period, since they are narrower than for the general population. Care must be taken to exclude ATAs in confusing cases *(100)*. In patients with hyperthyroidism, GD should be excluded by measurement of TRAbs or by RAI uptake (which will be low in PPTD because it is a destructive thyroiditis).

Immunopathogenesis

PPTD is an organ-specific syndrome that has been regarded as a model of aggravation of the autoimmune state *(101)*. Thyroid ultrasonography has demonstrated diffuse or multifocal echogenicity reflecting the abnormal thyroid morphology *(102)* and consistent with the known lymphocytic infiltration of the thyroid characterized by a positive perchlorate discharge test *(103)*. The destructive nature of the thyroiditis is also shown by the increase in urinary iodine excretion in the hyperthyroid as well as in the hypothyroid phase of the syndrome *(104)*, in addition to an early rise in serum Tg *(105)*. There is no evidence that ambient iodine concentrations affect the incidence of PPTD, and iodine administration to marginally iodine-deficient pregnant women will not prevent the onset of PPTD *(106)*. Although it is accepted that anti-TPO antibodies are a useful marker of the syndrome, it is unlikely that they are the sole pathogenetic agents for it *(91)*. Studies of antibody functional affinity and IgG subclass distribution suggest strongly that, as in other autoimmune diseases,

activation of the complement system is an etiologic factor in PPTD, especially as this correlates with the severity of the thyroid dysfunction *(107)*. Studies of the cellular arm of the immune system have shown that women who develope PPTD have a significantly higher CD^{4+}/CD^{8+} ratio and T-cell activation than do normal anti-TPO-antibody-negative controls *(108)*. In an animal model, the severity of experimental autoimmune thyroiditis was reduced in pregnancy by enhancement of the TH2 phenotype as evidenced by interleukin-4 production *(109)*. Further work in this area is required to elucidate factors that trigger PPTD, as well as those that are resposible for its transitory nature in the majority of patients.

Follow-Up and Course of the Syndrome

Although the hyperthyroidism of PPTD always resolves, several long-term studies of the hypothyroid phase have documented persistence of hypothyroidism in 20–30% of cases *(91)*. Longer follow-up (up to 7 yr) shows that 50% of patients who have developed thyroid dysfunction postpartum will subsequently become hypothyroid, as compared to only 5% of antibody-positive but PPTD-negative women *(110)*. Recurrence of transient PPTD has been observed by several workers in small numbers of patients *(111)*, and a 70% chance of developing recurrent PPTD after a first attack, with a 25% risk even for women who were only anti-TPO-antibody-positive without thyroid dysfunction during the first postpartum period, has been documented *(112)*.

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15 Goitrogens in the Environment

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Contents

INTRODUCTION

No less than 200 million members of the world's human population have the thyroid enlargements known as goiters and associated disorders, resulting in a public health and socioeconomic problem of major proportions *(1,2)*. It is clear that the greatest goitrogenic factor among the world's population is iodine deficiency. Seventy-five percent of people with goiter live in less developed countries where iodine deficiency is prevalent. The role of iodine deficiency as an environmental determinant in the development of endemic goiter is firmly established. However, iodine deficiency does not always result in endemic goiter *(3)*, and iodine supplementation does not always result in complete eradication and prevention of goiter *(1,3–9)*. Even in the presence of extreme iodine deficiency there is an unequal geographic distribution of goiter.

With 25% of people with goiter living in more developed countries where goiter occurs despite iodine prophylaxis, it is also clear that there are other factors, beyond iodine deficiency, that may play a role in this etiopathogenesis of endemic goiter. Naturally occurring dietary agents, such as a cyanogenic glucoside from cassava *(10–12)* and flavonoids from millet *(13–18)*, have been well studied and shown to magnify the severity of goiter endemia. A large number of both naturally occurring and human-made chemical agents in the environment are known in the laboratory to interfere with thyroid-gland morphology and function, and may pose the danger of thyroid disease. Some of these have been shown also to interfere with human thyroid-gland morphology and function.

Environmental exposures to disease-causing factors occur through inhalation, ingestion, or dermal exposure. Environmental exposures to goitrogens are generally only known to occur through ingestion, primarily in either food or water. As in all toxicology, the words of Paracelsus that "the dosage makes the poison" apply to goitrogens. Whether the

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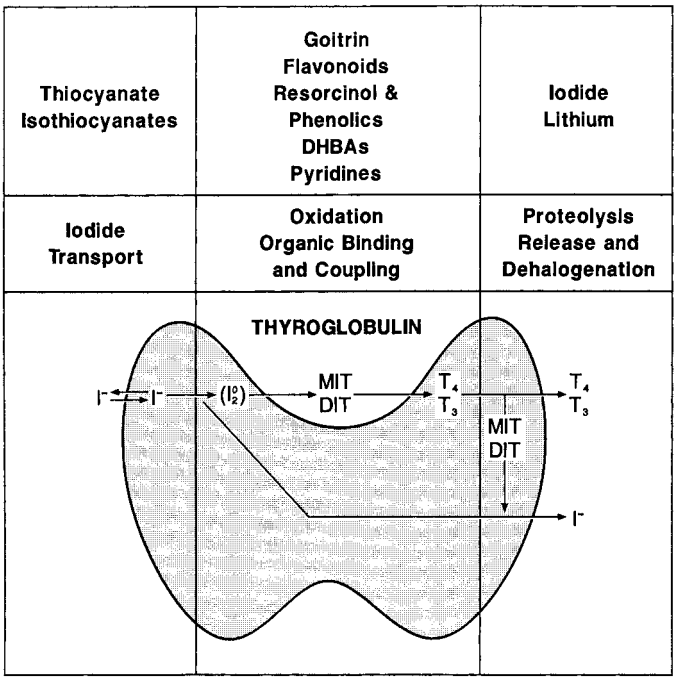

Fig. 1. Environmental antithyroid/goitrogenic compounds and their site of action in the thyroid gland. Abbreviations: goitrin, L-5-vinyl-2-thiooxazolidone; DHBAs, dihydroxybenzoic acids; I^-, iodide; MIT, monoiodotyrosine; DIT, diiodotyrosine; T4, thyroxine; T3, triiodothyronine. Reprinted, with permission, from the *Annual Review of Nutrition*, Volume 10, © 1990 by Annual Reviews, www.AnnualReviews.org.

environmental exposure to specific goitrogens is sufficient to have an effect, or an adverse effect, on the thyroid must be examined on a case-by-case basis. Goiter is the most prominent effect of antithyroidal agents. These agents may cause the goitrous condition by acting directly on iodine uptake by the thyroid gland (Fig. 1), by interfering with thyroid hormone (TH) synthesis or release, by altering the regulatory mechanisms of the thyroid gland, and by affecting the peripheral metabolism and excretion of TH *(4,19,20)*.

Figure 1 illustrates the three main steps in the production of TH, showing some of the environmental agents that may act directly on the thyroid gland by interfering with the process of hormone synthesis at various steps. While the mechanisms leading to increases or decreases in TH production are fairly well known, the mechanism that induces the trophic changes leading to goiter is not well understood. Thyrotropin or thyroid-stimulating hormone (TSH) is the primary hormone related to thyroid stimulation, but other humoral, paracrine, and autocrine growth factors appear to be involved in the goitrogenic process *(21–23)*. Environmental agents may enter into the food, water, and air exposure pathways, becoming an additional antithyroid and/or goitrogenic factor in humans *(4,19,20)*. Their effects may be additive to those of iodine deficiency, making more severe the intensity of the manifestations of goiter, hypothyroidism, and iodine-deficiency disorders (IDD) (cretinism, congenital hypothyroidism (CH), and various degrees of impairment of growth and mental development). In iodine-sufficient areas, these environmental agents may be responsible for the development of some "sporadic" goiters. Their potential roles in other thyroid disorders, such as autoimmune thyroiditis, hypothyroidism, hyperthyroidism, and, possibly thyroid carcinoma *(1,2,24)*, deserve consideration.

ENVIRONMENTAL GOITROGENS OF INTEREST OR CONCERN

Iodine, and possibly selenium, are the two environmental goitrogens whose effect is demonstrated by their absence or deficiency. The other goitrogens demonstrate their effect by interfering with the thyroidal system through direct action. For convenience, we have grouped such studied environmental goitrogens into: (1) thiocyanate-like and thiourea-like substances derived generally from dietary vegetation (e.g., goitrin and flavonoids); (2) organic chemicals (often from combustion or chlorination) that are generally industrially-related; and (3) inorganic chemicals generally present in groundwater. Most of the chemicals considered to be environmental goitrogens are chemicals found in the environment and shown in in-vitro studies to have antithyroid effects; others have been demonstrated to have the antithyroid and/or goitrogenic effects in in-vivo laboratory studies; and a few have been demonstrated in humans to have such effects. The last group includes thiocyanate, goitrin, resorcinol, 2,3-dinitrophenol, and the inorganic substance iodine, lithium, nitrate, and perchlorate.

Dietary Goitrogens

Sulfurated Organic Compounds: Thiocyanate, Isothiocyanates, and Thio-oxazolidone (Goitrin)

Thiocyanates and isothiocyanates have been demonstrated as goitrogenic principles in plants of the Cruciferae family. Goitrin itself was isolated from yellow turnips and from brassica seeds. Cyanogenic glycosides (thiocyanate precursors) have also been found in several staple foods (cassava, maize, bamboo shoots, sweet potatoes, lima beans) native to Third World countries. After ingestion, these glycosides can be readily converted to thiocyanate by widespread glycosidases and sulfur transferase. Isothiocyanates not only use the thiocyanate metabolic pathway, but also react with amino groups to form derivatives that have thiourea-like antithyroid effects. Thus, the actual concentration of thiocyanates or isothiocyanates in a given foodstuff may not represent its true goitrogenic potential, nor does the absence of these compounds negate a possible antithyroid effect, because inactive precursors can be converted into goitrogenic agents both in the plant itself and in animals after being ingested. Thioglycosides undergo a rearrangement to form isothiocyanate derivatives and, in some instances, thiocyanate. Therefore, the amount of thiocyanate in the urine is a good indicator of the presence of thioglycosides in food. Ingestion of progoitrin, a naturally occurring thioglycoside, elicits antithyroid activity in rats and humans because of its partial conversion by intestinal microorganisms into goitrin, which has more potent antithyroid ativity. This ability of plants and animals to readily convert inactive precursors into goitrogenic agents must be considered when the possible etiologic role of dietary elements in endemic goiter is being investigated *(12,19,20,25)*.

Several goiter endemias have been attributed to the presence of sulfurated organic compounds in foodstuffs *(2,10–12,19,20,25)*. The best documented are in some areas of Zaire, where as many as 60% of the population are affected by goiter. Cassava, a staple food in these areas, has definite antithyroid effects in humans and experimental animals, and daily consumption of cassava, in the presence of severe iodine deficiency, is thought to be the cause of endemic goiter and cretinism in these areas of Zaire. The goitrogenic action of cassava is a result of endogenous release of thiocyanate from linamarin, a cyanogenic glucoside present in cassava, particularly in the tuberous roots. A number of studies have demonstrated that high thiocyanate levels from cabbage-eating in Sicily *(41)* or cassava consumption in Mozambique *(42)* and Zaire *(43)* showed that adequate iodine in the diet

prevented a goitrogenic effect. Thiocyanate is also present in pearl millet (*Pennisetum americenium* [L] Leeke, also known as *P. typhoides* or *P. americanum*), the staple food of people living in areas of iodine deficiency endemic goiter in the western Sudan. Pearl millet is rich in C-glycosylflavones, which, in combination with thiocyanate, exert additive and complementary antithyroid and goitrogenic effects *(13)*.

Thiocyanate. Thiocyanate or thiocyanate-like compounds primarily inhibit the iodine-concentrating mechanism of the thyroid, and their goitrogenic activity can be overcome by iodine administration (Fig. 1). Thiocyanate at low concentrations inhibits iodide transport by increasing the velocity constant of iodide efflux from the thyroid gland. At high concentrations, the iodide efflux is greatly accelerated, whereas the unidirectional iodide clearance into the gland is inhibited. Thiocyanate at high concentrations also inhibits the incorporation of iodide into thyroglobulin (Tg) by competing with iodide at the thyroid peroxidase (TPO) level *(25)*. Thiocyanate is rapidly converted to sulfate in the thyroid gland. Administration of thyroid-stimulating hormone (TSH) increases the intrathyroidal catabolism of thiocyanate and is capable of reversing the block of iodide uptake produced by the thiocyanate ion. TSH probably accelerates the oxidation of thiocyanate to sulfate.

Thiocyanate is also found in high concentrations (1 g/L) in wastewater effluents of coal-conversion processes, and in body fluids as a metabolite of hydrogen cyanide gas consumed while smoking *(19)*. Goiter and hypothyroidism were documented in patients receiving long-term thiocyanate treatment for hypertension *(25a)*. This goitrogenic effect of thiocyanate is more evident in the presence of iodine deficiency. Several observations suggest that thiocyanate crosses the human placenta and may cause both goiter and neonatal hypothyroidism *(26–28)*. The thiocyanate ion contains a linear SCN group in which the double-bond character of the S-C linkage reflects the existence of two tautomeric structures: S–C–N and –N=C=S. Thiocyanate, like nitrate and cyanide ions, is ambident, since the negative charge can be located either on S or N. This tautomerism explains the existence of two series of covalent derivatives, the thiocyanates and isothiocyanates *(25)*.

The explanations for the effects of cigarette smoking on the thyroid gland are unclear. Cigarette smoke, besides containing thiocyanate, contains a variety of goitrogenic resorcinol derivatives, flavonoids, and hydroxypyridines *(19)*. As mentioned earlier, cigarette smoking may produce goiter *(19,29)*. Recent studies indicate that smoking increases the severity and metabolic effects of hypothyroidism, probably by altering both thyroid function and hormone action *(30,31)*.

Serum thiocyanate levels are clearly increased in smokers, and thiocyanate has been shown in the laboratory to be the only potent antithyroidal agent in smoking products *(32)*. Smoking was associated with an increase in clinically overt thyroid disease in a Danish twin study *(33)*. A Swedish study found that the prevalence of both nontoxic goiter and toxic diffuse goiter was significantly higher in smoking women than in nonsmoking women, and suggested that thiocyanate might be the goitrogenic factor responsible for the increased prevalence of nontoxic goiter *(34)*. A Japanese study of female patients with Hashimoto's thyroiditis (HT) showed a marked increase in hypothyroidism among those who smoked *(35)*. A 1994 review of cigarette smoking and the thyroid concluded that TH levels and TSH-receptor autoantibodies (TRAbs) were not affected by smoking, but that serum TSH levels were slightly reduced *(36)*. A Belgian study indicated that maternal cigarette smoking could contribute to neonatal thyroidal enlargement *(28)*, and an Italian study indicated that passive (paternal) smoking contributed a goitrogenic effect (elevated serum Tg level) during pregnancy (cord blood sample) but not during infancy (sera at 1 yr of age), in spite of increased plasma thiocyanate levels *(37)*. This latter observation contributed to the

conclusion that "it seems appropriate to look beyond thiocyanate to explain the multiple thyroidal effects of smoking" *(38)*.

Isothiocyanates. The isothiocyanates and cyanogenic glycosides act on the thyroid mainly by their rapid conversion to thiocyanate. However, isothiocyanates, as previously mentioned, not only use the thiocyanate metabolic pathway but also react spontaneously with amino groups to form thiourea derivatives, which produce a thiourea-like antithyroid effect. Isothiocyanates also have intrinsic antithyroid activity, as demonstrated by in vitro inhibition of iodide uptake in the case of methyl- and allyl-isothiocyanates, and of both iodide uptake and organification in the case of butylisothiocyanate *(25,39)*.

Thio-Oxazolidone (Goitrin). The thionamide or thiourea-like goitrogens interfere in the thyroid gland with the organification of iodide and formation of the active TH, and their action usually cannot be antagonized by iodine. Naturally occurring goitrin (L-5-vinyl-2-thio-oxazolidone), a potent antithyroid compound is representative of this category of substances (Fig. 1). Long-term administration of goitrin to rats results in increased thyroid weight and decreased radioactive iodine (RAI) uptake and hormone synthesis by the thyroid gland *(25)*. The thionamide-like antithyroid effects of goitrin have been confirmed in vitro by marked inhibition of both TPO *(40)* and iodine organification *(39)*. Indeed, goitrin has 133% of the potency of propylthiouracil (PTU) in humans. Goitrin is unique in that it is not degraded like thioglycosides. Additive antithyroidal effects of thiocyanate, isothiocyanate, and goitrin also occur with combinations of these naturally occurring goitrogens *(25)*.

Disulfides

The small aliphatic disulfides (R–S–S–R; R = methyl- ethyl-, *n*-propyl-, phenyl-), major components of onion and garlic, exert marked thiourea-like antithyroid activity in the rat *(19,20,25)*. *n*-Propyl disulfide also suppresses RAI uptake by the thyroid in rats on a low-iodine diet. None of these disulfides inhibits TPO in vitro *(44)*, but fractions containing sulfur-bearing organic compounds, possibly including aliphatic disulfides from the goitrogenic well supplying a Colombian district in which goiter is endemic, inhibited I-organification examined with thyroid slices in vitro *(5,6)*.

Disulfides are also present in high concentration (0.3–0.5 g/L) in aqueous effluents from coal-conversion processes, and have been identified as water contaminants in the United States *(19,20,25,44)*. The most frequently isolated disulfide compounds in the United States are dimethyl, diethyl, and diphenyl disulfides.

Flavonoids

Flavonoids are important stable organic constituents of a wide variety of plants. Flavonoids are universally present in vascular plants and in a large number of food plants. Because of their widespread occurrence in edible plants such as fruits, vegetables, and grains, flavonoids are an integral part of the human diet *(4,46–48)*. They are present in high concentrations in polymeric (tannins) and oligomeric (pigments) forms in various staple foods of the Third World, such as millet, sorghum, beans, and ground nuts.

Flavonoids are polyhydroxyphenolic compounds with a C_6–C_3–C_6 structure *(14,47–49)*. Because mammalian organisms are unable to synthesize the flavone nucleus, flavonoids are strictly exogenous food components, of exclusively vegetable origin. They have high chemical reactivity, with multiple important biologic implications *(47,48)*. Most flavonoids are present as β-glucosides that cannot be absorbed in tissues. No mammalian enzymes have been found that deglycosylate these compounds to their bioactive aglycone species. Following their ingestion by mammals, flavonoid glycosides are hydrolyzed to flavonoid

aglycones by intestinal microbial glycosidases. These aglycones may be absorbed and undergo metabolism by mammalian tissues, or may be further metabolized by intestinal microorganisms to undergo β-ring hydroxylation and middle-ring fission, with production of various metabolic monomeric compounds, including phenolic acids, phloroglucinol, resorcinol, and garlic acid *(14)*. Each metabolic step is characterized by a marked increase in antithyroid effects *(14)*.

Because flavonoids are quickly metabolized by intestinal microorganisms, they are not found in normal tissue constituents *(14)*. Flavonoid aglycones, such as the apigenin and luteolin present in Fonio millet (*pigitaria exilis*) *(18)*, and a variety of flavonoid metabolites (phloroglucinol, resorcinol, phenolic acids), are several times more potent than the parent glycosides glucosylvitexin, glucosylorientin, and vitexin present in pearl millet (*P. americanum* [L] leeke) *(13,46)* as inhibitors of TPO, the enzyme catalyzing iodide oxidation and hormone synthesis in the thyroid gland. This greater inhibitory effect is further enhanced by the additive effects exerted by mixtures of flavonoid aglycones and flavonoid metabolites, which are formed after ingestion of mixtures of flavonoid glycosides present in many plant foodstuffs. In addition, these metabolic products may exert adverse effects on other parameters of thyroid function not observed with the glycosides. As a result, the antithyroid effects of flavonoid glycosides in foodstuffs may be greatly enhanced by metabolic alterations after ingestion by mammals, as in the case of the flavonoids present in pearl millet, the staple food of people living in areas of endemic iodine-deficiency goiter in western Sudan, which make a major contribution to and are primarily responsible for the antithyroid and goitrogenic effects of pearl millet *(13,15)*. Furthermore, antithyroid effects in vivo of vitexin, one of the three major flavonoids in pearl millet, have recently been demonstrated *(15)*, providing direct experimental evidence that C-glycosylflavones are the goitrogens in this cereal grain. It is of interest that a significant portion of the flavonoids isolated from Fonio millet *(18)*, the staple food of people living in the severely affected endemic-goiter area of Guinea in west Africa, are already present as the aglycones apigenin and luteolin, which, as noted earlier, have more potent antithyroid activity than their parent glycosides.

Flavonoids not only inhibit TPO but, acting on iodothyronine deiodinase enzymes, also inhibit the peripheral metabolism of TH. Flavonoids also affect serum TH binding and TSH regulation. Furthermore, polymers of the flavonoid phloretin interact with TSH, preventing its action on thyroid cells *(4,47–50)*. The flavonoid quercetin, an inhibitor of heat-shock protein (humic substances HSP-70) messenger RNA (mRNA), has also been shown to increase iodide uptake in FRTL-5 thyroid cells, and the quercetin glycoside rutin has been shown to have a similar effect in porcine thyroid slices *(14,51)*. The isoflavone genistein, a specific tyrosine kinase inhibitor, blocks the epidermal growth factor (EGF) desensitization of TSH-adenylate cyclase "crosstalk" in thyroid cells *(52)*. Additionally, the flavonoid luteolin present in Fonio millet depresses cyclic adenosine monophosphate (cAMP) phosphodiesterase, implying a concomitant overproduction of TSH-dependent cAMP *(18)*. Thus, these experimental observations indicate that flavonoids alter TH economy in a complex manner.

There is substantial epidemiologic and experimental evidence indicating that: (1) various millet species used as staple food by populations in the semiarid tropics are rich in flavonoids; (2) flavonoids have potent and diverse antithyroid properties; and (3) that under the appropriate environmental dietary conditions of low iodine and protein-calorie intakes, which are prevalent in most countries of the Third World, flavonoids become an important etiologic determinant of endemic goiter and hypothyroidism *(2,13–18,24,53–55)*. As in the case of cassava, the goitrogenic staple food in Zaire, more plant-biotechnology research is necessary to identify and produce a superior variety of millet with a low flavonoid content

and which can endure various environmental hazards, such as weather, insects, pathogens, and herbicides, while producing optimum crop yields. Modern genetic engineering is finding increased use in plant biotechnology, with significant potential to modify and improve crop plants *(2,56)*.

Soy Protein

In the United States, the consumption of diets with a high soy content as health products has recently increased. Soy products form an important part of the diet of vegetarians. The goitrogenic effect of soybeans was first observed in rats in the 1930s *(57)*. The report of goiter in infants receiving soy-based infant formulas in the United States led to the increased supplementation of such formulas with iodine in the mid-1960s *(58)*. Nonetheless, some hypothyroid children still showed thyroxine (T_4) refractiveness *(59)*. A Japanese study of healthy adults with adequate iodine intake demonstrated that a diet including 30 g/d of soybeans led to thyroid enlargement and an increased serum level of TSH *(60)*. A rebound increase in free thyroxine (FT_4) was observed after cessation of the soy consumption.

Soybean extract yields the isoflavones genistein and daidzein, which have been shown to inhibit TPO catalyzed reactions essential to TH synthesis. In the presence of iodide ion, genistein and daidzein blocked TPO-catalyzed tyrosine iodination by acting as alternative substrates for TPO, yielding mono-, di-, and tri-iodoisoflavones *(61)*. In an experiment with human goiter-derived Tg as a substrate, genistein and daidzein inhibited the formation of thyroxine in a concentration-dependent manner with the inhibitory concentration for a 50% reduction in thyroxine formation (IC_{50}) value being 2.0 and 8.8 μM, respectively. Consumption by humans of two servings of a soy-protein beverage per day for 14 d yielded mean plasma thyroxine levels of 0.6 and 0.3 μM, respectively *(62)*.

Industrial and Geologic Organic Chemicals

Polyhydroxyphenols and Phenol Derivatives

Coal is a source of a wide variety of antithyroid and goitrogenic compounds, including phenol, resorcinol, and other dihydroxyphenols, substituted dihydroxybenzenes, thiocyanates, disulfides, phthalic acids, pyridines, and halogenated and polycyclic aromatic hydrocarbons (PAHs) *(4,19,20,63–66)*. Most of these compounds have been identified in drinking water from iodine-sufficient goitrous areas of Kentucky and Colombia *(4,66)*. Phenolics are the major organic pollutants in wastewater effluents from various types of coal-treatment processes. Resorcinol, substituted resorcinols, and other antithyroid phenolic pollutants are present at levels of as high as 5 g/L in coal-derived effluents. Up to 8% of shale bitumen is also composed of phenols.

Phenol, dihydroxyphenols, trihydroxyphenols, and halogenated phenols are readily absorbed from the gastrointestinal tract; phenol, resorcinol, and catechol, in suitable preparations, can be absorbed through human skin. Essentially all phenols, polyhydroxyphenols, and halogenated phenols are readily absorbed after injection *(67)*. A major route of metabolism of polyhydroxyphenols, polyhydroxyphenolic acids, and halogenated phenols is by conjugation to glucuronic or sulfuric acids. The pattern of conjugation varies with animal species. Although the polyhydroxyphenols and their derivatives, including halogenated dihydroxyphenols, have more than one hydroxyl group capable of undergoing metabolic conjugation, only one group is conjugated. The major route of excretion of these compounds is the urinary tract, and various amounts of the free parent compound and its monoglucuronide and monosulfate conjugates are excreted in the urine *(67)*.

Resorcinol, the prototype of the polyhydroxyphenol group of compounds and their derivatives, is antithyroid and goitrogenic both in humans and experimental animals *(4,19,20)*. In the early 1950s, the goitrogenic effect of resorcinol was demonstrated when patients applying resorcinol ointments for the treatment of varicose ulcers developed goiter and hypothyroidism. Several observations also suggested that resorcinol crossed the human placenta and could cause both goiter and neonatal hypothyroidism *(27)*. Resorcinol has been shown both in vivo and in vitro to inhibit thyroidal organification of iodide *(4)*. A comparison of the antiperoxidase activity of resorcinol (1,3-dihydroxybenzene), catechol (1,2-dihydroxybenzene), and hydroquinone (1,4-dihydroxybenzene) indicates the importance of hydroxyl groups in the *meta* position of the benzene ring for maximal activity *(44)*. Furthermore, the net antiperoxidase effects of mixtures of dihydroxyphenols, as well as of dihydroxyphenols and thiocyanate—another coal-derived pollutant—are as great as or greater than the sum of the effects produced by individual compounds of this type, indicating that the true goitrogenic potential of the major water-soluble compounds present in coal and shales comes from the combined effects of the individual constituents, rather than from any single compound *(44)*. The in vivo and in vitro demonstration of antithyroid and goitrogenic activities of coal-water extracts from iodine-sufficient areas of endemic goiter *(68)* indicates that shale- and coal-derived organic pollutants appear to be major contributors to the high prevalence of goiter and associated disorders observed in certain areas with aquifers and watersheds rich in these organic rocks *(4,7,19,20,66)*.

Studies of the physical state of organic goitrogens in water indicate that the active compounds form dissociable complexes, and that they are part of larger organic molecules, possibly humic substances *(4,19,20,69)*. Furthermore, resorcinol and other parent antithyroid phenolic and phenolic–carboxylic compounds are monomeric byproducts of the reduction, oxidation, and microbial degradation of humic substances. Humic substances, which are high-molecular-weight complex polymeric compounds, are the principal organic components of soils and waters. More than 90% of total organic matter in water consists of humic substances, which are also present in coals and shales. Decaying organic matter becomes the substrate of lignin and flavonoid types of humic substances during the process of fossilization or coalification. Indeed, cyanidin, a naturally occurring flavonol used as the model subunit of flavonoid-humic substances, yields by reductive degradation the antithyroid compounds resorcinol, phloroglucinol, orcinol, and 3,4-dihydroxybenzoic acid (DHBA) *(19)*. The demonstration in vivo and in vitro of antithyroid effects of vitexin *(15)*, a major C-glucosylflavone in pearl millet, provided direct evidence of flavonoid structures as a link between phenolic goitrogens in foodstuffs (e.g., millet) and those present in coals, shales, soils, and water, all of which are an obligatory step and integral part of the biogeochemic cycling of organic-phenolic goitrogens in nature *(20,67)* (Fig. 2).

The presence of halogenated organic compounds with known or potential harmful effects has awakened public-health and environmental concerns. These compounds are produced by the chlorination of water supplies, sewage, and power-plant cooling waters. Present in microgram-per-liter concentrations (parts per billion) in treated domestic sewage and cooling waters, 4-chlororesorcinol and 3-chloro-4-hydroxybenzoic acid have antithyroid activities as inhibitors of TPO and thyroidal iodide organification *(19,67)*. Whether these pollutants exert additive or synergistic antithyroid effects, and/or act as "triggers" of autoimmune thyroiditis, requires investigation, particularly because more than 60 soluble chloroorganic substances have been identified in the primary and secondary effluents of typical domestic sewage-treatment plants.

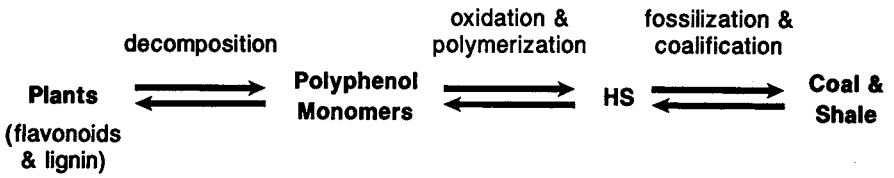

Fig. 2. Simplified scheme of the biogeochemical cycle of goitrogens and interrelationships among plant flavonoids (e.g., millet), polyhydroxyphenols, humic substances, coals, and shales. Reprinted, with permission, from the *Annual Review of Nutrition*, Volume 10, © 1990 by Annual Reviews, www.AnnualReviews.org.

Derivatives of 2,4-dinitrophenol (DNP) are widely used in agriculture and industry. An insecticide, herbicide, and fungicide, DNP is also used in the manufacture of dyes, to preserve timber, and as a chemical indicator; it is also a byproduct of the ozonization of parathion. DNP is readily absorbed through intact skin and the respiratory tract; it causes toxicity by uncoupling oxidative phosphorylation in the mitochondria of cells throughout the body. Administration of DNP to human volunteers resulted in rapid and pronounced decline of circulating TH. A decrease in TSH secretion results in decreased synthesis and release of thyroxine and triiodothyronine (T_3), and possibly in involution of the thyroid gland. The antithyroid effect of DNP is due in part to an inhibition of the pituitary TSH mechanism. Once thyroxine and triodothyronine are released into the circulation, they are instantaneously bound to serum carrier proteins. DNP also interferes with thyroxine binding, further decreasing the serum thyroxine concentration. In addition to inhibiting the TSH mechanism and interfering with thyroxine binding, DNP also accelerates the disappearance of thyroxine from the circulation, thus further reducing the serum concentration of this hormone *(19,67)*. The public-health impact of DNP on the thyroid is still unknown.

Pyridines

In addition to phenols and the other substances named in the hydroxypyridines also occur in aqueous effluents from coal-conversion processes, as well as in cigarette smoke *(19,20, 44,63,67)*. Dihydroxypyridines and 3-hydroxypyridine are potent inhibitors of TPO, producing effects comparable to or greater than those of PTU *(44)*. After ingestion, mimosine, an amino acid occurring naturally in the seeds and foliage of the tropical legume *Leucaena leucocephala*, is metabolized to 3,4-dihydroxypyridine (3,4-DHP), a potent antithyroid agent that produces goiter in mice, rats, sheep, and cattle *(19,20,70)*. 3,4-DHP crosses the placental barrier, producing goitrous offspring. The phenolic properties of the 3-hydroxy group in various hydroxypyridines are reflected in the metabolism of these compounds in vivo. 3-Hydroxypyridine fed to rabbits is converted to ethereal glucuronide and sulfate conjugates. 3,4-DHP glucuronide and sulfate conjugates account for the majority of 3,4-DHP in the blood of cattle grazing on *L. leucocephala*. The ring structure of dihydroxypyridines does not appear to be broken down in the body, and also appears to be relatively stable to bacterial degradation *(70)*.

Phthalate Esters and Metabolites

Phthalic acid esters, or phthalates, are ubiquitous in their distribution and have been frequently identified as water pollutants *(19,20,71–73)*. Dibutyl phthalate (DBP) and dioctyl phthalate (DOP) have been isolated from water supplying areas of endemic goiter in western Colombia and eastern Kentucky *(4,19,20,66)*. Although phthalate esters are most commonly the result of industrial pollution, they also appear naturally in shale, crude oil, petroleum,

plants, and fungal metabolites, and as emission pollutants from coal-liquefaction plants *(19,20,63–66,73)*.

Phthalate esters are well absorbed from the gastrointestinal tract. Prior to their intestinal absorption, they are hydrolyzed to the corresponding monoester metabolite. This is particularly true of the longer-chain derivatives, such as DOP. Phthalates are widely distributed in the body; the liver being the major, initial repository organ. Clearance from the body is rapid. Short-chain phthalates can be excreted unchanged or following complete hydrolysis to phthalic acid. Before excretion, most long-chain phthalate compounds are converted, by oxidative metabolism, to polar derivatives of the monoesters. The major route of phthalate-ester elimination from the body is urinary excretion *(71–73)*.

Phthalate esters are commonly used as plasticizers to impart flexibility to plastics, particularly polyvinylchloride (PVC) polymers, which have a wide variety of biomedical and other uses including building and construction, home furnishings, cars, clothing, and food wrappings. A small fraction of phthalate esters are used as nonplasticizers for pesticide carriers, oils, and insect repellents. Phthalates may be present in concentrations of up to 40% of the weight of a plastic *(71,72)*.

Phthalate esters are known to leach out from finished PVC products into blood and physiologic solutions. The entry of these plasticizers into a patient's bloodstream during blood transfusion, intravenous fluid administration, or hemodialysis has become a matter of concern among public-health officials and the medical community *(19,71–73)*. A high incidence of goiter has been reported in patients receiving maintenance hemodialysis *(73a)*. Whether phthalate-ester metabolites, contaminants in the water entering a patient's bloodstream, or middle molecules (e.g., hydroxybenzoic and vanillic acids), which accumulate in uremic serum and are poorly removed by hemodialysis, are responsible for such goiter remains to be determined *(19,73)*. Although phthalate esters and phthalic acids do not have intrinsic antithyroid activity, they undergo degradation by gram-negative bacteria to form dihydroxybenzoic acid (DHBA) *(19,20,73)*. DHBAs are known to possess antithyroid properties *(19,69,73)*. The 3,4- and 3,5-DHBAs also inhibit TPO in vitro, as well as the incorporation of iodide into TH. The proven effective role of gram-negative bacteria in phthalate biodegradation may in part explain the relationship between frequency of goiter and bacterial contamination of water supplies *(19,20,73,74)*. Furthermore, marked ultrastructural changes in the thyroid gland, similar to those seen after administration of TSH, and a decreased serum thyroxine concentration, have been observed in rats treated with phthalic acid esters *(75)*. Thus, phthalates may become goitrogenic under appropriate conditions. Moreover, phthalates are actively concentrated and metabolized by several species of fish. Whether these widely distributed pollutants exert deleterious effects on the thyroid glands of humans has not been investigated. A study in Puerto Rico found measurable phthalate levels in 23% of the control population *(76)*. The Centers for Disease Control and Prevention has measured phthalate concentrations in blood samples collected by the National Health and Nutrition Examination Survey (NHANES III) in the United States. This would provide a reasonable database in which to seek an association between phthalate levels and thyroid function, although no such study has yet been reported.

Polychlorinated and Polybrominated Biphenyls

The polychlorinated biphenyls (PCBs) and polybrominated biphenyls (PBBs) are aromatic compounds containing two benzene nuclei with two or more substituent chlorine or bromine

atoms. They have a wide variety of industrial applications, including use in electric transformers, capacitors, and heat transformers *(19,77,78)*.

There is growing evidence that atmospheric transport is the primary mode of global distribution of PCBs from sites of their use and disposal *(77)*. Plant foliage accumulates the vapor of PCBs from the atmosphere. The occurrence of these substances in surface water (e.g., rivers and lakes) generally comes from runoff from industrial sites or industrial applications. PCBs have also been detected in drinking water *(79)*. Perhaps the most significant human exposures are limited to individuals consuming freshwater fish from PCB-contaminated streams and lakes, and to occupational exposure of industrial workers. PCBs can also be found in the milk of nursing mothers who have eaten large amounts of sport fish or who have been occupationally exposed *(19,78,79)*. At present, PCBs are targeted by bioremediation strategies, and some strains of *Pseudomonas* spp. (*Pseudomonas cepacia*) can degrade these stable aromatic pollutants *(80,81)*.

PCBs and PBBs have high lipid solubility and resistance to physical degradation. They are slowly metabolized, and their excretion is limited. Long-term, low-level exposure to the organohalides results in their gradual accumulation in fat, including the fat of breast milk. PCBs have been found in the adipose tissue of 30–45% of the general population *(78,79,82)*. The levels in humans have declined during the past two decades *(83)*, such that in the United States, PCB levels are now mainly below the limit of detection *(84)*. The biologic and toxicologic properties of PCB mixtures vary according to their isomeric composition. Oral administration of PCBs to various mammals results in their rapid and almost complete (90%) intestinal absorption. The degradation and elimination of PCBs depend on the hepatic microsomal enzyme system *(79)*, and the excretion of PCBs is related to the extent of their metabolism. PCBs with greater chlorine contents have a correspondingly longer biologic half-life in mammals. This resistance to metabolism is reflected in the deposition of these substances in adipose tissue. The PCBs, however, have very low acute toxicity in all animal species tested, and PBBs have biologic properties similar to those of PCBs.

PCBs are potent hepatic microsomal enzyme inducers *(19,78,79)*. Rats exposed to PCBs exhibit a greatly enhanced biliary excretion of circulating thyroxine. The thyroxine is excreted as a glucuronide that is then lost in the feces *(19,78)*. This response is probably secondary to induction of hepatic microsomal T_4–uridine diphosphate–glucuronyl transferase. The enhanced peripheral metabolism and reduced binding of T_4 to serum proteins in PCB-treated animals results in markedly decreased serum thyroxine concentrations. These low levels stimulate the pituitary–thyrotropin–thyroid axis, eventually resulting in goiter formation. Although PCB-treated animals exhibit decreased serum thyroxine concentrations, their triiodothyronine levels are unchanged. The relative iodine deficiency brought about by the accelerated metabolism of thyroxine may induce increased thyroidal triiodothyronine secretion as well as increased peripheral deiodination of thyroxine to triiodothyronine. PBBs appear to act similarly to PCBs. There is, however, some indication that they may also interfere directly with the process of hormone synthesis in the thyroid gland *(19)*.

Despite the lack of evidence that dietary PCBs and PBBs have deleterious effects on the thyroid, there is growing concern and uncertainty about the long-range effects of bioaccumulation and contamination of the ecosystem with these chemicals. The uncertainty extends to the potential harmful effects of these pollutants on the thyroid. A study of workers at a plant manufacturing PBBs and PBB oxides reported four workers with primary hypothyroidism *(85)*. These workers had elevated titers of microsomal anti-TPO antibod-

ies, which may indicate that their hypothyroidism was a manifestation of lymphocytic autoimmune thyroiditis, perhaps a PBB-induced pathogenic autoimmune response, or an exacerbation of underlying subclinical disease.

A number of studies have been done on the relationship of PCBs to thyroid function in humans, some finding negative and some finding weakly positive results. An occupational study in the United States did not find a significant correlation between thyroxine and either the logarithm of adipose PCB or that of serum PCB concentrations *(86)*. Workers in a factory that produced PCBs in Slovakia were compared with controls in regard to thyroid volume, thyroxine, and TSH. Although thyroid volume was significantly increased in the workers, there was no difference in thyroxine levels or in the percentage of those with elevated TSH levels *(87)*. In a Dutch study, infants exposed to PCBs in milk had significantly higher TSH levels at 2 wk and 3 mo after birth. Total thyroxine levels were increased at 2 wk but not at 3 mo *(88)*. All thyroid measures were in the normal range, and at the age of 3 mo there were no differences in the infants' plasma total triiodothyronine, total thyroxine, or free thyroxine (FT4). In a Swedish study of fishermen's wives, PCB-153 was negatively correlated with total triiodothyronine but not with free triiodothyronine (FT_3), total thyroxine, or FT_4 concentrations *(89)*. In a Swedish study of male fish eaters, no relationship was found between PCB levels and any of the thyroid hormones *(90)*.

An examination of TH and liver enzyme levels in residents of a village near an organochlorine-compound factory in Catalonia compared their associations with hexachlorobenzene (HCB) and PCB blood levels *(91)*. HCB and PCB levels were much higher in residents who had been employees of the factory than in residents who had not. Neither TH nor liver enzyme levels were found to be associated with PCB levels in the adjusted regression models, though occasional associations with HCB did fit the models.

Similarly, a study of the thyroid function of 320 children living near a toxic-waste incinerator in northern Germany and their body burdens of lead, mercury, cadmium, and PCBs showed that of the heavy metals, neither lead nor mercury affected TH levels, and that blood cadmium concentrations were associated with increasing TSH and diminishing FT_4 levels *(92)*. One specific PCB congener (PCB-118) was positively associated with TSH levels, and five specific PCB congeners (PCB-138, -153, -180, -183, and -187) were negatively associated with FT_3 levels. No blood PCB congener level affected FT_4 levels, the primary measure of thyroid function. Among newborns, an analysis of cord serum TH levels with respect to *in utero* PCB exposures determined for North Carolina newborns from maternal serum and breast milk PCB levels showed no strong relationship between PCB exposure and any of the thyroid measures *(45)*. In particular, the FT_4 levels were similar for each PCB exposure stratum.

Other Organochlorines

2,2-Bis-(*p*-chlorophenyl)-1,1,1-trichloroethane (DDT) is polychlorinated and nondegradable. This substance is practically insoluble in water and resistant to destruction by light and oxidation. Its stability has created difficulties in residue removal from water, soil, and foodstuffs. The dominant degradative reaction of DDT is dehydrochlorination to form 2,2-bis-(*p*-chlorophenyl)-1,1-di-chloroethylene (DDE), which, like its precursor, has the same low solubility in water and high lipid–water partitioning. This substance is almost undegradable, both biologically and environmentally *(93)*.

Dieldrin is one of the cyclodiene insecticides. It is almost insoluble in water and, like DDT and DDE, is very stable both environmentally and biologically *(93)*. DDT has been used extensively, both in malaria control and in agriculture, throughout the world.

Because of biomagnification and persistence, DDT and its breakdown products, DDE and dichlorodiphenyldichloroethane (DDD), are ubiquitous contaminants of water and of virtually every food product. Most of fish from Lake Michigan in North America contain DDT residues. DDT is also present in milk, and since humans are at the top of the food pyramid, human milk is especially contaminated. The situation is basically similar for dieldrin, which is found in surface waters virtually everywhere. Dieldrin is heavily bioconcentrated in the lipids of terrestrial and aquatic wildlife, humans, and foods, especially animal fats and milk. Global distribution of high concentrations of organochlorines including DDT, DDE, DDD, and dieldrin were recently found not only in developing countries but also in industrialized countries, which continue to be highly contaminated even though the use of many of these compounds is restricted *(94)*.

DDT is reductively dechlorinated in biologic systems to form DDE and DDD. DDE, the predominant residue stored in tissues, reaching about 70% of all residues in humans, is much less toxic than DDT. DDE is slowly eliminated from the body; little is known about its degradation pathway. DDT is also slowly eliminated from the human body through reduction to DDD and other more water-soluble derivatives *(82,93)*.

DDT is known to cause marked alterations in thyroid-gland structure, such as thyroid enlargement, follicular epithelial-cell hyperplasia, and progressive loss of colloid in birds; DDD is known to cause goiter and increased hepatobiliary excretion of TH in rats *(78)*. All of these compounds (DDT, DDE, DDD, and dieldrin) induce microsomal enzyme activity that may affect TH metabolism in a similar way to that of the polyhalogenated biphenyls and polycyclic aromatic hydrocarbons (PAHs) *(19,95)*. The impact of these pollutants on the human thyroid is unknown.

Dioxin (tetrachlorodibenzodioxin, TCDD), one of the most toxic small organic molecules, is a contaminant in the manufacturing process of several pesticides and herbicides, including Agent Orange. Also a potent inducer of hepatic microsomal enzymes, TCDD markedly enhances the metabolism and biliary excretion of thyroxine-glucuronide *(19,78)*. Rats treated with TCDD concomitantly develop hypothyroxinemia, increased serum TSH concentrations, and goiter, probably as a result of loss of thyroxine in the bile *(19)*. The impact on the thyroid of humans exposed to this agent is unknown, and evaluation of thyroid-gland function and studies of TH metabolism are necessary in those affected.

PAHs

Polycyclic aromatic hydrocarbons (PAHs) have been found repeatedly in food and domestic water supplies, and in industrial and municipal waste effluents *(19,20,78,93)*. They also occur naturally in coal, soils, ground water and surface water, and in sediments and biota. One of the most potent of the carcinogenic PAH compounds, 3,4-benzo[a] pyrene (BaP), is widely distributed and, as in the case of other PAHs, is not efficiently removed by conventional water-treatment processes.

The PAH carcinogens, BaP and 3-methylcolanthrene (MCA), accelerate thyroxine metabolism and the excretion of thyroxine-glucuronide by enhancing hepatic uridine diphosphate–glucuronyltransferase and glucuronidation, reducing serum thyroxine concentrations, activating the pituitary–thyrotrophin–thyroid axis, and eventually leading to goiter formation *(19,20,78)*. There is also indication that methylcholanthrene (MCA) interferes directly with the process of hormone synthesis in the thyroid gland. Furthermore, MCA, as well as 7,12-dimethylbenzanthracene, also induces goitrous thyroiditis in the BUF rat *(96)*. Thus, MCA exerts its deleterious effects on the thyroid gland by at least three different mechanisms. Finally, methylanthracene (MA), a coal-derived PAH identified in drinking

water from the goitrous coal-rich district of eastern Kentucky *(13,20,66)*, was found to produce goiter in the BUF rat without alteration of hormone synthesis or lymphocytic infiltration of the thyroid gland *(20)*.

Inorganic Chemicals

Excess Iodide

The effects of iodine excess are discussed in Chapter 17.

Lithium

Studies in Venezuela documented higher lithium concentrations in the water supply in localities with a high incidence of endemic goiter than in nearby communities in which goiter is nonendemic. Experimental observations in rats indicated that lithium at those high concentrations can be goitrogenic, but this effect is conditioned by dietary protein and iodine intake *(19,97)*.

Lithium carbonate in larger amounts is definitely goitrogenic, as demonstrated by development of goiter with and without hypothyroidism in lithium-treated manic/depressive patients *(97,98)*. Lithium also crosses the placenta, with potentially adverse effects on fetal thyroid function *(26)*.

Nitrates

Nitrate ion (NO_3^-) is the thermodynamically stable form of combined nitrogen for terrestrial, oxygenated aqueous systems. Accordingly, there is a tendency for all nitrogenous materials in natural water to be converted into nitrate. Major point sources of combined nitrogen are municipal and industrial waste waters, refuse dumps, animal feed lots, and septic tanks. Diffuse sources include runoff or leachate from manured or fertilized agricultural lands, urban drainage, and biochemical nitrogen fixation. Nitrate is highly water-soluble. The Maximum Contaminant Level (MCL) set by the U.S. Environmental Protection Agency (EPA) is 10 mg/L of nitrate expressed as nitrogen. In the United States, 1% of public water-supply wells and 9% of domestic wells exceeded this standard. An appreciable amount of nitrates are ingested in the diet. Dietary intakes of nitrates have been determined from various European surveys; for Finland this was 77 mg/d *(99)*, for the United Kingdom 54 mg/d *(100)*, and for the Netherlands 80 mg/d *(101)*.

Nitrate at concentrations of 0.0001 *M* and 0.1 *M* can competitively inhibit both iodine accumulation in thyroid tissue and competitively inhibit the organic binding of iodine, respectively *(102)*. Nitrate has been shown to affect thyroid function or structure in the pig *(103)*, in the rat *(104,105)*, and in foals (CH) *(106)*. Administration of iodine can compensate for the effect of the nitrate ion *(107)*. Goiter prevalence was found to be increased in local populations with high (70–90 mg/L) levels of nitrate in their water in Germany *(108)* and in Bulgaria *(109)*. The Bulgarian study reported that goiter prevalence was reduced after antigoiter prophylaxis was increased to 1 mg weekly for children 3–10 yr old and to 2 mg weekly for children 11–14 yr old. A study in the Netherlands, where there was no iodine insufficiency, showed a nitrate dose-dependency for thyroid volume, with the development of hypertrophy at nitrate levels exceeding 50 mg/L *(110)*.

Perchlorate

Because perchlorate is a most potent competitive inhibitor of iodine uptake by the sodium-iodide symporter (NIS), it has been used for the past 50 yr to treat hyperthyroidism *(111)*. Early data (1954) had shown about an 80% inhibition of iodine uptake with perchlorate

treatment at 400–600 mg/d *(112)*. At present, perchlorate is primarily used medically as an effective treatment for iodine-associated, amiodarone-induced thyrotoxicosis, with potassium perchlorate beginning at a dose of 800–1000 mg/d and continuing for 4–6 mo at lower doses *(113)*. Perchlorate is a more potent inhibitor of iodide uptake than is thiocyanate or nitrate. Perchlorate does not block the synthesis of TH at pharmacologic levels. Perchlorate does displace thyroxine from plasma proteins *(114)*.

In 1977, the California Department of Health Services increased the sensitivity of the assay for perchlorate in drinking water from 0.4 ppm to 4 ppb [μg/L]. At a detection level of 4 ppb, perchlorate was found to be present in the drinking water of most of Southern California (5–8 ppb) and Southern Nevada (5–24 ppb). Thus, perchlorate entered the list of antithyroidal agents found in the environment.

A succession of studies then followed to determine the human health effects of occupational and environmental exposures to perchlorate. Two occupational studies showed no adverse health effects on workers' thyroidal or nonthyroidal systems. The first occupational study showed no exposure-related effects of either acute exposure or chronic exposure on thyroid, bone-marrow, liver, or kidney function *(115)*. The second occupational study simultaneously measured respiratory and inhalation exposure to perchlorate, pre- and postexposure urinary excretion, and thyroid status (TSH, thyroxine, triiodothyronine, FTI [free thyroxine index], THBR [thyroid hormone binding ratio], TPO, and physical examination) in four groups of workers *(116)*. The workers had mean absorbed perchlorate dosages of 1, 4, 11, and 34 mg perchlorate per day, and serum perchlorate levels of 0.00, 0.11, 0.41, and 1.62 μg/mL, respectively. No differences in thyroid-function parameters were found among the four groups of workers.

Perchlorate has been found to be a persistent and pervasive low-level contaminant in groundwater and in public drinking water. At least 14 states have confirmed perchlorate in ground or surface water, usually from sites of perchlorate manufacture or use, and possibly from fertilizer used in agriculture *(117)*. Epidemiologic studies have been conducted to determine whether perchlorate-containing drinking waters were associated with any increase in thyroid diseases. CH was found to be no more prevalent in the California and Nevada counties with perchlorate in their water supply than in the rest of the state *(118)*. Neonatal thyroxine *(119)* and neonatal TSH *(120)* levels in the areas of Nevada with perchlorate in the drinking water (9–15 ppb) did not differ from those in other areas. An initial finding *(121)* in Arizona of higher neonatal TSH levels in an area with perchlorate in the drinking water at 6 ppb was later found to indicate a regional difference rather than a difference in exposure. A study in Chile examined the thyroid status of both newborns and 6-yr-old schoolchildren in communities with drinking-water perchlorate levels of 0.0 (nondetectable: <4 ppb), 5–7 ppb, and 100–120 ppb, and found no differences in neonatal or childhood TSH or in goiter prevalence *(122)*. Serum thyroxine levels were increased rather than decreased in the community with higher perchlorate levels. This study concluded that no evidence had been found that perchlorate in drinking water at up to 120 ppb was associated with thyroid suppression in newborns or school-aged children. Furthermore, there was no evidence of adverse effects on thyroid, bone-marrow, liver, or kidney function. Subsequent studies in Nevada showed no increase in the prevalences of specific thyroid diseases *(123)* or of neurobehavioral diseases of childhood *(124)* in the area with perchlorate in the drinking water.

Inhibition of iodine uptake is the known mechanism by which perchlorate produces its effect on the thyroid. It can be postulated that studies of RAI uptake represent the most sensitive and specific method of ascertaining a no-effect level for perchlorate concentration in the water supply. Two laboratories have conducted volunteer studies to examine the

magnitude of inhibition of iodine uptake with differing levels of perchlorate in drinking water. The Boston group found a 38% inhibition ($p<0.01$) of iodine uptake at a 10 mg/d perchlorate dosage after a 2-wk exposure *(125)*, and a 10% decrease (p = ns) at a 3 mg/d dosage *(126)*. Log-linear extrapolation of these data would predict a no-effect level of approximately 2 mg/d. The Oregon group gave perchlorate dosages of approximately 0.5, 1.4, 7, and 35 mg/d (0.007, 0.02, 0.1, and 0.5 mg/kg/d) for 2 wk and developed data yielding a log-linear regression prediction of a no-effect level for inhibition of iodine uptake at 0.5 mg/d, which they then confirmed *(127)*. A dosage of 0.5 mg/d would be equivalent to a 2-L daily consumption of water containing 250 ppb (μg/L) perchlorate. The Oregon study, like the occupational study described above, found no effect on serum thyroxine levels at an exposure of 35 mg/d perchlorate (0.5 mg/kg/d). These human studies indicate that the no-effect level for perchlorate on inhibition of iodine uptake is about an order of magnitude above observed drinking-water levels, and that the no-observed-adverse-effect level for perchlorate on serum thyroxine levels is about two orders of magnitude above that *(128)*. A number of laboratory toxicologic studies of perchlorate, prepared for consideration by the EPA in assessing perchlorate risk, are beginning to reach the peer-reviewed literature *(129–131)*.

Chlorate

Sodium chlorate is formed as a byproduct of the use of chlorine dioxide in water disinfection. Chlorates may be found at levels as high as 2.0 mg/L in finished drinking water. Sodium chlorate is used as an oxidizing agent in the tanning and leather industry, in the manufacture of dyes, explosives and matches, and in the paper industry as a bleaching agent. Chlorate is a weak inhibitor of iodide uptake and a weak blocker of TH synthesis *(102)*. Male rats showed follicular-cell hyperplasia in fewer than 10% of follicles after 90 d of sodium chlorate intake at a concentration of 1 g/L, and at lower concentrations showed only significant colloid depletion and hypertrophy *(132)*. Sodium chlorate showed no suppression of serum thyroxine levels in African green monkeys after 8 wk of consumption of drinking water containing 400 mg/L *(133)*. In the same study, chlorine dioxide suppressed serum thyroxine levels after 6 wk of chlorine dioxide at 100 mg/L, while a different study [in the same paper] reported no thyroid effect on humans exposed for 3 mo to water disinfection with chlorine dioxide at approx 1 mg/L chlorine dioxide.

PREVENTION, CONTROL, AND CLINICAL CONSIDERATIONS OF ENVIRONMENTAL GOITROGENESIS

The multifactorial nature and complex interactions of host factors (age, sex) with region-specific environmental conditions in the pathogenesis of endemic goiter constitute a major challenge to the understanding and control of the problem of goitrogenic substances in areas endemic for goiter. Besides iodine deficiency and environmental goitrogens, protein–calorie malnutrition (PCM) also results in various alterations of thyroid gland morphology and function *(2,20)*. PCM and endemic goiter frequently coexist, and poor nutrition appears to increase the risk of goiter in susceptible groups of the population (infants, children, and pregnant women). Studies show that malnourished individuals have the same thyroid-gland abnormalities that have been shown in experimental animals to favor enlargement of the thyroid gland. A low-protein diet in rats impairs the thyroidal transport of iodine, decreases iodine concentration in the thyroid, and is accompanied by an enlargement of the thyroid. Under these circumstances, the goitrogenic effect of antithyroid agents is enhanced. The administration of protein reverses these alterations and decreases the action of such goitrogenic agents. Therefore, to control and prevent this important public-health problem

of endemic goiter and associated disorders, the most obvious but difficult initial step requires substantial socioeconomic improvements in the affected areas of the Third World, including provision of efficient iodine-supplementation programs, diversification of dietary constituents with adequate daily protein–calorie intake, and institution of proper sanitary conditions with effective water treatment to eliminate organic and bacterial pollutants. This last intervention is also a requirement for controlling and preventing goiter in the more developed iodine-sufficient countries of the world. A more comprehensive view and understanding of the biogeochemical cycle of organic goitrogens in nature should provide the basis for development of devices and interventions at various steps in the cycle to prevent exposure to or the presence or action of these organic compounds as well as to pollutants that constitute serious environmental health hazards. All of this together will permit the development of cost-effective medical and public-health measures to prevent and/or treat these frequent and deleterious thyroid disorders at the community, household, or individual level. Agricultural genetic engineering *(2,9,56)*, bioremediation processes *(80,81)*, water-purification systems *(56)*, and optimal treatment(s) and disposal of waste-water effluents from coal-conversion processes *(44,65)* are among the interventions to be investigated and implemented. At present, medical or surgical treatments for the individual with goiter, but not measures for its prevention and control, are being applied, when available, in iodine-sufficient areas of goiter. Until preventive measures are available, the physician will be restricted to observation, and the administration of levothyroxine, antithyroid drugs (propylthiouracil, methimazole, carbimazole, and/or radioiodine (^{131}I)) needle aspiration, or surgery for treating goiter.

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16 Iodine Deficiency and Its Elimination by Iodine Supplementation

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Contents

INTRODUCTION

Iodine is an essential component of the thyroid hormones (TH), thyroxine (T_4) and 3,5,3′-triiodothyronine (T_3). Its deficiency causes inadequate production of these hormones, which in turn leads to the so-called iodine deficiency disorders (IDDs). We review here iodine metabolism by the thyroid gland, and the metabolic, clinical, and public-health consequences of iodine deficiency and its correction. Aspects of these topics have already been reviewed extensively in recent years *(1–5)*. Here we build on previous summaries, point out new developments, and try to relate physiology to public health.

NORMAL IODINE METABOLISM AND THE EFFECTS OF IODINE DEFICIENCY

Many reviews and textbooks trace the major steps of iodine metabolism in the thyroid *(6–20)*, and we will only highlight them here. Virtually all iodine enters the body by oral ingestion. In the gastrointestinal tract it is actively absorbed into the circulation, mainly in the chemical form of iodide. The thyroid concentrates iodide from the circulation through the action of the sodium/iodide symporter (NIS). This glycoprotein is located in the basal membrane of the thyrocyte, and couples the transfer of sodium ions with the influx of iodide *(10,11)*. Once in the thyroid cell, iodide is oxidized in a complex series of reactions involving:

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(1) pendrin, an iodide/chloride transporter of the apical membrane (genetic mutations lead to Pendred syndrome); (2) thyroid peroxidase (TPO), a glycoprotein synthesized in the thyroid cell; (3) hydrogen peroxide, whose production requires both calcium and reduced nicotinamide adenine dinucleotide phosphate (NADPH), the latter from the action of NADPH oxidase; and (4) thyroglobulin (Tg), a large glycoprotein, made in the thyroid, that provides the peptide backbone for TH synthesis *(7)*. At the apical membrane of the thyrocyte, iodine attaches to tyrosyl residues within Tg to yield monoiodotyrosine (MIT) and diiodotyrosine (DIT). Next, two iodotyrosyl residues couple in another TPO-mediated reaction to form an iodothyronine, T_4 or T_3, leaving dehydroalanine or a derivative (alanine or pyruvic acid) at the site of the donor tyrosyl *(12)*. We and others have identified the major hormonogenic tyrosyls of Tg in a number of species *(13–15)*. In human Tg the two major hormone-forming sites are at residue 5, close to the N-terminus of the molecule, and at residue 2553, some 200 residues from the C-terminus. In other species and to a lesser degree in humans, residue 1290 is a T_4-forming site, and residue 2745, three residues from the C-terminus, is prominent as a T_3- or T_4-forming site, particularly in rabbits and guinea pigs *(13,14)*. In vitro iodination of low-iodine human Tg has shown that certain tyrosyls residues are favored for early iodination *(15)*. Some of these subsequently contribute the inner iodothyronine ring, but others do not, and thus are attractive candidates for donors of the outer ring. $Tyrosyl_{130}$ appears to be the outer-ring donor for T_4 formation at $tyrosyl_5$, the most important hormonogenic site *(16)*. We have described several consensus sequences surrounding tyrosyls that favor iodination and/or hormonogenesis *(15)*.

In addition to the role of iodine as constituent of the TH, the process of iodination is also associated with cleavage of discrete peptide bonds in Tg. One such cleavage in human Tg leaves a 26-kDa N-terminal fragment, which on further iodination becomes an 18-kDa N-terminal peptide *(17)*. Similar distinctive cleavage patterns with iodination occur in all vertebrate species so far examined *(18)*. Additional iodine cleavage sites probably exist, particularly in the C-terminal region.

Iodination of Tg takes place at the apical microvilli of the thyroid cell, where TPO is concentrated. Iodinated Tg is then secreted into the follicular lumen and stored as colloid. For retrieval of hormone, Tg is returned to the cell and digested with lysosomal proteases. Three exopeptidases—cathepsins L, B, and D—cleave the Tg polypeptide chain at distinctive sites *(19)*. Cathepsin L acts mainly in the C-terminal region, whereas cathepsin B cleaves at both the C-terminus and the N-terminus. At the N-terminus, cathepsin B also has an exopeptidase action, producing a T_4-Glu peptide at residue 5, the most important T_4-forming site. The exopeptidase lysosomal dipeptidase I (LDPI) can act in coordination with cathepsin B to yield free T_4 from this dipeptide in vitro *(20)*. The thyroid is rich in another exopeptidase, dipeptidyl peptidase II (DPPII). Its principal site of action on Tg has not yet been identified, but may well be in the C-terminal region. After release, free T_4 is secreted from the thyroid into the circulation, where it is bound to carrier proteins for peripheral distribution. Thyroidal deiodinases remove iodine from the nonhormonal iodoamino acids, MIT and DIT, and recycle their iodide within the thyroid. This is an important mechanism of iodine conservation, and persons with congenital inability to perform this step are at risk for iodine deficiency; treatment with moderately high doses of iodine can restore them to homeostasis *(21)*.

Thyroid-stimulating hormone (TSH) affects most of these steps. Several thyroid transcription factors regulate synthesis of the NIS, TPO, and Tg, and all are influenced by effects of TSH *(6–8)*. Physiologic effects of TSH are to increase thyroidal concentration of iodide from the circulation, enhance Tg iodination and hormonogenesis, alter the priority of hormonogenic-site utilization within Tg to favor T_3 production, and stimulate the action

of the cysteine proteinases, cathepsins L and B, in Tg degradation. The net effects are to produce more TH and to use iodine more efficiently.

Iodine availability also affects thyroid metabolism. Some of this action results from stimulation by TSH, which accelerates in response to inadequate production of TH. In addition, the thyroid adapts to iodine deficiency by greatly increasing its uptake of iodide from the circulation. Iodine deficiency favors the production of MIT over DIT in Tg, and when iodotyrosyl coupling occurs, promotes T_3 formation over that of T_4. The iodine-deficient thyroid typically has an increased iodine turnover, with little colloid accumulation, and rapid degradation of hormone for release into the circulation.

CONSEQUENCES OF IODINE DEFICIENCY

Several publications and organizations have proposed 150 μg of iodine as the recommended daily intake for nonpregnant, nonlactating adult humans *(22,23)*. Additional recommendations are 90 μg/d for young children, 120 μg/d for older children, and 200–280 μg/d for pregnant and lactating women. The recommendation for adults is based on iodine levels found in areas without endemic goiter, calculations from thyroxine-turnover studies, and replacement doses of thyroxine for athyreotic individuals. Such estimates put daily iodine needs on the order of 50–100 μg *(24)*. The figure of 150 μg/d provides a margin of safety for variations to cover the range within a population.

All established consequences of iodine deficiency stem from inadequate production of TH and attempts to compensate for it. These consequences—the IDDs—are damaged reproduction, including complications of pregnancy, increased fetal and infant mortality and developmental retardation; goiter; hypothyroidism; and socioeconomic deprivation. All have been discussed at length in various publications *(1–5,25,26)*, and are summarized briefly below. Before reviewing them, note is made of two less established but intriguing possible additional and extrathyroidal roles for iodine. The first is in the breast, where treatment with iodine was reported to improve mammary dysplasia in an experimental rat model *(27)*, and correlated with limited clinical experience in humans suggesting that iodine treatment improves fibrocystic breast disease; in these studies treatment with molecular iodine (I_2) was more effective than with iodide *(28)*. The second possible nonthyroidal role for iodine is in the immune response. Limited studies have suggested that the immune response is impaired in iodine deficiency *(29)*. In laboratory experiments, iodine is an effective halogen donor for the myeloperoxidase of white blood cells, raising the possibility that iodine may aid the immune response. Although speculative, this issue has considerable potential significance, because infectious diseases and impaired immunity are the major causes of death in most of the developing world. Most of these same countries also have iodine deficiency, so correction of iodine deficiency may have benefits in addition to those for the established IDDs.

Damaged reproduction is the worst consequence of iodine deficiency *(25,30)*. For the mother, the reproductive damage includes anovulation, infertility, increased first-trimester abortions, stillbirths, gestational hypertension, and abnormal presentations. The consequences for the fetus are more devastating, beginning with increased mortality. Several studies have shown that correction of iodine deficiency before or during pregnancy improves birth weight and survival. For example, addition of iodine to irrigation water in western China over a period of several years decreased neonatal death by about 65% *(31)*. A similar reduction occurred when very young infants in an iodine-deficient area of Indonesia were treated with iodized oil *(32)*. Studies from Congo *(33)* and Papua New Guinea *(34)* also attest to the beneficial effects of iodine sufficiency on both short-term and long-term child survival.

Even if they survive, the children of iodine-deficient mothers risk permanent neurologic damage. TH is essential for normal development of the central nervous system (CNS), particularly during the period of rapid myelination occurring in gestation and lasting at least into the first 2 yr of life *(35)*. The developmental retardation ranges from mild subclinical features to frank cretinism. At the mild end, affected subjects show only subtle mental dulling, but at the other, cretins have severe mental retardation and a host of other developmental problems including deaf-mutism, skeletal deformities, and short stature. Over the past several decades it has become apparent that the effects of iodine deficiency on development of the CNS cover a broad continuum, without clear demarcation between normal and affected. Careful examination of iodine-deficient populations shows substantial numbers of individuals with subclinical hypothyroidism and assorted developmental defects. The key event appears to be maternal hypothyroxinemia rather than hyperthyrotropinemia *(36)*. Low maternal thyroxine levels can occur in frank hypothyroidism in iodine-sufficient areas, but are even more likely in areas with iodine deficiency, because TH production is shifted away from thyroxine toward triiodothyronine.

The public-health implication of maternal hypothyroxinemia is enormous. It means that in areas with significant iodine deficiency, the majority of the population could have some degree of retardation, and virtually everyone is at risk. A metaanalysis by Bleichrodt and Born *(37)* showed that on average, iodine deficiency appeared to lower intelligence quotient (IQ) by about 13 points relative to iodine-sufficient groups who were otherwise comparable. In addition, mild deficits in hearing, educability, and motor performance are easily overlooked without specific testing for them.

Overt cretinism is becoming increasingly rare as iodine nutrition improves globally, but it still occurs. Two phenotypes—"neurologic or myxedematous"—have been described *(35)*. The former is attributed to lack of sufficient TH early in the second trimester, a critical period in fetal development. The myxedematous type is thought to come from sustained hypothyroidism in late fetal and early postnatal life, and its typical features are mental retardation and severe clinical hypothyroidism. Careful examination of cretins shows frequent overlap in these two phenotypes.

Goiter begins as an adaptation to iodine deficiency, probably mainly from increased stimulation by TSH. As the thyroid secretes inadequate amounts of hormone, the pituitary responds by releasing more TSH, with all its various effects including thyroid enlargement. Goiter, then, is one of the first signs of iodine deficiency. Initially, the goiter is diffuse and reflects generalized hyperplasia, so-called "simple goiter." At first, the compensation may be adequate, but with continued iodine deficiency or renewed demand for increased TH, such as in pregnancy or adolescence, the cycle may begin anew, with increased TSH and further thyroid enlargement. Over years or decades, the hyperplasia becomes more focal, and nodules or adenomas develop, resulting in a multinodular goiter. The nodules may be adenomas, cysts, collections of colloid, or, less commonly, follicular cancer.

Some adenomas are autonomous and may eventually hyperfunction and produce hyperthyroidism. This potential for excess hormone production is greatly increased if fairly large amounts of iodine suddenly become available. This iodine-induced hyperthyroidism (IIH) can be considered as another IDD, because it occurs chiefly in subjects who were initially iodine-deficient *(38)*. It typically involves an older subject having a longstanding goiter that has developed autonomous nodules. Previously, such a gland has avidly sought every available atom of iodine. If now sufficient iodine becomes available, the nodular thyroid will react by synthesizing and secreting inappropriately large amounts of TH and causing hyperthyroidism. Some occurrences of this consequence are unavoidable, but their impact

can be lessened by appropriate measures. These include careful monitoring of salt (or other iodine-supplementation sources), to ensure that its levels of iodine are not significantly above those recommended in the national program, and measuring of representative urinary iodine levels to see if they are in an appropriate range. If the estimates used to calculate the desired levels of iodine addition to salt were incorrect, they should be adjusted. The medical sector and public at large should be aware of the clinical features of hyperthyroidism so that they seek and receive treatment promptly. The most important manifestations are cardiac, including tachycardia; arrhythmias, particularly atrial fibrillation; and even heart failure and death *(39,40)*. Prompt recognition of these features can lead to appropriate treatment and usually prevents or corrects most of the damage. Treatment for the hyperthyroidism is the same as in iodine-sufficient communities and consists of the use of radioactive iodine (RAI), antithyroid drugs (ATDs), or surgery. The increased incidence of hyperthyroidism may peak after 1 or 2 yr of increased iodine intake, and typically then returns to preexisting or lower levels.

If the iodine deficiency is not severe and if the thyroid can compensate satisfactorily, the only consequence may be goiter. However, if the response is inadequate, then hormone production becomes insufficient and hypothyroidism ensues. For the adult, this means the usual clinical stigmata seen from other causes of hypothyroidism, including decreased energy, somnolence, and torpor. These features are usually reversible with iodine or thyroxine treatment. The consequences for the developing human are much more dire, as described above.

A further consequence of iodine deficiency is socioeconomic retardation *(41)*. Iodine-deficient subjects are less educable, make poorer employees, and are less productive than their iodine-sufficient peers. Iodine deficiency also affects domestic animals, leading to decreased production of meat, wool, eggs, and milk, and these effects also reduce the productivity of the local economy. In more developed countries, such as in much of Europe, iodine deficiency also incurs a financial cost through increased need for thyroid diagnostic tests, medical procedures, and lost work time. Estimates of annual financial losses from these thyroid-related complications alone amount to more than $1 billion for Germany *(42)* and $160 million for Italy *(43)*.

ASSESSMENT OF IODINE DEFICIENCY

The World Health Organization (WHO), the International Council for the Control of Iodine Deficiency Disorders (ICCIDD), and the United Nations International Children's Education Fund (UNICEF) have carefully considered the available indicators for assessing iodine nutrition *(23)*. These can be categorized as follows: most useful, urinary iodine concentration; also useful, thyroid size (especially as measured with ultrasound), neonatal TSH screening, and serum Tg; and less useful, serum TSH (other than in neonates), T_4, T_3, and RAI uptake. These statements refer to assessment in populations rather than in individuals.

Urinary Iodine Concentration

About 90% of ingested iodine eventually appears in the urine, and as a result its excretion there correlates well with iodine nutrition. Urine samples are obviously easier to obtain than blood samples, and are more acceptable and feasible in field surveys. Simple colorimetric methods allow processing of large numbers of samples at relatively low cost *(23,44–48)*. Two approaches are now in general use. One, so-called Method A, digests urine with ammonium persulfate at 100°C, then measures the reduction of yellow-colored ceric ammonium sulfate

Table 1
Urinary Iodine Concentration as Indicator of Iodine Nutrition

Median urinary I (μg/L)	*Iodine nutrition*
<20	Severe deficiency
20–49	Moderate deficiency
50–99	Mild deficiency
100–199	Optimal
200–299	More than adequate
>300	Excessive

Adapted from ref. *23*.

to the colorless cerous form in the presence of arsenious acid; this is the Sandell–Kolthoff reaction, and since iodide is a catalyst for it, the speed of color change is proportional to the iodide content of a sample. Details of this have been published *(23)*. A more automated approach digests urine in a special sealed envelope and then employs the Sandell–Kolthoff reaction on microtiter plates *(23,47)*. A further promising simplification uses the redox indicator ferroine to recognize color change in the Sandell–Kolthoff reaction *(44,48)*. This method can be done quantitatively, or more rapidly, semiquantitatively, to group samples within ranges based on assay results rather than to give absolute values. For most epidemiologic purposes the range is sufficient. Older methods related urinary iodine to urinary creatinine excretion, but this has been shown to be unnecessary, occasionally misleading, and more expensive *(46)*. Expression of urinary iodine as the median concentration, in micrograms per liter, is adequate for populations. Although individuals may vary in degree of hydration, this effect is damped by obtaining sufficient samples within a community.

Table 1 relates median urinary iodine concentration to community iodine nutrition *(23)*. Although these designations are arbitrary, they provide a useful framework for gauging the severity of the iodine deficiency in a population and the urgency for its correction. This stratification is particularly valuable in deciding whether implementation of effective iodization can be delayed until a program based on iodized salt can be successfully introduced (usually requiring several years), or whether more immediate intervention is needed, such as with iodized oil.

Thyroid Size

Because thyroid enlargement is one of the earliest and most sensitive clinical manifestations of iodine deficiency, it has traditionally been a principal assessment tool. Typically, examiners palpate the necks of subjects in convenient groups, such as schoolchildren, and assign them to an arbitrary category of thyroid size. Several classification schemes have been endorsed by the WHO and others over the years. The most recent classification describes thyroid glands as not enlarged (“no goiter”), enlarged according to palpation alone (group 1, “palpable goiter”), or enlarged according to visual inspection (grade 2, “visible goiter”) *(23)*. Older schemes subdivided these classifications to distinguish thyroid enlargement that is visible when the neck is extended but not in the normal position (grade IA) and goiters that are grossly enlarged on inspection (grade III). The simplified version was adopted because of

recognized difficulties in distinguishing some of these subcategories. Inherent to this scheme is a satisfactory definition of normal thyroid size, and this has never been established. The WHO standard in 1960 defined that each lobe of a normal thyroid should be no larger than the terminal phalanx of the thumb of the subject being examined *(49)*. Experience indicates that this definition is neither anatomically valid nor clinically correct.

Recently, ultrasonography has become the method of choice for quantitation of thyroid size *(50–54)*. The technique is simple, rapid, and feasible. Instruments can be used in the field, each determination requires no more than several minutes, and results are quantifiable and precise. Several publications have tabulated medians and 97th percentiles for adults and children at various ages *(52,53)*. Body surface and height are usually better reference markers than age or weight. Ultrasonography is particularly useful for tracking the effects of various interventions in longitudinal studies, and should replace palpation of the thyroid whenever possible. Palpation is still valuable for large goiters. It is much less reliable for smaller thyroids, and its inadequacy becomes an increasing problem as thyroid volume decreases in response to various iodine-supplementation programs. In addition, the examiners are often inexperienced and produce results with considerable interobserver error. Even in the best hands, palpation will misclassify about 30% of smaller thyroids when compared to results of ultrasonography. For thyroid assessment, children provide the best test subjects because their thyroid condition reflects fairly recent iodine nutrition and they are conveniently assembled in schools *(4,23)*.

Serum Thyroid-Related Hormones

The most useful measurement for thyroid-related hormones is the serum or blood-spot TSH, because iodine deficiency threatens the thyroid with hypothyroidism, and the pituitary responds with increased TSH secretion. In practice, serum TSH measurement can be helpful in moderately severe iodine deficiency, but is less useful in milder degrees of such deficiency, where the median levels may be within the normal range although higher than in iodine-sufficient areas *(54)*. In contrast to these conclusions about TSH in children and adults, the neonatal TSH titer can be a sensitive indicator. When screening is universally applied to all newborns in an iodine-deficient area, the incidence of transient hypothyroidism, as detected by increases in neonatal TSH, is increased, because the neonatal period is particularly sensitive to effects of iodine. This transient hypothyroidism increases the recall rate in screening. For example, an assessment in 1986 of several European cities showed that Stockholm, where the mean urinary iodine concentration was 110 μg/L, had a recall rate of 0.07%, but Freiberg, Germany, where urinary iodine levels were 12 μg/L, had a recall rate of 0.89% *(55)*. Most developing countries do not have universal screening programs for iodine sufficiency, but this approach is useful in iodine-deficient areas of Europe *(56)*.

Serum TH levels change in a characteristic pattern with iodine deficiency, typically showing a low thyroxine and a normal or increased serum triiodothyronine concentration. However, the tests for these are more cumbersome and expensive, and do not have much epidemiologic sensitivity; therefore, they are generally not recommended for routine assessment. Tg in serum is a nonspecific but sensitive marker for increased activity of thyroid-derived tissue, including the thyroid hyperplasia that typifies iodine deficiency. The Tg concentration can be determined in dried blood spots, making it feasible to obtain samples in the field, particularly if such specimens are being gathered for other assessment purposes *(57)*. Serum Tg correlates fairly well with other measures of iodine deficiency such as thyroid size, and is more useful than serum TSH, thyroxine, or triiodothyronine *(54)*.

Other

The thyroidal uptake of RAI correlates inversely with iodine availability, and so is a sensitive marker for iodine deficiency. It can be a useful indicator when normal ranges are already established as part of general endocrinologic assessment in a community. However, it will almost never be practical for primary assessment in the field; other methods are simpler, safer, and cheaper.

Use of Tests

Of the assessment methods discussed above, the urinary iodine concentration assay, the measurement of thyroid size (preferably by ultrasound), and the serum or blood-spot Tg assay are the three most valuable. The urinary iodine assay indicates very recent iodine intake (within days), while thyroid size and serum Tg reflect iodine nutrition over months or years. In adults, longstanding goiters, particularly those with nodules, may never show appreciable diminution in size, even when urinary iodine levels are brought into the normal range. For survey purposes, school visits for rapid examination by palpation and preferably ultrasound, and collection of urine samples for subsequent iodine measurement in a laboratory, constitute the usually recommended evaluation program. The sampling design is critically important, and must especially not overlook the poor remote areas that are most likely to have resistant iodine deficiency. The WHO/ICCIDD/UNICEF manual provides guidelines for survey conduction *(23)*. An international network of regional referral laboratories is currently being developed by the CDC, WHO, ICCIDD, and other parties to provide adequate facilities for measuring urinary iodine in developing countries. As urinary iodine measurements become more available, thyroid examination, especially by palpation, becomes less important.

TREATMENT OF IODINE DEFICIENCY

Iodine is the obvious treatment for iodine deficiency. Several good ways for introducing iodine have been reviewed extensively elsewhere *(1–5,58–61)*. Here we will summarize them and their application. The general criteria for an optimal vehicle for delivering iodine are that it: (1) provide adequate iodine in daily physiologic amounts; (2) reach all of the affected population, including the poor, the isolated, women and children; and (3) be practical, feasible, effective, and affordable. The fulfillment of these conditions can vary greatly from one country to another, even among those with similar cultural, ethnic, and geographical features.

Iodized Salt

Iodized salt is the overwhelming favorite vehicle for general iodine supplementation. Salt is a nutritional essential, its sources are frequently limited and controllable, and the iodization technology is simple *(58)*. The level of iodine addition to salt can be easily calculated for a particular population from the baseline iodine consumption, daily salt intake, and estimated losses of iodine between production and consumption. A general target is 150 μg/d iodine for adults, 200–280 μg/d for pregnant women, and 90 μg/d for children *(22,23)*. For example, if the average adult in a community has a current iodine consumption of 50 μg/d, she needs to be targeted for an additional 100 μg. If the daily salt consumption is 10 g, an additional 10 μg iodine per gram of salt is needed. Additionally, if 25% of the iodine is lost by the time of consumption, the optimal level of iodine to add to the salt would be 12.5 ppm. (KI is 77% iodine, KIO_3 is 59%; the regulation should specify iodine content rather than the total compound content, to prevent confusion).

Most countries have set the iodization level for salt at 20–40 ppm. Several considerations influence this choice: (1) iodine excess is less damaging than iodine deficiency; (2) the level must be adequate for covering all in the population, including pregnant and lactating women and those in poor and remote areas; (3) salt moves across national borders, and regional agreement on salt-iodization levels simplifies international trade; (4) for most countries, it is more practical to have regulations that apply to the entire country, including iodine-sufficient regions; this greatly simplifies the distribution of iodized salt, and the small amount of additional iodine from this source is unlikely to cause any harm to those already receiving enough; (5) the cost of iodized salt should be competitive with that of any available noniodized salt; (6) salt for domestic animals should also be iodized, because they also experience the ravages of iodine deficiency, and many households use the same salt for humans and animals; and (7) the use of iodized salt in prepared foods needs attention; in developing countries, most salt is added in the kitchen, but in Western countries, most salt intake comes from prepared food, which is not typically iodized (Switzerland is an exception); thus, in the United States, only about 15% of the daily salt intake is added at the table *(3)*; furthermore, some iodine may be lost from salt added during cooking, more from KI than from KIO_3.

Iodine is added either as KIO_3 or KI. The latter is cheaper and more soluble, but less stable in the presence of impurities, poor packaging, heat, or humidity. Most countries use iodate, but some with pure salt, good packaging, and temperate climate use iodide (e.g., Switzerland, Canada, United States). In places where the sources of salt are limited and sophisticated production facilities exist, addition of iodine is simple and incurs only a trivial increase in price. In contrast, introducing iodized salt into countries with many small producers, each with local regional markets, is much more difficult and expensive, and will take a longer time to be effective.

Iodized Oil

The most widely used iodized oil preparation is Lipiodol (Guerbert, Paris) *(59)*. It contains 480 mg/mL iodine as iodinated poppy-seed oil. A single intramuscular administration provides sufficient iodine for 2–3 yr. Capsules containing 200 mg provide adequate iodine for 6 mo, and 400 mg provides adequate iodine for 12 mo, in school-age children *(54)*. Relative to iodized salt, iodized oil is more cumbersome because it requires direct administration to each target, and the iodine levels are uneven, being supraphysiologic shortly after administration and declining steadily thereafter. On the other hand, iodized oil can be administered immediately without waiting the several years usually required to effectively introduce iodization into the salt trade. Thus, iodized oil is appropriate to use as a short-term measure in areas with moderate to severe iodine deficiency while simultaneously working on the implementation of iodized salt.

Iodized Water

Water, like salt, is a daily necessity. Several systems have been developed to add iodine to drinking water to correct iodine deficiency *(61)*. These include: (1) simply adding drops of KI daily to school drinking water *(62)*; (2) diverting some piped water through a canister containing iodine crystals, then reintroducing it into the main stream *(63)*; (3) placing porous polymers containing KI into well water, with slow diffusion of iodide into the water over periods lasting up to a year *(64)*; and (4) iodization of irrigation water *(31,65)*. The last of these methods has the additional advantage of simultaneously correcting iodine deficiency in plants and animals, with consequent benefits to agricultural productivity as well as to

humans. In general, the practical application of iodized water depends on local conditions, such as the existence of central water supplies, how much of the iodized water is used for drinking, and whether reliable supervision is available. Iodine added in the form of molecular iodine (I_2) provides antibacterial effects as well as iodine supplementation. Polluted drinking water and iodine deficiency often coexist in many developing countries.

Iodine Tablets or Solutions

Tablets of KI, or appropriate solutions of KI or Lugol's iodine, have occasionally been used to supplement particular population groups at risk of iodine deficiency *(66)*. Examples are adolescent schoolchildren in Bolivia (A. Pardo, personal communication) and Romania *(67)*, and pregnant women in Sweden *(68)*, Sudan *(68)*, and Belgium *(69)*. Such programs usually supplement inadequate iodized-salt programs. Many highly industrialized countries in Europe are still iodine deficient (e.g., Germany, Italy), and such supplements have been feasible and effective. KI is increasingly being included in vitamin preparations for use in pregnancy in Europe and the United States. One study compared different doses of KI to iodine-deficient schoolchildren in Zimbabwe, and concluded that a dose of 30 mg iodine monthly or 8 mg every 2 wk provided satisfactory prophylaxis *(66)*. The approach of daily or frequent regular iodine supplementation is physiologically sound, but relies on individual responsibility in seeing that the iodine is actually ingested on a regular basis.

Other Vehicles

A host of other vehicles for iodine supplementation have been employed in different parts of the world. Examples are brick tea in Western China *(70)*, sugar in the Sudan *(71)*, and bread in the Netherlands and Russia *(72)*. Occasionally these are practical vehicles for providing iodine, but such food items are not essential in the way that salt and water are, raising concern that the most vulnerable groups in a population may not be included in the intervention, particularly in developing countries.

Organization of IDD Control Programs

Efforts to eliminate iodine deficiency almost always fail unless the intervention is part of a clear and sustainable program *(5,73)*. Organizational schemes vary among countries in accordance with local conditions and customs. Usually the program against iodine deficiency is administered within a country's ministry of health, but it should be closely linked to other government agencies including those for education, commerce, and agriculture. Multisectoral involvement, especially of salt manufacturers and the iodine-deficient communities, is essential and can be provided through a national advisory committee or similar structure. The program itself should include education at all levels, ranging from government officials to health personnel, salt workers, and the iodine-deficient community.

The most critical element of sustainable success of an iodine supplementation program is monitoring *(3,5,23)*. This should include process markers, particularly the level of iodine in salt at the consumer level, and also biologic indicators. The latter are the same as described earlier for the assessment of iodine sufficiency. In monitoring prophylaxis programs, the most useful indicators are the urinary iodine concentration and a measure of thyroid size. The issue of sustainability is receiving increasing scrutiny. We advocate mandated regular monitoring, both of salt iodine and biologic indicators, with required public reporting of the results. Failure to have such a system will result in complacency about the threat of iodine deficiency, and relaxation of monitoring *(73,74)*. Under such circumstances, iodine deficiency promptly recurs. Examples from the past of this undesirable course have

Table 2
Guidelines for Sustainable Elimination of IDD as a Public Health Problem

Iodine nutrition	Median urinary iodine is at least 100 μg/L.
Iodized salt	At least 90% of households use adequately iodized salt.
Sustainability	
(Evidence for following)	
Government	Responsibility, commitment, legislation, public education, regular monitoring (urine and salt), public reporting.
Salt industry	Commitment, quality control
National coalition	All stakeholders included; advocacy and awareness; regular review and reporting of results

Adapted from ref. *23*.

occurred in Guatemala, Colombia, and Thailand; all had good initial iodization programs that subsequently failed because of inadequate monitoring. China provides an excellent example of the role of monitoring in a successful IDD program *(3)*. The government has set up an extensive monitoring network, including determination of urinary iodine concentration; every 2 yr a national meeting reviews results and plans any necessary corrections. Because the median urinary iodine concentration was recently found to be higher than 300 μg/L, the level of iodization in salt was reduced from 50 to 35 ppm. The key to the success of China's IDD program has been strong government support, an effective monitoring system, and appropriate responses to its findings.

CRITERIA FOR OPTIMAL IODINE NUTRITION

The WHO/ICCIDD/UNICEF guide offers indicators for the sustainable elimination of iodine deficiency *(23)*. They are summarized in Table 2. If iodized salt is the main iodine-supplementation method, as is almost always the case, the availability and consumption of adequately iodized salt (>15 ppm iodine) must be guaranteed, as demonstrated by its use in more than 90% of households. The guidelines further state that a country must have adequately iodized salt to cover the potential human demand, that 95% of salt for human consumption must be iodized at the production or importation levels, and that adequate monitoring of the iodine content of salt already exist. The same guidelines recommend that the median urinary iodine concentration be at least 100 μg/L in a representative sample of the population, and that monitoring data exist for the last 2 yr.

The guidelines also address sustainability. Although conditions vary among countries, the following are typical key elements of an IDD elimination program: (1) clear responsibility for the program, usually within the ministry of health, with support by a national advisory/coordinating committee that represents all relevant stakeholders; (2) effective legislation; (3) political commitment to the elimination of iodine deficiency; (4) an effective program of periodic monitoring of iodine nutrition and of iodized salt; (5) appropriate public education and social mobilization; (6) commitment from the salt industry for quality control; and (7) regular recording of monitoring data, especially on urinary iodine and salt iodization, with mandatory public reporting.

In addition, optimal iodine nutrition should avoid iodine excess, and a median urinary iodine excretion of >300 μg/L should prompt inquiry and corrective action.

International organizations, especially ICCIDD, WHO, and UNICEF, recognize that iodine deficiency is a chronic geochemical fact, and that its elimination requires continuing

surveillance. In this sense, the elimination of iodine deficiency differs fundamentally from other public-health projects such as the eradication of smallpox or polio. Several initiatives can be used to address the issue of sustainability. One is developing national coalitions that can maintain long-term national interest in iodine nutrition. The formation of such coalitions will vary with social and political features of individual countries, but will typically be at least partly independent of the government, although cooperating closely with it, and will include all sectors with an interest in iodine nutrition. Some of these sectors are health, the salt industry, agriculture, education, and most importantly, consumers. Endocrinologists have an important role in such an initiative because they deal with the consequences of iodine nutrition in their daily practice, and will be among the first to recognize problems and the need for action. Another initiative is partnership evaluations of national programs. ICCIDD, WHO, and UNICEF are currently conducting such evaluations at the request of countries, and provide constant advice and encouragement for their programs.

In addition, several international activities focus on monitoring for iodine sufficiency. Among them are the development of regional referral laboratories for quality control and training in techniques of measuring iodine in urine and salt. Another is the ThyroMobil, a joint project of ICCIDD and the E. Merck Company, that applies a standardized methodology for measurement of urinary iodine and of thyroid size by ultrasound, for comparison with international standards *(2)*.

OUTCOMES

Correction of iodine deficiency prevents new cases of cretinism and endemic deaf-mutism, decreases goiter prevalence, improves educability, and advances economic productivity. Case examples abound. One frequently quoted example is from Jixian village in China *(75)*, where, after 8 yr of effective iodization, goiter prevalence decreased from 80% to 4.5%, no new cretins were born, the school ranking in the district improved from last (14th) to third, the school failure rate dropped from more than 50% to 2%, and per capita income rose more than 12-fold. Another example is Switzerland *(3)*, where goiter was endemic throughout the entire country and cretins were frequent before the introduction of iodized salt in the 1920s, whereas today all manifestations of deficiency have disappeared. Much of the midwestern United States was also iodine-deficient early in the 20th century, and goiter was the most frequent cause of rejection for military service in upper Michigan in the First World War *(76)*; through the use of iodized salt and, later, "silent prophylaxis," iodine deficiency has disappeared and goiter does not occur from this cause.

WHO, ICCIDD, and UNICEF have tracked iodine-nutrition status in recent years (Table 3). Overall, progress has been quite good, but much remains to be done. A summary prepared by WHO, with extensive input from ICCIDD, showed that of the world's 191 countries, IDD was a public-health problem in 130, but another 41 had insufficient data *(77)*. Only 20 countries could be classified as no longer having IDD. Over 2-billion people, or 38% of the 5-billion people living in the 130 countries in which IDD was a problem, were at risk for IDD, based on total goiter rate. Predictably, the problem was most severe in the least developed countries, especially in Africa, Central Asia, and Southeast Asia. (For more details, see the ICCIDD country database, at www.iccidd.org). ICCIDD has more recently compiled information from its regional coordinators (Table 3 *[3]*) and from other sources. In that informal summary of the world's 156 largest countries, 35% appear to be iodine-sufficient on the basis of median urinary iodine concentrations >100 μg/L. In 21% of countries, at least 90% of households use iodized salt. Adequate monitoring of urine is present in only 23% of countries, and of salt, in only 17%.

Table 3
Recent Estimates of Global IDD Status

	Africa	*Americas*	*China/ Western Pacific*	*Europe*	*Middle East*	*Southeast Asia*	*Total*
WHO (1999)							
Countries included (no.)	46	35	27	51	22	10	191
Population with goiter (millions)	124	39	124	130	152	172	740 (13%)
Population at risk (millions)	295	196	513	275	348	599	2225 (38%)
Household iodized salt consumption (%)	63	90	76	27	66	70	68%
ICCIDD (2001)							
Countries included (no.)	46	25	3	53	16	13	156
UI > 100 μg/L (no.)	13	20	1	11	6	3	54 (35%)
Iodized salt use >89%	9	12	0	5	5	1	32 (21%)
Adequate UI monitoring (no.)	10	6	1	13	3	3	36 (23%)
Adequate salt monitoring (no.)	10	5	1	6	2	3	27 (17%)

Table entries from the two reports differ in: (1) geographic grouping of countries; (2) number of countries (the ICCIDD report omits smaller nations); (3) emphasis on populations (WHO) vs countries (ICCIDD); and (4) use of indicator (goiter for WHO, UI for ICCIDD).

Sources: WHO report (ref. *31*); ICCIDD (ref. *3*).

UI, urinary index.

Review of the current status of iodine nutrition prompts several observations. The reduction in IDD in the past 10 yr has been enormous. Median urinary iodine concentrations are increasing, goiters are becoming smaller, and severe iodine-deficient cretinism may have vanished. At the same time, a great deal more needs to be done. Less than half of the world's countries have achieved iodine sufficiency. Monitoring systems are nonexistent or weak in most countries. The crucial need is to build in each country a self-sustaining program that will guarantee optimal iodine nutrition, and the world is still a long way from achieving this objective.

SUMMARY

Iodine is an essential component of the thyroid hormones, and its deficiency leads to varying degrees of hypothyroidism. The consequences are increased reproductive damage; abnormal development, particularly of the brain; goiter; adult and neonatal hypothyroidism; and retarded economic productivity. The best assessment tools for iodine sufficiency are urinary iodine concentration, thyroid size (preferably by ultrasound), serum or blood-spot Tg, and neonatal TSH levels. The principal iodine-supplementation method is through iodized salt, but iodized oil, iodized water, iodine tablets, and occasionally other iodization vehicles also have a role. Once a successful program for iodine sufficiency has been established, effective monitoring is the key to its sustainability. Currently, only about 35% of the world's 156 largest countries appear to be iodine-sufficient, and <25% have adequate monitoring of urine or iodized salt. With properly designed programs, elimination of global iodine deficiency is feasible but requires intensified commitment and vigor to be achieved.

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17 Iodine-Induced Thyroid Disease

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Contents

INTRODUCTION

Iodine is an essential requirement for thyroid hormone (TH) synthesis. The recommended daily iodine intake is variable, depending on the age of the subject; it is 40 μg/d during the neonatal period and 150 μg/d in the adult. The iodine requirement increases to approx 200 μg/d during pregnancy. Severe iodine deficiency, leading to various degrees of cretinism, is the major cause of mental and physical retardation in many developing countries, and its eradication is currently addressed by the International Council for Control of Iodine Deficiency Disorders (ICCIDD), United Nations International Children's Education Fund (UNICEF), and World Health Organization (WHO). Mild to moderate iodine deficiency, ranging from 40–80 μg iodine daily, also is prevalent, including in many areas of continental Western Europe. In the United States, the average iodine intake in the 1988–1994 population was approx 150 μg/d, a decrease of more than 50% relative to 1971–1974 *(1)*. A low iodine intake (<50 μg/d) was found in 11.7% of the population. Although the findings are not indicative of iodine deficiency in the overall population of the United States, the observed downward trend in iodine intake requires further close monitoring of iodine intake.

The thyroid gland has intrinsic regulatory mechanisms, which maintain normal thyroid function even in the presence of iodine excess. When large amounts of iodine are given to subjects with normal thyroid function, a transient decrease in TH synthesis occurs for approx 48 h. This acute inhibitory effect of iodine on TH synthesis is called the acute Wolff–Chaikoff effect, and is attributable to increased intrathyroid iodine concentrations *(2)*. TH synthesis then resumes normally despite continued administration of pharmacologic quantities of iodine *(3)*, thereby maintaining euthyroidism. The most likely explanation for

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this escape from or adaptation to the acute Wolff–Chaikoff effect is a decrease in the thyroid iodide trap, thereby decreasing the intrathyroidal iodide concentration *(4)*.

The expression of the recently cloned thyroid iodide trasnporter (the sodium/iodide symporter, or NIS) *(5,6)* is indeed diminished by excess iodide *(7)*; this effect is probably mediated by iodinated lipid inhibitors such as iodolactones and iodoaldehydes *(8)*.

Excess iodine ingestion (up to 150 mg/d) also decreases the release of thyroxine (T_4) and triiodothyronine (T_3) from the thyroid, resulting in small decreases in serum T_4 and T_3 concentrations with compensatory increases in basal and TRH-stimulated TSH concentrations, all values remaining well within the normal range *(9–14)*. Subjects so treated with iodine remained euthyroid although they ingested excess iodide and serum TH, and their TSH values returned to basal levels when the iodide was discontinued. These subtle changes in thyroid function were accompanied by increased thyroid volume as assessed by echography *(13,14)*, and by a decrease in thyroid blood flow determined by color Doppler flow imaging *(15)*.

The smallest quantity of iodine exceeding that consumed with the diet in the United States that does not affect thyroid function is 500 μg/d *(16)*. Administration of 1 mg/wk iodine for 6 wk, followed by the administration of 2 mg iodine weekly for another 6 wk, did not affect thyroid function *(17)*. Other studies have suggested that the administration of 500 μg/d iodine induced a small but significant increment in basal and thyrotropin-releasing hormone (TRH)-stimulated serum TSH concentrations *(18,19)*. Ingestion of 1500 μg iodine/d for 15 d to euthyroid subjects invariably resulted in a significant decrease in serum free T_4 (FT_4) concentrations and the FT_4 Index, with a significant compensatory increase in basal and TRH-stimulated serum TSH concentrations *(16,18,19)*.

Pharmacologic quantities of iodine almost always result from administration of inorganic and organic iodine-containing compounds, which are often used for therapeutic and diagnostic purposes. A list of these substances containing different amounts of iodine is given in Table 1 *(20,21)*. Despite the highly effective regulatory mechanism described here, iodine excess can induce hypothyroidism, with or without goiter, and hyperthyroidism in susceptible patients. These conditions are discussed in the following sections.

IODINE-INDUCED HYPOTHYROIDISM AND/OR GOITER (TABLE 2)

Patients Without Underlying Thyroid Disease

During Fetal and Neonatal Life

The human fetal thyroid gland begins to accumulate iodine at approx 10 wk of pregnancy *(22,23)*, but probably escapes from the acute Wolff–Chaikoff effect only during the last 4 wk of a term pregnancy *(22)*. Therefore, excess iodine exposure of the fetus and premature neonate can induce goiter and hypothyroidism, especially in areas of iodine deficiency. Excess iodine administered directly to the fetus is rare; in the past this was due to amniofetography with X-ray contrast agents containing iodine. However, maternal ingestion of drugs containing iodine is the main source of iodine excess in the fetus, since iodine freely crosses the placental barrier. Neonatal hypothyroidism and goiter owing to maternal ingestion of iodine have been reported in the past *(23)*. Goiter and hypothyroidism occurred in a single newborn whose mother was treated with the iodine-rich drug amiodarone during pregnancy *(24)*, and in another patient, transient neonatal hypothyroidism occurred *(25)* (see the following discussion of iodine-induced hyperthyroidism and amiodarone-associated thyroid dysfunction). In recent years, maternal iodine excess has been reported to be associated with transiently increased cord-blood TSH concentrations *(26–28)*. Also, topical

Table 1
Commonly Used Iodine-Containing Drugs

Drugs	*Iodine Content*
Oral or local	
Amiodarone	75 mg/tab
Caclium iodide (Calcidrine Syrup)	26 mg/mL
Diiodohydroxyquin (Yodoxin)	134 mg/tab
Echothiophate iodide ophthalmic solution (Phospholine)	5–41 μg/drop
Hydriodic acid syrup	13–15 mg/mL
Iodochlorhydroxyquin (Entero-Vioform)	104 mg/tab
Iodine-containing vitamins	0.15 mg/tab/25 mg/mL
Iodinated glycerol (Organidin[a], Tuss Organidin[a], Iophen)	15 mg/tab/25 mg/mL
Idoxuridine ophthalmic solution (Herplex)	18 μg/drop
Isopropamide iodide (Darbid.Combid)	1.8 mg/tab
Kelp	0.15 mg/tab
KI (Quadrinal)	145 mg/tab/24 mg/mL
Lugol's solution	6.3 mg/drop
Niacinamide hydroiodide + KI (Iodo-Niacin)	115 mg/tab
Ponaris nasal emollient	5 mg/0.8 mL
SSKI	38 mg/drop
Parenteral preparations	
Sodium iodide, 10% solution (recently withdrawn)	85 mg/mL
Topical Antiseptics	
Diiodohydroxyquin cream (Vytone)	6 mg/g
Hair dye	?
Iodine tincture	40 mg/mL
Iodochlorhydroxyquin cream (Vioform)	12 mg/g
Iodoform gauze (NuGauze)	4.8 mg/100 mg/gauze
Povidone iodine (Betadine)	10 mg/mL
Radiology contrast agents	
Diatrizoate meglumine sodium (Renografin-76)	370 mg/mL
Iodized oil	380 mg/mL
Iopanoic acid (Telepaque)	333 mg/tab
Ipodate (Oragrafin)	308 mg/cap
Iothalamate (Anglo-Conray)	480 mg/mL
Metrizamide (Amipaque)	483 mg/mL before dilution

[a]Iodine was removed from Organidin and Tuss Organidin in 1995. Adapted from ref. *21*.

application of antiseptic agents containing iodine to the skin of neonates, and the injection of small amounts of iodinated contrast dye through nonradiopaque silastic catheters, increased serum TSH concentrations, especially in neonates of very low birthweight *(29–31)*. All of these findings were primarily made in areas of mild iodine deficiency. We have not observed any significant changes in thyroid function in premature, sick babies exposed to locally applied povidone–iodine in Worcester, MA, an area of iodine sufficiency *(32)*.

Iodine contamination during perinatal life is a frequent event, occurring in 17.6% of 79,871 neonates in one series *(33)*, and is the most frequent cause of transient neonatal hypothyroidism *(34)*, accounting for 3% of recalls at screening for neonatal hypothyroidism,

Table 2
Iodine-Induced Hypothyroidism or Goiter

No underlying thyroid disease
Fetus and neonate, mostly preterm
Secondary to transplacental passage of iodine and exposure of newborn infants to topical or parenteral iodine-rich substances.
Infant
Occasionally reported in infants drinking iodine-rich water (China)
Adult
Frequently reported in Japanese subjects with high iodine intake (Hashimoto's thyroiditis has been excluded)
Elderly
Reported in elderly subjects with possible defective organification and autoimmune thyroiditis
Chronic nonthyroidal illness
Cystic fibrosis
Chronic lung disease (Hashimoto's thyroiditis was not excluded)
Chronic dialysis treatment
Thalassemia major
Anorexia nervosa
Underlying thyroid disease
Hashimoto's thyroiditis
Euthyroid patients previously treated for Graves' disease with ^{131}I, thyroidectomy, or antithyroid drugs
Subclinical hypothyroidism, especially in the elderly
After transient post partum thyroiditis
After subacute, painful thyroiditis
After hemithyroidectomy for benign nodules
Euthyroid patients with a previous episode of amiodarone-induced destructive thyrotoxicosis
Euthyroid patients with a previous episode of interferon-α-induced thyroiditis
Iodine plus other potential goitrogens
Sulfisoxazole: cystic fibrosis
Lithium
Sulfadiazine (?)

Adapted from ref. *21*.

in comparison with a 0.1% recall rate in neonates not exposed to iodine *(35)* in areas of mild iodine deficiency. Following iodine withdrawal, thyroid-function tests became normal.

In contrast to these studies demonstrating vulnerability of the thyroid gland to excess iodine exposure during the perinatal period, Momotani et al. *(36)* reported that the administration of 6–40 mg/d iodine to pregnant women with Graves' hyperthyroidism did not affect neonatal thyroid function. This study, however, did not exclude the possibility that these fetuses also had autoimmune thyroid hyperfunction, or that the high ambient iodine intake in Japan may result in different findings than in Europe and North America. Administration of a single dose of 15 mg of potassium iodide (KI) to 3214 Polish newborns on the second day of life, to prevent thyroid radiation from the Chernobyl reactor accident, resulted in a transient increase in serum TSH concentrations in only 12 infants (0.37%) *(37)*.

During Infancy

Iodine-induced hypothyroidism has been reported in children living in a village in central China who drank water containing 462 μg/L iodine. These children did not have positive

antimicrosomal or TSH-binding inhibitory antibodies. However, only those who developed goiter had positive thyroid growth-stimulating antibodies *(38)*. This observation, in our opinion, requires further study. Iodine-induced hypothyroidism has been observed in 20% of children chronically treated with amiodarone *(39)*. In contrast, the administration of a single dose of 50–70 mg of KI to children, to prevent radioactive contamination of the thyroid from the Chernobyl reactor accident, did not induce significant changes in serum TSH concentrations *(37)*. These differences may be attributable to the larger quantity of iodine and thyroid-inflammatory changes in the amiodarone-treated children (*see* "Amiodarone-Induced Thyroid Disease" following).

During Adult Life

In the past, iodine-induced goiter has been observed in 10% of healthy adult subjects residing in Hokkaido, Japan *(40)*. These subjects consumed large quantities of kombu, an iodine-rich seaweed that supplies approx 200 mg/d iodine. In these patients, however, hypothyroidism was a rare event. Hypothyroidism defined by serum TSH concentrations of >5 mU/L has been found in 12.1% of Japanese subjects with a morning urinary iodine excretion of >75 μmol/L; in contrast, subjects with a lower urinary iodine excretion had only a 2.3% incidence of hypothyroidism *(41)*. In another study, half of the subjects who developed iodine-induced hypothyroidism had lymphocytic thyroiditis but half did not *(42)*. Furthermore, when iodine intake was decreased, hypothyroidism disappeared *(43)*.

Water-purification units can be another source of iodine excess. Among 96 Peace Corps Volunteers working in Niger, West Africa, 33 had thyroid dysfunction (serum TSH was increased in 29 and suppressed in 4) and 44 had goiters (of whom 30 had normal thyroid function), obviously related to a very high urinary iodine excretion of 1105 μg/dL *(44)*. The iodine excess originated from a water-purification system in which iodine-resin containing filters were applied to inactivate bacteria and viruses. Drinking-water samples contained an average of 10 mg/L iodine, which led to a daily iodine intake of at least 50 mg, or 330 times the recommended daily allowance of 0.15 mg. Astronauts in the United States Space Program used water disinfected with iodine, which resulted in excess iodine ingested during their time in space. They experienced a small increase in serum TSH upon landing on Earth, but no increase in thyroid disease. This small rise in serum TSH was eliminated in 1998 when the iodine was removed from astronauts' drinking water by an anion-exchange resin just prior to consumption *(44a)*.

During Old Age

In elderly patients receiving excess iodine, hypothyroidism and goiter may also occur, especially in subjects positive for antithyroid antibodies. Interestingly, it has been reported that slightly increased serum TSH concentrations were more frequent in elderly patients residing in Worcester, MA, than those residing in Parma, Italy *(45)*. The two groups of patients differed not only because positive antithyroid antibody and iodine-perchlorate discharge tests were more frequent in subjects residing in Worcester, but also because the Worcester patients had a higher iodine intake than subjects residing in Parma, an area of mild iodine deficiency. Subsequent population-based studies among 66–70-yr-old subjects have also demonstrated a preponderance of hypothyroidism in areas with a relatively high iodine intake (as in Iceland), and a preponderance of hyperthyroidism in areas with a relatively low iodine intake (as in Jutland, Denmark) *(46)*. It is likely that increased serum TSH concentrations in elderly subjects exposed to excess iodine would be observed in those with silent autoimmune thyroiditis and/or defective iodine organification, which increases in frequency with age *(47)*. The administration of 262 mg/d iodine for 10 d to elderly subjects

slightly increased TRH-stimulated serum TSH concentrations, but not basal TSH levels, and only in those 80–89 yr old *(48)*.

In Subjects With Nonthyroidal Illness

In general, nonthyroidal illnesses are not associated with underlying thyroid disorders. However, patients with nonthyroidal illnesses may develop iodine-induced hypothyroidism. This condition has been observed in adult subjects with chronic respiratory disease treated for 1 mo to 8 yr with iodine *(49)*, and in children with cystic fibrosis, especially when iodine was given along with sulfisoxazole *(50)*. Whether lung disease is a predisposing factor to iodine-induced hypothyroidism is unclear. Hashimoto's thyroiditis (HT) was not excluded in the adult patients with pulmonary disease *(49)*. Iodine-induced hypothyroidism has also been observed in children and adults with thalassemia major requiring chronic blood transfusions *(51)*. It is likely that hemosiderosis of the thyroid is the predisposing factor in such cases. Hypothyroidism has often been observed in dialyzed patients with chronic renal failure. These subjects do not have evidence of thyroid autoimmunity, and restriction of iodine has resulted in the normalization of serum TSH concentrations in a large majority of such patients *(52–54)*. Iodide-induced hypothyroidism is also reported in patients with anorexia nervosa who eat oshaburi–kombu as a low-calorie food (one package contains approximately 13.4 mg of iodine) *(55)*. Iodine restriction may reverse hypothyroidism in such patients: serum TSH decreased by 50% in 6.1 ± 3.1 d upon institution of a normal iodine intake in patients with normal thyroid echogenecity and no antithyroid antibodies *(56)*.

Patients with Underlying or Previous Thyroid Disease

Euthyroid patients with underlying or previous thyroid disease are particularly prone to iodine-induced hypothyroidism.

Hashimoto's Thyroiditis

Patients with HT frequently develop hypothyroidism as a result of the chronic autoimmune destruction of the thyroid and the presence of TSH-receptor-blocking antibodies in this disease. In euthyroid patients with HT, the administration of 180 mg/d iodine induced hypothyroidism in 60% of the patients *(57)*. The iodine-perchlorate discharge test was positive in those patients who developed hypothyroidism *(57)*. Since euthyroid patients with HT frequently have positive iodine-perchlorate discharge tests, indicating an impaired thyroidal organification of iodide, it is not surprising that these patients will often develop iodine-induced hypothyroidism. Increased dietary iodine intake may induce hypothyroidism, as reported in Japanese patients with HT *(42,43)*. In these patients, dietary restriction of iodine resulted in normalization of the serum TSH concentration. Even small amounts of supplementary iodine may affect thyroid function: in euthyroid subjects with anti-thyroid peroxidase (anti-TPO) antibodies and/or thyroid hypoechogenecity on ultrasound examination, daily administration of 250 μg KI caused (subclinical) hypothyroidism in 7 of 40 patients after 4 mo but in only 1 of 43 patients who had not received KI *(58)*. In a group of women with Sjögren's syndrome and HT, the application of an iodine-rich hair dye induced mild hypothyroidism, which disappeared when the hair dye was discontinued *(59)*. Apparently, iodine excess accelerates autoimmune thyroiditis in autoimmune-prone individuals. Studies with experimental animals have shown direct stimulating effects of iodine on immune cells such as macrophages, dendritic cells, and T and B lymphocytes *(60)*.

The pathogenic role of antibodies directed against NIS, which have been detected in the serum of some patients with autoimmune thyroid disease, is currently unclear *(61)*.

Postpartum Lymphocytic Thyroiditis

Postpartum thyroiditis is an autoimmune disorder, most often occurring 2–8 mo after parturitition. The disease may cause either transient thyrotoxicosis followed by hypothyroidism or hypothyroidism only. In general, the thyroiditis is self-limited, and normal thyroid function returns, although permanent hypothyroidism may occur. This disorder is almost certain to recur after subsequent pregnancies *(62)*. We have reported that euthyroid women with a previous episode of postpartum thyroiditis who were given 10 drops of saturated solution of KI (SSKI; containing approx 300 mg iodine) for 3 mo developed hypothyroidism, some with goiter. When SSKI was discontinued, normal thyroid function resumed *(63)*. In these women, positive iodine-perchlorate discharge tests were highly predictive (86%) for the development of iodine-induced hypothyroidism.

Graves' Disease

The administration of SSKI for a few weeks after ^{131}I therapy for Graves' disease (GD) may induce transient hypothyroidism, which abates after the SSKI is discontinued *(64)*. We have observed that the iodine-perchlorate discharge test may be abnormal in euthyroid patients years after ^{131}I therapy, subtotal thyroidectomy, and antithyroid drug (ATD) treatment. Such previously treated patients are susceptible to iodine-induced hypothyroidism that disappears when iodine is discontinued *(65,66)*. The iodine-induced hypothyroidism is especially severe in patients previously treated with ^{131}I.

Subacute Thyroiditis

The usual clinical course of painful, subacute thyroiditis is transient thyrotoxicosis followed by transient hypothyroidism, and then euthyroidism. Occasional patients will remain permanently hypothyroid. The administration of SSKI for 120 d to euthyroid patients who recovered years earlier from an episode of subacute thyroiditis resulted in a significant increase in basal and TRH-stimulated TSH concentrations and, in a few patients, in the symptoms and signs of hypothyroidism *(67)*. All patients became euthyroid after SSKI was discontinued. A positive iodine-perchlorate discharge test was highly predictive (75%) of the development of iodine-induced hypothyroidism in these patients.

Partial Thyroidectomy

Euthyroid patients who have had a lobectomy for benign nodular thyroid disease years earlier almost always maintain normal thyroid function. It has been reported that many such euthyroid patients with a history of nodulectomy or lobectomy for benign disease without HT developed iodine-induced hypothyroidism when given pharmacologic doses of iodine *(68)*. The patients became euthyroid again when the excess iodine was discontinued.

After Interferon-α Therapy

The therapeutic administration of interferon (IFN)-α for the treatment of chronic active hepatitis may induce autoimmune thyroiditis, hyperthyroidism or hypothyroidism *(69,70)*, and a positive iodide-perchlorate discharge test *(70)*. Pharmacologic quantities of iodine administered to euthyroid patients who had previously developed IFN-α-induced thyroid dysfunction resulted in subclinical hypo- or hyperthyroidism in some of these patients *(71)*.

However, simultaneous treatment with IFN-α and 350 mg/d iodine has shown no synergistic effect on the development of abnormal thyroid-function test results *(72)*.

After Amiodarone Therapy

As noted below, amiodarone may induce a form of thyroiditis resulting in thyrotoxicosis. Therefore, it is not surprising that large doses of iodine administered to euthyroid patients long after withdrawal of amiodarone after the development of amiodarone-induced thyrotoxicosis resulted in subclinical hypothyroidism *(73)*.

IODINE-INDUCED HYPERTHYROIDISM (TABLE 3)

Iodine-induced hyperthyroidism, or Jod–Basedow disease, was first described by Coindet in 1823, following the consumption of dried seaweed in an iodine-deficient region of France. The presence of autonomous function in patients with large goiters caused by iodine deficiency has been reported in many studies showing either a scintigraphic pattern of hyperfunctioning adenomas *(74)* or low or suppressed basal and TRH-stimulated serum TSH concentrations *(75)*. Although the etiology of iodine-induced hyperthyroidism remains unclear, it is probably caused by excessive production and release of TH when autonomous nodules are exposed to excess iodine *(76)*.

Outbreaks of iodine-induced hyperthyroidism have been reported in the past when iodine prophylaxis was introduced to treat populations with endemic iodine-deficiency goiter. These outbreaks were usually self-limited, and the prevalence of hyperthyroidism would decrease with a few years of adequate iodine intake. Iodine-induced hyperthyroidism has been reported in the past in areas such as Tasmania and Brazil when iodine-deficient populations were given iodine supplements or were exposed to a normal iodine intake *(76)*. More recently, the introduction of iodized salt or oral iodized oil in endemically iodine-deficient areas such as Spain and Zaire resulted in an increased incidence of self-limited hyperthyroidism *(77,78)*. Very recently, Bourdoux et al. reported that after 2 yr of iodine prophylaxis with iodized salt (up to 148 ppm) in Zaire, resulting in urinary iodine excretion of 200–500 μg daily, 29 of 190 subjects had undetectable serum TSH values, and 14 of them developed clearly elevated serum T_4 and T_3 concentrations and symptoms of thyrotoxicosis requiring ATD treatment *(79)*. Another outbreak of severe thyrotoxicosis occurred in Zimbabwe after the introduction of iodized salt (30–90 ppm) to treat endemic iodine-deficiency goiter *(80)*. In contrast to these studies, the administration of oral iodized oil in goiter-endemic regions of Senegal did not induce hyperthyroidism *(81)*. Although prevention of iodine deficiency goiter, mental and growth retardation, and cretinism is essential, careful monitoring of populations given iodine supplementation is indicated in view of the possible induction of iodine-induced hyperthyroidism *(82)*.

The induction of hyperthyroidism in iodine-sufficient regions such as the United States upon the administration of pharmacologic doses of iodine is far less common than in iodine-deficient areas, but does occur, especially in patients with nontoxic nodular goiter *(46,83–85)*. Hyperthyroidism may also occur in elderly patients following the use of iodine-rich X-ray contrast agents in both iodine-sufficient *(86)* and iodine-deficient *(87)* areas. In a prospective study of the effect of nonionic agents in elderly patients, Conn et al. also reported that frank hyperthyroidism was uncommon and that subclinical hyperthyroidism was observed *(88)*. Likewise, only 2 of 788 unselected euthyroid patients living in an iodine-deficient area developed hyperthyroidism within 12 wk after coronary angiography *(89)*. It has been suggested that a prophylactic course of methimazole (MMI) or perchlorate given the day before and for 2 wk after X-ray contrast studies to patients with thyroid autonomy

Table 3
Iodine-Induced Hyperthyroidism

Iodine supplementation for endemic iodine-deficiency goiter
Iodine administration to patients with euthyroid Graves' disease, especially those in remission after antithyroid drug therapy
Iodine administration to patients with underlying thyroid disease (more common in areas of marginal iodine sufficiency)
Nontoxic nodular goiter
Autonomous nodule
Nontoxic diffuse goiter
Iodine administration to patients with no recognized underlying thyroid disease.

Adapted from ref. *21*.

decreases the incidence of iodine-induced hyperthyroidism *(90)*. However, prophylactic therapy is not generally recommended because the risk of side effects is perhaps even greater than the risk of iodine-induced thyrotoxicosis *(89)*. Rarely, hyperthyroidism might be aggravated or induced in patients with functioning metastatic differentiated thyroid cancer following studies using iodinated contrast media *(91)*. A recent study from Switzerland reported a prevalence of iodine-induced thyrotoxicosis of 20% among thyrotoxic patients in a large urban hospital *(92)*. Causes of iodine excess were mainly iodinated radiocontrast agents, besides amiodarone and kelp tablets. The clinical presentation was dominated by tachyarrhythmias and heart failure. Most patients had goiter; thyroid antibodies were absent. Treatment consisted of MMI with frequent addition of beta-blockers, lithium, or prednisone, and euthyroidism was restored on average after 6.4 wk.

AMIODARONE-INDUCED THYROID DISEASE

Amiodarone, a lipid-soluble benzofuranic drug, contains 75 mg iodine per 200 mg tablet, has a prolonged half-life of many months, and releases approx 9 mg of inorganic iodine per day during its metabolism. Amiodarone administered chronically to euthyroid subjects without underlying thyroid disease results in increased serum T_4, FT4 and rT3 concentrations, lower serum T_3 concentrations, and usually normal serum TSH concentrations *(93)*. These changes are mainly the result of the drug's potent inhibition of 5′-deiodinase activity, the selenoprotein enzyme that deiodinates T_4 to T_3 and rT3 to 3,3′T2. Despite these alterations in peripheral hormone metabolism, patients treated with amiodarone usually remain euthyroid. However, patients treated with amiodarone may also develop hypothyroidism or thyrotoxicosis *(94,95)*. The incidence of amiodarone-induced thyrotoxicosis (AIT) is higher in areas with a low environmental iodine intake than in areas with a high iodine intake (11.9% and 1.7% respectively); in contrast, the incidence of amiodarone-induced hypothyroidism is higher in regions with a high than with a low iodine intake (13.2% and 6.4%, respectively) *(96–98)*.

Hypothyroidism is thus more common in patients residing in iodine-replete areas such as the United States, especially patients with underlying HT *(96)*. The development of hypothyroidism is probably the consequence of a permanent block of thyroidal iodine organification owing to a failure of escape from the acute Wolff–Chaikoff effect. This hypothesis is consistent with the observation that when potassium perchlorate, which is a competitive inhibitor of the thyroid iodine pump, is administered to patients with amiodarone-induced hypothyroidism, normal thyroid function resumes, since excess intrathyroidal iodide is reduced *(99,100)*. After amiodarone withdrawal, patients with hypothyroidism

usually become euthyroid, but this may take months, owing to the prolonged half-life of the drug. However, amiodarone is usually continued and the patient is treated with appropriate doses of L-thyroxine (levothyroxine) to normalize the serum TSH concentration. The dose of levothyroxine may be higher than usual since, as noted above, amiodarone is a potent inhibitor of T_4 to T_3 conversion and T_3 is the active hormone. However, caution should be used since we have observed a patient receiving amiodarone who developed subclinical hypothyroidism and later destructive thyrotoxicosis (*see* next paragraph) *(101)*.

Thyrotoxicosis has frequently been observed in patients treated with amiodarone, especially those residing in continental western Europe, where mild iodine deficiency is often present *(96,98)*. In these patients, it is likely that the iodine released from amiodarone increases TH synthesis in glands with underlying areas of autonomy *(76,94)*. However, thyrotoxicosis has also been described in patients without evident underlying thyroid disease. In these patients, the thyrotoxicosis is apparently due to a destructive process in the gland, most likely caused by amiodarone *per se*—a form of drug-induced thyroiditis—and not to iodine *(102,103)*. In vitro studies have confirmed that amiodarone has a cytotoxic effect on thyroid follicles *(104)*, possibly mediated by increased interleukin (IL)-6 production by thyrocytes *(105)*. The amiodarone-induced IL-6 production is inhibited in vitro by prednisolone. Electronmicroscopic examination reveals specific ultrastructural changes different from those induced by iodine excess alone; specific changes include marked distortion of thyroid architecture, necrosis and apoptosis, inclusion bodies, lipofuscinogenesis, and markedly dilated endoplasmatic reticulum *(105,106)*. The data indicate a direct cytotoxic effect of amiodarone on thyrocytes, mediated through disruption of subcellular organelle function. The main features of this form of thyrotoxicosis (called AIT type II) are the lack of underlying thyroid disease, the severity of the thyrotoxicosis, the pathologic findings on fine-needle aspiration biopsy or in surgical specimens, increased serum concentrations of IL-6, and absence of thyroid hypervascularity on color flow Doppler sonography *(94,95,107)*. Patients with destructive thyrotoxicosis owing to amiodarone may later develop primary hypothyroidism *(102)*. Furthermore, these patients are likely to develop iodine-induced hypothyroidism when exposed to excess iodine years after arniodarone withdrawal *(73)*.

Distinction of the two forms of amiodarone-induced thyrotoxicosis is relevant for the choice of treatment (Table 4). In AIT type I, patients usually are still hyperthyroid 6–9 mo after discontinuation of amiodarone *(108)*. Treatment with MMI or propylthiouracil (PTU) is not very effective because of the decreased efficacy of ATDs in the presence of excess iodine *(98)*. Effective control of AIT type I thus remains difficult, also because ^{131}I therapy is often not feasible in view of a suppressed thyroidal RAI uptake. Total thyroidectomy is an option, but the surgical risk may be high in patients with cardiac disease. It is, however, reassuring that the outcome of total thyroidectomy is very good, with low postoperative morbidity and no mortality reported so far *(98,109–112)*. Nonsurgical alternatives are fortunately available. Addition of potassium perchlorate (500 mg of $KClO_4$ twice daily) to ATDs blocks further entry of iodine into the thyroid gland, thereby depleting the thyroid of its intrathyroidal iodide stores and enhancing the efficacy of the ATDs. The combined regimen restored euthyroidism within 15–90 d in all patients with AIT type I except one *(108)*. Agranulocytosis is a serious side effect of potassium perchlorate, but so far it has not been encountered in the small series of reported patients (possibly attributable to the rather low daily dose of 1 g and the short treatment period of 2–3 mo).

In AIT type II, euthyroidism is usually restored within 3–5 mo after discontinuation of amiodarone *(108)*. ATDs are of little use owing to the destructive nature of AIT type II, and the same holds true for ^{131}I therapy. Treatment with prednisone (20–80 mg/d for 7–12 wk) can

Table 4
Features of Amiodarone-Induced Thyrotoxicosis (AIT)

	AIT type I	*AIT type II*
Underlying thyroid abnormality	Yes	No
Pathogenetic mechanism	Excessive thyroid hormone synthesis owing to iodine excess	Excessive thyroid hormone release owing to destructive thyroiditis
Goiter	Diffuse or multinodular goiter usually present	Occasionally small, diffuse, firm goiter
Thyroid radioiodine uptake	Low, normal, or elevated	Low
Thyroid ultrasound	Nodular, hypoechoic, increased volume	Normal
Color flow Doppler sonography	Thyroid vascularity present or increased	No thyroid hypervascularity
Serum IL-6	Normal or slightly elevated	Markedly elevated
Preferred treatment	Thionamides and potassium perchlorate	Glucocorticoids
Subsequent hypothyroidism	Unlikely	Possible
Effect of excess iodine administration following the thyrotoxic phase	Probable iodine-reinduced hyperthyroidism	Possible iodine-induced hypothyroidism

Modified from refs. *93* and *95*.
AIT, autoimmune thyroiditis; IL-6, interleukin-6.

be very effective *(94,98,102,103)*. However, early discontinuation of steroids after 2–3 wk is associated with recurrent thyrotoxicosis, necessitating the reintroduction of steroids *(113)*. Lithium carbonate (900–1350 mg/d) might be tried in severe cases: normalization of thyroid-function tests after discontinuation of amiodarone occurred in 10 wk with or without PTU treatment, but in 4 wk with combined PTU and lithium treatment *(114)*. In view of the self-limiting nature of AIT type 2 it might not be absolutely necessary to stop amiodarone treatment. Indeed, some cases have been reported in which, after a limited course of ATDs, euthyroidism was maintained despite continuation of amiodarone treatment *(115)*.

In summary, AIT type I is best treated by discontinuation of amiodarone and instituting a combined regimen of MMI and potassium perchlorate. AIT type II is best treated by discontinuation of amiodarone (although its continuation might be considered in selected cases) and instituting a course of prednisone. However, the distinction between AIT type I and type II can be difficult, and mixed forms can be present. Exacerbation of thyrotoxicosis may occur in both types, and may require the addition or increased dosage of glucocorticoids and ATDs *(113)*. Despite all efforts, some patients do not respond to multidrug treatment with thionamides, potassium perchlorate, and steroids; of six patients with life-threatening AIT in one series, four died of thyroid storm *(98)*. In serious cases of AIT total thyroidectomy should be considered. Plasmapheresis has occasionally been used in patients with resistant AIT in an attempt to remove the excess of TH from the body, but failures are reported *(116)*.

CONCLUSIONS

The effects of excess iodine on various aspects of thyroid function in normal volunteers and patients with underlying thyroid disease have been evaluated. The responses to excess

iodine vary, depending on the underlying thyroid disorder and the ambient iodine intake. Thus, euthyroid individuals with HT and those with a history of treated GD, postpartum lymphocytic thyroiditis, and subacute thyroiditis may develop iodine-induced hypothyroidism. However, euthyroid subjects without underlying thyroid disease remain euthyroid even when exposed to large quantities of iodine, probably because of a decrease in the thyroid iodide trap, thereby defending the gland against increased concentrations of iodide, which can potentially inhibit TH synthesis. In contrast, iodine-induced hyperthyroidism is more common in older patients with euthyroid nodular goiter, especially in areas of iodine deficiency. The iodine-rich drug amiodarone can induce hypothyroidism or thyrotoxicosis, the latter owing either to excess iodine or drug-induced thyroiditis. Thus, thyroid dysfunction following to amiodarone administration is encompassed by the spectrum of thyroid disorders.

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18 Clinical Relevance of the Thyroid Sodium/Iodide Symporter

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CONTENTS

INTRODUCTION

The first step in the formation of thyroid hormones (TH) involves the active accumulation of iodide from the extracellular fluid across the basolateral membrane and into the thyroid follicular cell. The protein responsible for this was previously described as the iodide trap or iodide pump. Because the transport into the follicular cell of iodide against its concentration gradient is coupled with transport of sodium, the protein was named the sodium/iodide symporter (NIS). The gene for NIS was cloned by Carrasco's group in 1996 *(1)*, paving the way for a whole new field of research not only involving thyroidal physiology and pathology, but now extending into breast cancer and gene therapy for nonthyroidal cancer. Regulation of NIS gene expression is being uncovered, and there is increasing knowledge of the structure of NIS protein. NIS distribution has been found outside the thyroid, with diagnostic as well as therapeutic implications. Mutations in *NIS* are now known to be responsible for a subtype of congenital hypothyroidism (CH) attributable to defects in iodide transport. The mechanism of the escape from the acute Wolff–Chaikoff effect, first postulated almost 40 yr ago, has now been demonstrated to be caused by iodide-induced downregulation of NIS messenger RNA (mRNA) and protein. The controversial area of NIS and thyroid autoimmunity remains hotly contested, and the role of anti-NIS antibodies is still debated.

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The potential clinical implications of NIS are probably most significant in the field of oncology. Thyroid cancers may become dedifferentiated with time and lose their ability to take up radioiodine (RAI). The prognosis for patients with metastatic thyroid cancer who do not show RAI uptake is very poor. Manipulation of NIS expression could potentially restore RAI uptake and greatly improve the prognosis for this group of patients. NIS is found not only in the lactating breast, but also in a large proportion of estrogen-receptor-positive breast cancer. The use of RAI as a diagnostic and therapeutic modality remains an exciting possibility. Ultimately, and hopefully in the not too distant future, NIS may play a role in the still elusive goal of targeted gene therapy for nonthyroidal cancer.

NIS STRUCTURE, REGULATION, AND DISTRIBUTION

Rat *NIS (rNIS)* was cloned by Dai et al. *(1)* in 1996, through functional screening of a clonal DNA (cDNA) library from a rat-thyroid-derived cell line (FRTL-5) expressed in *Xenopus* oocytes. The cDNA for *rNIS* was 2839 bp in length, with an open-reading frame of 1854 nucleotides that encoded a protein of 618 amino acids. The protein was initially predicted to be an intrinsic membrane protein containing 12 transmembrane domains, with both its amino and carboxy termini facing the cytoplasm. Further testing of the secondary-structure model of the protein showed that rNIS had 13 transmembrane segments, with the amino-terminus facing the extracellular milieu *(2)*. Although there are three N-linked glycosylation sites, absence of glycosylation at these sites did not seem to affect the function or stability of NIS *(2)*. Human *NIS (hNIS)* was subsequently cloned by Smanik et al. *(3)*. The nucleotide sequence of *hNIS* showed an open-reading frame of 1929 nucleotides encoding a 643-amino-acid protein that had 84% identity and 92% similarity to *rNIS*. *hNIS* was mapped to chromosome 19 and consisted of 15 exons and 14 introns *(4)*. NIS activity was found to be sodium dependent and involved cotransport of two sodium cations per one iodide anion. In addition to iodide, NIS was found to be capable of transporting other anions. Perchlorate, however, which is the most potent inhibitor of NIS, was not transported *(5)*.

In the thyroid, NIS is predominantly regulated by thyroid stimulating hormone (TSH). TSH, via a cyclic adenosinie monophosphate (cAMP)-mediated pathway, causes a rapid increase in NIS mRNA, followed by a slower increase in NIS protein *(6–8)*. Iodide has also been shown to regulate NIS in the form of autoregulation. Iodide decreases both NIS mRNA and NIS protein in vivo, at least in part by a transcriptional mechanism *(9)*. In addition, iodide has been shown to decrease NIS protein in vitro by a posttranscriptional mechanism *(10)*. Follicular thyroglobulin (Tg) also appears to be a negative regulator of *NIS* expression *(11)*, possibly as a counterbalance to the action of TSH. Cytokines, too, may play a role in modulating NIS gene expression, and suppression of *NIS* expression by cytokines may be partly responsible for diminished RAI uptake in thyroiditis *(12,13)* and aging *(14)*. The promoter region of both *hNIS* and *rNIS* has been sequenced and characterized *(15–18)*. Binding sites for thyroid transcription factor-1 (TTF-1) *(19)* and Pax-8 *(18)*, two transcription factors implicated in thyroid-specific gene transcription, have been identified in the promoter region of *rNIS*. In addition, a novel TSH-response element (TSH-RE) was identified in the *rNIS* promoter, and the putative binding protein for this site was termed NIS TSH-response factor-1 (NTF-1) *(20)*. Additionally, Ohno et al. found an *rNIS* enhancer that mediated thyroid-specific gene expression. This enhancer was termed NIS upstream enhancer (NUE), and consisted of two Pax-8 binding sites and a degenerate cAMP-response element (CRE) *(18)*.

NIS is most abundantly found within the thyroid gland. Within the thyroid follicle, NIS expression is heterogeneous, with uneven distribution among the follicular cells *(21)*. Only about 20% of the follicular cells are positive for NIS by immunohistochemistry *(22)*. In

Graves' disease (GD), NIS expression is increased *(21,22)*, while NIS expression is generally decreased in thyroid cancer *(21,23–27)*. Iodide uptake is not limited to the thyroid gland, and other organs like the salivary glands, stomach, placenta, and lactating mammary glands have been found to concentrate iodide *(28)*. Using Northern blot analysis, NIS mRNA has been detected in the parotid gland *(29)*. Using the reverse transcriptase–polymerase chain reaction (RT–PCR), NIS mRNA expression has been detected in the stomach, salivary gland, mammary glands, pituitary, pancreas, testis, ovary, adrenals, heart, thymus, lungs, extraocular muscles, and colon *(4,29,30)*. Positive immunostaining for NIS protein has been detected in salivary gland, gastric mucosa, mammary glands, rectum, lacrimal glands, and pancreas *(31,32)*. It is not known whether iodide is organified within these extrathyroidal tissues. NIS expression in lactating mammary glands presumably functions to supply iodide to the newborn, but the role of iodide transport in other nonthyroidal tissues remains speculative.

NIS MUTATIONS AND HYPOTHYROIDISM

Iodide transport defect (ITD) is a rare disorder that is characterized by the absence or diminution of iodide transport within the thyroid gland. It generally presents as congenital goitrous hypothyroidism although it shows considerable phenotypic variability. The first case was reported over 40 yr ago *(33)*, and to date, about 40 patients from over 20 families have been documented, with more than half the cases from Japan. The diagnostic criteria are: (1) absent or poor RAI uptake; (2) defective iodide concentration in the salivary glands, with a low saliva-to-serum iodide ratio; and (3) goiter with hypothyroidism or compensated hypothyroidism.

With the cloning of NIS, the molecular basis of the ITD is being elucidated. The first case of *NIS* mutation giving rise to ITD was reported by Fujiwara et al. in 1997 *(34)*. This was a homozygous missense mutation, T354P, in the NIS gene of a patient who had consanguineous parents. This mutation was shown to produce a complete loss of iodide-transport activity in transfection experiments. Subsequently, five patients from three unrelated families with ITD were shown to harbor the same mutation, suggesting that T354P could be a recurrent mutation in a hot spot, and a major cause of ITD *(35)*. Seven other mutations in *NIS* have been detected. One was a nonsense mutation resulting in loss of the C-terminal of the NIS protein *(36)*. Another was a frameshift mutation leading to a premature stop codon as well as to low levels of the mature NIS gene transcript *(37)*. The other five mutations were missense mutations located within the transmembrane domains of the *NIS* gene *(37–40)*. The precise mechanisms for loss of function of the NIS mutations remain to be fully characterized. Experiments have shown that T354P is expressed and properly targeted to the membrane, but the lack of a hydroxyl group near the β carbon of the residue at position 354 may play a role in the loss of NIS function in this mutation *(41)*. Using a monoclonal antibody against the extracellular domain of NIS, it was demonstrated that two mutations, Q267E and S515X, have faulty membrane targeting *(42)*.

The documented *NIS* mutations seem to be of a recessive nature, with only homozygotes or compound heterozygotes affected. The clinical features of ITD are heterogeneous *(43)*. Some patients are hypothyroid or even show features of cretinism, whereas others are euthyroid. Most, but not all, have goiter. Even among patients with the same mutation there is phenotypic heterogeneity. One patient homozygous for the T354P mutation was hypothyroid and was identified in early childhood *(34)*, while the second case homozygous for T354P was euthyroid, and presented with a huge goiter only at the age of 18 yr *(44)*. The high dietary intake in the second patient could possibly have compensated for the patient's

iodide-transport defect. Identification of new *NIS* mutations and increased understanding of the relationship between the structure and function of NIS will in the future provide further insight into the mechanisms of NIS mutations in ITD.

NIS AND IODIDE AUTOREGULATION

Autoregulation in the thyroid refers to the regulation of iodide metabolism within the thyroid gland, independent of TSH. In the presence of acute iodide excess, the thyroid is able to shut off hormone synthesis, presumably to prevent a surge in TH formation. This iodide-induced inhibition of TH synthesis is known as the acute Wolff–Chaikoff effect *(45)*. The mechanism responsible for the acute Wolff–Chaikoff effect has been postulated to result from inhibition of the organification of iodide into organic iodocompounds within the thyroid. Wolff and Chaikoff subsequently demonstrated that this inhibitory effect was transient, and that the thyroid escaped from or adapted to prolonged iodide excess, resuming near-normal hormone synthesis *(46)*. This escape mechanism allows the resumption of normal TH synthesis, thus preventing prolonged hypothyroidism. In certain pathologic thyroid states such as chronic autoimmune thyroiditis, this escape mechanism seems to be faulty, and chronic exposure to high levels of iodide can lead to prolonged hypothyroidism. Braverman et al. suggested that adaptation to the acute Wolff–Chaikoff effect was caused by a decrease in iodide transport into the thyroid, which then reduced the intrathyroidal iodide load to concentrations that were insufficient to sustain the decreased organification of iodide *(47)*. This postulate of the escape mechanism was reinvestigated by Eng et al., who showed in vivo that escape from the acute Wolff–Chaikoff effect was associated with a decrease in NIS mRNA and protein, and that the decrease in NIS was likely to be at least in part transcriptional *(9)*. In vitro experiments in FRTL-5 cells showed that in the presence of excess iodide, NIS protein but not NIS mRNA decreased *(10)*. This suggested that iodide may also partly modulate NIS at the posttranscriptional level, possibly be inducing an increase in protein turnover.

NIS AND AUTOIMMUNE THYROID DISEASES

Autoimmune thyroid diseases (AITDs) are associated with the formation of antibodies towards thyroidal antigens such as Tg, thyroid peroxidase (TPO), and the TSH receptor (TR). While some of these antibodies such as anti-Tg and anti-TPO antibodies indicate the presence of thyroid autoimmunity, the anti-TR antibody (TRAb) is pathogenic and can lead to the development of GD or hypothyroidism. NIS, a membrane protein that plays a crucial role in iodide uptake into thyroid follicular cells, could potentially be a target of autoimmunity in AITD, and its function could potentially be influenced by NIS autoantibodies (NISAbs). The first indication that NIS has a role in AITD came before the molecular cloning of NIS. Raspe et al. reported the drastic inhibition of TSH-induced iodide uptake in dog thyrocytes by the serum of a patient suffering from Hashimotos's thyroiditis (HT), autoimmune gastritis, and rheumatoid arthritis *(48)*. Because of unavailability of this serum, the study could not conclusively prove that the effect was antibody-mediated. However, the inhibitory effect on iodide uptake could be reproduced to a lesser degree with a mouse monoclonal antibody obtained from immunizing mice with a thyroid-membrane preparation.

Since the cloning of rNIS *(1)*, several groups of investigators, using various methods, have explored the putative role of NIS as a novel antigen. By slot-blotting, Endo et al. reported immunoreactivity against an NIS fusion protein in 84% (22 of 26) of patients with GD and

15% (3 of 20) of patients with HT *(49)*. Endo et al. also showed that 4 of 34 HT sera that were positive in their slot–blot binding assay had inhibitory activity against iodide transport. With Chinese hamster ovary (CHO)-K1 cells stably expressing rNIS, the IgG preparations from these four HT patients inhibited iodide accumulation by 14–62%. With synthetic peptides, the study mapped the binding epitope of two of these antibodies to a region located in the 6th extracellular loop of NIS *(50)*. The common occurrence of anti-NIS antibody in AITD, particularly GD, was echoed in another study done by Morris et al. *(51)*. They measured binding of IgG to the 21 synthetic rNIS peptides replicating the entire sequence of the extramembranous domains of these peptides, using an enzyme-linked immunosorbent assay. Sixty-three percent of GD IgG bound to extramembranous domain 12, and 25.9% of HT IgG bound to extramembranous domains 12 and 13, but none bound to control IgG. In 1998, the secondary structure of rNIS was revised *(2)*, and in the current model, some of these reported antigenic epitopes are located intracellularly. Although it can be argued that NISAbs directed against intracellular epitopes could have arisen from thyroidal damage, the mechanism by which they affect iodide uptake remains unexplained. Moreover, CHO cells transfected with NIS are capable of only a low level of maximum iodide uptake *(50,52)* and do not provide a robust bioassay for assessing NIS activity. Therefore, the data presented above should be interpreted with caution. In addition, species specificity inherent in such assays when they are used with xenogenic antigens remains an issue. Indeed, with the cloning of hNIS, Morris et al. found, in contrast to their earlier study of rNIS, that the binding of IgG from GD patients to synthetic hNIS peptides was not significantly different from that of controls' IgG *(53)*.

Recently, two independent groups of investigators, using an in vitro transcription and translation (TNT) system and immunoprecipitation methods for detection of hNIS, made differing observations. Ajjan et al. showed a 22% (11 of 49 patients) prevalence of NISAbs in GD *(54)*, while Seissler et al. had a 10% rate of positivity in their assay *(55)*. With regard to HT, the two groups found a similar prevalence of NISAbs, of 24% by Ajjan et al. and 20.8% by Seissler et al. A number of factors could have contributed to the difference in GD despite similar methodology. First, calculations of antibody positivity differed in these two groups, and the controls utilized were also different. Second, Seissler and coworkers had screened a large cohort of normal and diseased subjects. While the prevalence of NISAbs in otherwise healthy subjects and in AITD remains uncertain, a large sample size for establishing baseline and disease states will overcome the problem of sampling error. Third, there may indeed have been a true difference in antibody prevalence in the two GD populations screened.

Another issue currently under debate is the effect of AITD sera on the functional activity of NIS. All studies reporting a high prevalence of NISAbs with functional-modulating effects examined small groups of patients (numbers not exceeding 100) and worked with CHO-NIS cell lines, with the inherent problems highlighted earlier *(50,52,54)*. With the establishment of a stable COS7 cell line expressing a high level of functional hNIS, a more robust bioassay with a huge capacity for iodide uptake was developed by Ho et al. in a 96-well format suited for mass screening *(56)*. In this study, only 14 of a total of 514 sera screened showed a diminution of iodide uptake to 2 SD below the mean of controls. Among these 14 sera, 7 available sera reevaluated after dialysis and/or IgG extraction lost their inhibiting activity on iodide uptake. This indicated that the effects were not antibody mediated, and that unknown serum factors were responsible. Therefore, with the evidence at hand, it would appear that the earlier studies with rNIS had overpredicted the significance of NISAbs in AITD. Results from hNIS research showed a lower prevalence of NISAbs in hNIS-binding

assays, although these antibodies appear to be more prevalent in HT than in GD. However, NISAbs capable of affecting NIS function are rare and do not contribute significantly to modulating thyroid function in AITD. The clinical and pathogenic importance of a potential autoimmune response to NIS remains to be determined. The measurement of NISAbs with the currently available assay systems does not contribute any additional diagnostic benefit to the detection of AITD.

NIS AND THYROID CANCER

Thyroid cancer, though accounting for less than 1% of all malignant tumors, is the most common endocrine malignancy and is responsible for more deaths than all other endocrine tumors combined. With the exception of medullary thyroid cancer derived from the parafollicular cells, and thyroid lymphoma, all thyroid cancers arise from the follicular cells. About 90% of nonmedullary thyroid cancers are classified as differentiated thyroid cancers (DTC). The majority of DTCs retain the ability to concentrate iodide (although at a lower level than normal thyroid tissue) and are amenable to treatment with RAI therapy. ^{131}I is used either as an adjunct to surgery to ablate the postoperative thyroid remnant or to treat metastatic disease. However, some DTCs lose their iodide-concentrating ability and become insensitive to subsequent ^{131}I therapy. It is known that 20–50% of metastatic thyroid cancers do not take up RAI, leading to a much poorer prognosis with a high rate of recurrence and decreased survival. Most DTCs appear to be hypofunctional on scintiscanning, implying decreased ability to take up RAI. This has been attributed to a decrease in NIS expression by the cancerous thyroid cells. NIS gene expression has been shown to be reduced in both rat and human thyroid-cancer cell lines. Using RT–PCR, Northern blotting, real-time PCR, Western blotting, and immunohistochemical techniques, most investigators have found either an absence of or decrease in NIS mRNA and protein expression in DTC as compared with normal thyroid tissue *(21,23–27)*. In contrast, Saito et al. reported an increased expression of NIS mRNA and protein in 7 of 17 cases of papillary thyroid cancer as compared with normal thyroid *(57)*. Very recently, Dohan et al. analyzed NIS protein expression in 57 thyroid-cancer specimens with high-affinity anti-NIS antibodies and found that 70% of the samples exhibited increased NIS expression with respect to normal thyroid *(58)*. NIS was found to be predominantly located intracellularly, in contrast to its basolateral-membrane location in normal thyroid. When membrane staining for NIS was detected, it was visible all around the cell and not just in the basolateral membrane. Thus, although the general consensus shows that NIS gene expression is decreased in thyroid cancer, the two reports just described suggest that lack of RAI uptake by some DTCs may be the result of impaired targeting of NIS protein or insufficient retention of NIS in the plasma membrane. Analysis of metastatic thyroid cancer also generally showed decreased expression of *NIS* in metastatic tissue as compared with the primary tumour *(26,59,60)*, possibly as a result of dedifferentiation during the process of metastasis. Even in metastatic lymph nodes that were positive for NIS gene expression, total body ^{131}I scanning did not show any RAI uptake, again suggesting that in addition to loss of NIS gene expression, defects in NIS protein structure, targeting, or activation may be involved.

Follow-up of thyroid cancer after therapy is complicated by numerous difficulties, including interference with the Tg assay by anti-Tg antibodies and absence of international standardization. Methods using RT–PCR to amplify and detect Tg mRNA from circulating thyroid cells after thyroid-cancer treatment have been shown to be more sensitive than measurement of serum Tg in the detection of metastatic or residual thyroid cancer. NIS mRNA measurement was also investigated as a possible marker for recurrent or residual

disease. Unfortunately, in contrast to Tg mRNA, detection of circulating NIS mRNA was neither sensitive nor specific for circulating thyroid tumor cells *(61)*. The detection of NIS mRNA did not improve the ability to detect active disease, and was not always concordant with Tg mRNA.

Because NIS gene expression is decreased or absent in certain thyroid cancers, and this may explain in part their loss of RAI-concentrating ability, strategies to upregulate NIS gene expression have been explored in the hope of restoring iodide uptake, and hence to make these cancers amenable to RAI therapy. One approach to this is to transfect the NIS gene into the thyroid cancers that lacked RAI-concentrating ability. This was first tried in malignant transformed rat thyroid cells (FRTL-Tc) *(62)*. FRTL-Tc cells could grow as a tumor in the subcutaneous tissues of Fisher rats, but did not concentrate RAI. When the FRTL-Tc cells were transfected with NIS expression vector, the resultant cell line, Tc-rNIS, could stably express NIS. Although tumors formed from Tc-NIS were able to accumulate RAI, there was failure to decrease tumor size. Possibly the Tc-rNIS tumors were unable to organify the RAI, resulting in a short effective half-life of RAI in the tumors. Smit et al. took a similar experimental approach with an NIS-defective human thyroid-cancer cell line, FTC 133, and were able to stably transfect hNIS into this cell line *(63)*. The hNIS-tranfected FTC 133 cells formed tumors in nude mice and were able to take up RAI.

A second approach to restoring RAI uptake is to try to upregulate endogenous NIS gene expression in dedifferentiated thyroid cancer. Simon et al. showed that RAI uptake could be restimulated in patients with non-RAI-accumulating thyroid tumors through the use of retinoic acid (RA), a well-characterized agent with differentiating properties *(64)*. The same group demonstrated that RA increased NIS mRNA levels in FTC133 cells *(65)*. In this in vitro system, however, there was no iodide accumulation, despite an increase in NIS mRNA and protein, possibly because of failure of NIS activation or targeting. Nearly half of all human genes contain CpG islands—stretches of nucleotides close to the transcription start site which are rich in the dinuceotide CG. Unmethylated CpG islands are seen in highly transcribed genes, and heavily methylated CpG islands inhibit transcription. In cancer, CpG islands in promoter regions of certain genes can become hypermethylated, resulting in loss of gene transcription. Chemical agents that can demethylate these regions could potentially restore gene expression. Venkataraman et al. treated seven human thyroid-cancer cell lines with 5-azacytidine or sodium butyrate, and were able to restore NIS expression in four cell lines and iodide uptake in two cell lines *(66)*. These results suggested that DNA methylation may contribute to loss of NIS expression in thyroid cancer, and that chemical demethylation therapy could be a potential therapeutic modality for dedifferentiated thyroid cancer. Acetylation of histones promotes the interaction of transcription factors with regions in promoter DNA, thus facilitating gene transcription. Histone deacetylase complexes antagonize this interaction and thus inhibit transcription. Depsipeptide, a novel histone deacetylase inhibitor undergoing phase 1 trials, acts by promoting histone acetylation and in turn gene expression. Kitazono et al. were able to increase NIS gene expression and increase iodine accumulation in four poorly differentiated human thyroid-cancer cell lines by using low doses of depsipeptide *(67)*. This could be another potential means for inducing RAI uptake in dedifferentiated thyroid cancer.

NIS AND BREAST CANCER

Iodine has been linked to breast cancer for more than 30 yr, and it has been thought that iodine may play a role in the prevention of breast cancer *(68,69)*. Almost as long ago, it was shown that breast cancers could be detected by RAI and ^{99m}Tc scintigraphy

(70,71). Recently, several investigators have explored the role of NIS as a breast-cancer tumor marker for diagnostic scanning of breast cancer, as well as its potential role in the treatment of breast cancer.

In the rat, mammary gland NIS (mgNIS) protein was found to be expressed during late gestation and lactation only, and was absent in the nubile mammary *(72,73)*. The expression of NIS corresponded to a phase of hormonally controlled glandular proliferation. Tazebay et al. also examined NIS protein expression in a different form of mammary proliferation, that of rat mammary tumors *(72)*. Using transgenic mice, either expressing the activated Ras oncogene or overexpressing the Neu oncogene, they demonstrated NIS protein in both *Ras* and *Neu* mammary tumors. NIS was absent in the contralateral nontumorous mammary tissue of the *Neu* mouse. In human breast-cancer specimens, 87% of 23 invasive carcinomas and 83% of 6 ductal carcinomas *in situ* expressed NIS, as compared to only 23% of 13 adjacent noncancerous tissue specimens *(72)*. None of the eight normal specimens taken from reduction mammoplasty were positive for NIS. The diagnostic value of NIS as a breast cancer marker thus compares well with breast-cancer marker *Her2*/*neu*, which is present in 33% of breast cancers only. The high frequency of NIS expression in breast cancer could be potentially utilized in the primary diagnosis of breast cancer. Conceivably, detection of NIS immunoreactivity in fine-needle aspiration biopsies could assist in cases with uncertain cytologic results.

Breast cancer has been shown to take up greater amounts of RAI than normal breast *(70)*, and the mechanism responsible for this could be the increased expression of NIS in breast-cancer tissue *(72)*. Hyuk Moon et al. recently showed that ^{99m}Tc-pertechnetate uptake correlated with the NIS mRNA expression in human mammary tumors *(74)*. Currently, it is unlikely that either ^{123}I or ^{99m}Tc-pertechnetate will replace conventional mammography supported by ultrasound examination for breast-cancer screening. NIS-based radioisotope scanning for breast cancer may, however, have a role in the evaluation of patients with abnormal mammograms.

An obvious goal of utilizing NIS in breast-cancer tissue would be the treatment of breast cancer with RAI, but several factors need to be considered in this context. One consideration would be the degree of RAI accumulation by the breast tumor. Regulation of NIS in the mammary gland is different from that in the thyroid. In the mammary, oxytocin upregulates NIS expression, and a threshold level of estrogen seems to be necessary for optimal expression of NIS. Prolactin augments the oxytocin-induced expression of NIS, but only in the presence of estrogen *(72)*. It is not known whether breast cancers are similarly regulated, but if so, NIS expression and hence RAI uptake could conceivably be augmented by hormonal manipulation. RA has been shown to induce NIS expression in the estrogen-positive human breast-cancer cell line MCF-7 *(75)*, and may be useful in breast-cancer therapy. A second consideration is whether RAI could be preferentially accumulated in mammary tumors. The difference in regulation between the thyroid and mammary NIS could be utilized. Thyroid hormone could be used to suppress TSH and hence to suppress thyroid NIS expression, thereby allowing uptake of RAI in the mammary tumor. Whether the thyroid suppression of NIS would be sufficient to allow tumor uptake of RAI, however, is not known. A third consideration is the effective half-life of the RAI in the tumor. Although lack of organification by TPO may theoretically lead to rapid efflux and a short effective half-life of RAI, it is not clear whether organification is absolutely necessary for effective RAI treatment. Although no organification of iodide was detected in the RA-treated MCF-7 cells described above—a breast-cancer cell line that cannot organify iodide—the iodide

efflux from these cells was slower than from FRTL-5 thyroid cells, possibly because of the absence of pendrin in the MCF-7 cells *(75)*.

Expression of NIS in breast-cancer tissue has opened an exciting avenue for potential new techniques in the diagnosis and treatment of breast cancer, and we anxiously await the results of future experiments and clinical trials in this field.

NIS GENE THERAPY FOR NONTHYROIDAL CANCER

The ability of thyroid cells to take up RAI leads to the effective treatment of most patients with thyroid cancer, even those with distant metastasis. This is in contrast to most other types of solid cancers, in which distant metastases are most often incurable. With gene therapy, NIS could be expressed in nonthyroidal cancers, and this could potentially allow their treatment with RAI.

Viruses can be used to transfer the NIS gene to the target cancer. This has been successfully achieved in various animal- and human-cancer cell lines. Mandell et al. demonstrated iodide accumulation in several cancer cell lines in vitro and in vivo, including melanoma, liver, colon, and ovarian cancer cells, after retrovirus-mediated transduction of the NIS gene *(76)*. Using a retroviral vector containing the NIS gene, Haberkorn et al. were able to infect a rat hepatoma cell line and establish modified hepatoma cells that expressed NIS and which showed iodide transport in vitro and in vivo *(77)*. Bolan et al. constructed a recombinant adenovirus vector encompassing the rNIS gene under the control of the cytomegalovirus promoter *(78)*. Infection of human cervical tumor cells, mammary-gland tumor cells, prostate tumor cells, and lung and colon tumor cells with this recombinant adenovirus resulted in perchlorate-sensitive iodide uptake. In addition, the infected tumor cells were selectively killed by exposure to ^{131}I, as revealed by clonogenic assay.

The expression of NIS could be limited to the target cancer through the use of tissue-specific promoters. Spitzweg et al. achieved tissue-specific expression of NIS in the human prostatic adenocarcinoma cell line LNCaP by designing an expression vector that coupled hNIS cDNA to the prostate-specific antigen (PSA) promoter *(79)*. Stable transfection of LNCaP cells expressing NIS driven by the PSA promoter showed perchlorate-sensitive and, more importantly, androgen-dependent iodide uptake. No iodide uptake was detected in androgen-deprived cells, and no iodide uptake was observed if the PSA promoter-NIS construct was transfected into prostate-cancer cell lines that did not express PSA. The same group later demonstrated selective killing of PSA-promoter–NIS-transfected LNCaP cells with ^{131}I in an in vitro clonogenic assay *(80)*. Xenografts of the PSA-promoter–NIS-transfected LNCaP cells established in athymic mice showed significant tumor reduction after a single intraperitoneal injection of therapeutic ^{131}I *(80)*.

One of the hurdles of NIS-gene therapy relates to the rapid efflux of RAI, owing to the inability of nonthyroidal tumor cells to organify RAI. This limits the effective biologic half-life of the RAI taken up by these cells. Huang et al. tried to overcome this by cotransfecting both NIS and TPO genes into a non-small-cell lung-cancer cell line *(81)*. This strategy resulted in increased retention of RAI, which was attributed to TPO-mediated organification of the RAI and hence decreased efflux of the RAI. The result was a greater degree of RAI-induced apoptosis in the NIS-and-TPO-cotransfected cells than in cells transfected only with NIS alone.

NIS-based gene therapy has significant potential for the treatment of nonthyroidal cancer. However, numerous practical issues still need to be addressed before this becomes a reality.

Efficient, safe, and tissue-specific in vivo gene-delivery systems need to be developed and tested clinically. Tumor-iodide efflux mechanisms need to be further studied. Strategies to enhance the RAI retention time of tumors, such as cotransfection with the TPO gene as described earlier, need to be designed. Further, RAI effects on NIS-expressing tissues need to be considered.

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